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ANNUAL GENERAL MEETING.

WEDNESDAY, 29th MARCH, 1950.

The Seventy-fifth Annual General Meeting was held in the Society's Rooms, Science House, Gloucester Street, Sydney, on Wednesday, 29th March, 1950.

Dr. R. N. Robertson, President, occupied the Chair.

The Minutes of the Annual General Meeting held on 30th March, 1949, were read and confirmed.

PRESIDENTIAL ADDRESS.

It is my privilege to deliver the 75th Presidential Address to this Society, instituted in 1874 "for the cultivation and study of the Science of Natural History in all its branches". Those of us who have derived social and intellectual pleasure and profit from the activities of the Society, owe a considerable debt to the wisdom and generosity of our predecessors. From the time of its inauguration, when it began with 124 members, the Society has gone on from strength to strength, fostering the study of Natural History. Not only have most local scientists interested in Natural History been active members, but, thanks to the generous benefaction of Sir William Macleay, the Society has been able to stimulate a considerable amount of research. It has survived difficult times—two economic depressions, two world conflicts, to say nothing of the disastrous Garden Palace fire of 1882 when it lost all its possessions and records. Despite these vicissitudes, the Society has established high standards of scientific work and its publications enjoy a high reputation.

It is pleasing to be able to report that the past session, despite some problems which have given the Council cause for concern, seems to have maintained the high standards of previous years. Our greatest problem is to meet the rising costs, particularly of publication, with the reduced income from the Society's investments, due to the decline in interest rates in recent years. We have already found it necessary to reduce the size of the PROCEEDINGS, and now that research in natural history has recovered from its temporary setback during the war, we are receiving more papers than we are able to publish. An approach to the State Government for an increase in the annual grant was not successful and, consequently, at recent special meetings, it has been decided that the subscription, which has been one guinea since 1893, should be increased to two guineas from 1st March, 1950.

The Society held only seven General Meetings during the year, as two meetings had to be cancelled because of lighting restrictions. The attendance at meetings averaged 35. If the interest shown by members is continued, rearrangement of the existing meeting room may need consideration, as the present maximum seating capacity is 50. It has been the Council's policy to arrange meetings to provide, as far as possible, for varied interests. Authors of papers have co-operated by presenting their papers in an interesting manner and a number of notes and exhibits have been arranged. The last meeting of the session took the form of a symposium, "The Surface of the Bacterial Cell", jointly with the New South Wales Society for Experimental Biology. The speakers were Dr. R. J. Swaby, Miss P. M. Rountree, and Mr. W. J. Scott, and a lively discussion followed. At the September meeting an interesting talk of considerable historical interest to the Society was given by Dr. N. W. G. Macintosh on "Crania in the Macleay Museum". Some of the crania described were collected by the late Baron Nicolai Nicolaevitch Miklouho–Maclay on his expeditions to northern Papua and the Philippines; others were from the collection of Sir William Macleay. Dr. Macintosh's paper is noteworthy as being the first anthropological paper presented to the Society for

many years. Our thanks are due to Mrs. P. Messmer, Mr. A. Musgrave, Mr. Ian Fraser and the Linnean Macleay Fellows for notes and exhibits at various meetings.

Exchanges received from scientific societies and institutions totalled approximately 1,480. A number of requests for exchanges was received and new exchanges were entered into with: Ecole Polytechnique de Jassy, Roumania; Anales del Jardin Botanico de Madrid; Matematisk–Naturvetenskapliga Biblioteket, Stockholms Hogskola, Sweden; Gotesborgs Kungl. Vetenskaps– och Vitterhetssamhalle, Sweden. The steady accumulation of periodicals received has caused the Library to become congested, and additional steel shelving has been ordered. Duplicate periodical parts have been disposed of to libraries in Australia and New Zealand, and lists of duplicate books for sale will be available shortly. The Library will require planning to allow for expansion in the future. Loans from the Library, particularly interstate inter-library loans, have increased.

Volume 74 of the Society's PROCEEDINGS, reduced in size because of increased costs, was all published during 1949; it consists of 236 + xxiv pages, 4 plates and 207 text-figures. This is approximately half the size of Volume 73. With the exception of a grant towards one paper, the total cost, £495, was borne by the Society. The PROCEEDINGS have now been placed on a fixed subscription basis for overseas and local non-member subscribers, and this is proving a most satisfactory arrangement. There has been an increased demand from abroad for reprints and parts of the PROCEEDINGS.

The nett return from Science House for the year was £547.

The numerical strength of the Society at 28th February, 1950, was: Ordinary Members, 218; Honorary Member, 1; Life Members, 15; Corresponding Members, 4; Total, 238. During the year 22 new members were elected, one member was lost by death and the names of four members were removed under Rule VII.

In May, the State Government called on organisations interested in fauna protection to nominate three members to serve on the Fauna Protection Panel, under the Fauna Protection Act (1948). The Society asked permission to nominate a member, and later Mr. E. Le G. Troughton was appointed. Other members of this Society who are on the Panel are Mr. J. R. Kinghorn and Professor P. D. F. Murray. The Society was invited by Unesco to send a representative to the International Technical Conference on the Protection of Nature, at Lake Success, in August, 1949. Mr. Troughton, who was abroad at that time, was able to attend this conference, as well as the meetings of the Uno Scientific Conference on the Conservation and Utilization of Resources embracing water, soil, minerals, flora and fauna, and fire control, which was held at the same time. Later in the year the Society was invited to advise the Fauna Protection Panel on the reservation of natural areas.

The Meteorological Bureau asked the Society to advise it on the compilation of lists of plants and animals suitable to use for making phenological observations. This request arose originally in 1947 from a conference of Directors of Meteorological Services. A committee consisting of Messrs. Musgrave, Zeck, Millett, Dr. H. S. McKee, Miss Morris and the Secretary, was formed. This committee has completed a list of plants, and has submitted it to the Meteorological Bureau; a list of insects is not yet finalized. The committee has offered to check the lists for northern Victoria and southern Queensland, and also to advise inland weather observers concerning the collection of insects.

Linnean Macleay Fellowships.

In 1948 the Council appointed four Fellows for 1949, viz., Miss M. Hindmarsh (Botany), Miss A. Millerd (Biochemistry), Miss M. Morris (Zoology) and Miss J. Balmain (Biochemistry). The first three Fellows made satisfactory progress in their investigations during the year. Miss Balmain resigned in June to go to England where she is now working at Dairy Research Laboratories, Reading. She was at the time of her resignation investigating inhibition of microbial dehydrogenase activity by a series of

compounds systematically related to phenol and benzoic acid. Good progress was made but no final results were achieved in the six months during which she held a Fellowship.

Miss Morris continued her survey of the seasonal variation of the plankton of the Hawkesbury Estuary for the first six months of the year. The collection of material was discontinued in May as a full twenty-four months' series of samples had then been collected. The material has been studied qualitatively and the quantitative estimations of the catches are now being carried out. It is hoped that this section of the work will be completed and prepared for publication early this year. A twelve months' series of samples collected by the Fisheries Division of C.S.I.R.O., from various stations from the mouth of the Hawkesbury Estuary to Windsor, has been studied, with the intention of determining to what extent the plankton population varies with changes in salinity. The qualitative and quantitative studies of these catches are now completed and the work is being prepared for publication. An investigation of the mode of action of the foot of the sandsnail, *Uber* (*Polinices*) *strangei*, was completed in March, 1949, and the paper accepted for publication by the Society.

Miss Hindmarsh carried out investigations of the processes concerned with mitosis in plant cells, by studying the relationship of certain compounds to the various stages of cell division. Work on the effects of sulphanilamide and p-aminobenzoic acid on meristematic cells has been continued. Sulphanilamide is known to inhibit cell division by preventing the initiation of prophase and the formation of the mitotic spindle. Earlier work showed that roots from onion bulbs had a very variable number of dividing cells. Some time was spent in improving techniques for growing onion roots which would give more consistent results. To produce aseptic and less variable material, onion seedlings were grown under modified culture conditions. Although untreated onion seedling roots have a fairly uniform number of dividing cells, roots treated with either sulphanilamide or p-aminobenzoic acid solutions are not so uniform. This variability is increased in roots treated with a mixture of the two substances. The conditions under which a typical sulphanilamide inhibition of cell division is produced were found to be different in bulb roots and seedling roots.

Miss Millerd was able to isolate the succinoxidase system from potato tuber (*Solanum tuberosum* L.). The system has been studied aerobically and anaerobically by the standard Warburg and Thunberg techniques. The effects of pH, redox dyes (2:6 dichlorphenolindophenol, thionine and methylene blue) and inhibitors (e.g., cyanide, phenyl-mercuric acetate), on succinic dehydrogenase and on the complete system, have been investigated. The enzymic properties of the system have been compared with those of the corresponding system from animal tissue. The results of these investigations are being prepared for publication.

The Council reappointed three Fellows for 1950: Miss Muriel Morris to a Fellowship in Zoology, Miss Mary Hindmarsh to a Fellowship in Botany, and Miss Adele Millerd to a Fellowship in Biochemistry. We wish them success in their investigations.

Macleay Bacteriologist.

The position of Macleay Bacteriologist was advertised in Great Britain, France, Denmark, Holland, the United States, and Australia, and five applications were received. The Council, on the recommendation of the Bacteriology Committee, considered that the application of Dr. Y. T. Tchan was outstanding and offered him the appointment for a five-year period. I am happy to be able to announce that Dr. Tchan has accepted this appointment and we hope that he will be able to commence his work in a few months. Dr. Tchan, who is of Chinese nationality, has had a distinguished career as a bacteriologist and is at present in charge of the laboratory work and demonstrations in soil microbiology, Institut Pasteur, and of the soils section of the French Colonial Ministry, Paris. Dr. Tchan has specialized in soil bacteriology and has a number of publications to his credit. Arrangements will be made for Dr. Tchan to continue this type of work at the University of Sydney. I am sure that I am speaking for all members of the Society when I say that we are looking forward to a happy and profitable association with Dr. Tchan.

Notes to Members.

We offer our congratulations to Mr. W. H. Maze, who has recently been appointed Registrar at the University of Sydney, to Dr. J. L. Still, who was appointed to the Chair of Biochemistry in the same University, and to Dr. E. V. Mercer, recently awarded the degree of Doctor of Philosophy at Cambridge.

Mr. J. M. Vincent and Dr. N. C. W. Beadle were on study leave in the United States and Great Britain during the year.

Obituary.

It is recorded with regret that Walter John Enright died on 27th September, 1949, aged 75, after having been a member of the Society since 1914. He was born in Maitland and was considered to be one of the finest criminal lawyers in the State, and a noted geologist. For nine years, including two years as Mayor, he served on the West Maitland Municipal Council. Mr. Enright graduated in 1893 as a Bachelor of Arts of the University of Sydney, where he was also one of the first students to graduate under Professor Sir Edgeworth David. Throughout his life he took a keen interest in geology and accompanied Professor David on his survey of the Maitland Coalfields. He was also an expert on aboriginal lore and spent some time living with the remnants of the Kuttung tribe at Port Stephens, and later, at Sawyer's Point on the Karuah River, was admitted as a member of the tribe. In recent years he accompanied Professor A. P. Elkin to the north coast of New South Wales to gain further knowledge. Mr. Enright had been a President of the New South Wales Anthropological Society, Fellow of the Anthropological Institute of Great Britain and Ireland, Fellow of the Royal Geographical Society of Great Britain, member of the Geographical Society of New South Wales, Member of the Royal Society of New South Wales, Member of the Royal Australian Historical Society, and of the Ornithologists' Union.

The Last Haunts of Demons: A Comparative Study of Secretion and Accumulation.

PROLOGUE.

In addressing you on this occasion, I am conscious of the changes in biological thought which the Society has witnessed during the 75 years of its existence. Two extracts from the first two Presidential Addresses by Mr. William, later Sir William, Macleay, will be sufficient to remind you of the state of biological knowledge 75 years ago. Of the theory of evolution, that topic of intense controversy, he said, "It is evident, I think, from the general tenor of scientific literature for some years past, that the evolution theory, long so unpopular, and which under Lamarck's teaching gained so few proselytes, has, under the superior fascinations of Darwin's admirable work, 'The Origin of Species', become the fashionable faith. But what may be generally believed is not necessarily true or worthy of belief". And a year later, after discussing the same topic, he goes on, "But the settlement of such a question as that of *spontaneous generation* is a matter of much more importance to the world at large than the most ingenious speculations upon subjects of which we know nothing, and are never likely to know much. The chief scientific discovery of the year is the entire disproval of the doctrine" (of spontaneous generation).

Subsequently the Society witnessed the acceptance of the theory of evolution and the gradual enlightening of biological thought by the glow of this brilliantly co-ordinated material. After the acceptance of the theory of evolution, perhaps the most marked changes in biological thought during the Society's existence were due to the continued success of explanations of living mechanisms in terms of the properties of matter. During the period there has been a receding of those troubled waters which have characterized the science of biology for generations, when biology was subjected alternately to the vitalistic flood and the mechanistic tide. We have gradually settled to something less turbulent. The biologist is prepared to continue his investigations without the unshakable convictions and furious polemics which characterized this age-old issue. I do not propose to address you on the vitalist-mechanist controversy but rather, in attempting an address sufficiently wide to interest more than one section of

our members and sufficiently specialized to be of some scientific value, to describe a physiological mechanism which shows similar principles in two widely separated organisms. Our Society started when comparative morphology was the fashion in biology: our acceptance of the theory of evolution has led us to look for similar principles underlying physiological mechanisms in different organisms. I wish to discuss this one particularly because it has only recently yielded to the physico-chemical approach.

One of my most distinguished predecessors in this Presidential Chair, Professor J. T. Wilson, devoted some portion of his Presidential Address of 1898 to the problem of how far mechanism, i.e., physico-chemical theories are capable of being utilized in the explanation of the phenomena of living activity. And in his own words a year later, "ventured to state the conviction that, in so far as a strictly scientific or natural-historical representation of these phenomena is the object aimed at, this can only be given in terms of physical cause, or mechanism". This early and clear warning as to the logically dangerous position of the vitalistic exponents is still worth reading today. In the subsequent year, Professor Wilson, who was still President, found it necessary to return to this topic, for meantime there was published, in the "Nineteenth Century", a paper by the great physiologist, J. S. Haldane, who stated the neovitalist position. To Haldane, the physiologist, it became unbelievable that the complexity of the processes undertaken by certain organs of animals could be explicable in terms of physics and chemistry. "To any physiologist", he writes, "who candidly reviews the progress of the last 50 years, it must be perfectly evident that, so far from having advanced towards a physico-chemical explanation of life, we are in appearance very much further from one than we were 50 years ago." In particular, Haldane found it difficult to believe that the explanations of secretion, growth, development, nutrition, heredity and excitability should be purely mechanical. You will be familiar with the progress that has been made in the physico-chemical explanations of some of these phenomena, but it is particularly with that of secretion that I wish to deal.

The Search for Demons.—Let me explain why secretory processes should present very special difficulty to a physico-chemical explanation. We know that the "no perpetual motion" law, the second law of thermodynamics, however this concept is stated, is obeyed by all the physical universe of which we have experience. This law, which means that energy cannot flow from a low level to a higher one, is obeyed in all its forms. Thus a warm body cannot grow warmer at the expense of a cooler body. A low source of electrical potential does not charge a cell with a higher electrical potential. Molecules do not diffuse from where they are in low concentration to where they are in high concentration, so secretion and accumulation, defined as movement of ions in biological systems against concentration gradients, would appear, at first sight, to negate the second law of thermodynamics. It is, perhaps, no wonder that some began to look for something special in the way of a force, not familiar to physics. We have the suggestion from Johnstone (1914) and from Lewis and Randall (1923) that there may be phases within a living cell in which the second law of thermodynamics is not obeyed. As late as 1940, in a discussion of secretion, R. W. Gerard wrote, "Can living things, then, transcend the second law? Is this a basis for 'vital' action? Some have thought so, and possibly the time has not yet come to consider the question closed. None the less, the probabilities are strongly against it".

How can the plant or animal cell bring about the movement of substances in such a direction as to reverse the spontaneous movement we should expect on statistical physical laws? Let me confess that I have always been fascinated, as have many others, with the idea of the demon of Clerk-Maxwell. He suggested that a minute being, able to see individual molecules, operating a frictionless door in a wall separating two compartments of a vessel filled with gas would be able to take advantage of the natural movement of the molecules to separate them into a compartment of fast moving molecules and a compartment of slow moving molecules. As every fast moving molecule approaches from the left the demon opens the door but closes it again to every molecule approaching from the right, so more fast moving molecules are accumulated on one side

than on the other. Thus, such a demon residing in the living cell would be able to open the surface doors to the desirable molecules, to shut them fast to those that were undesirable, and soon all required would be inside. The suggestion that the living cell in secretion or accumulation might operate by a mechanism which is unknown in physics is tantamount to postulating the existence of such a demon. It will be my purpose in this address to show that no such demon is necessary. Let me provide a clue at the outset. The Maxwellian demon could counteract the physical law only so long as he himself did not draw on any energy for his existence or for his work in the opening and shutting of doors. The living cell is not short of energy and our problem is one of how the energy is applied to secretion or accumulation.

Defining the Problem.—This property of living cells of transferring substances from a solution in which the concentration is low to a solution in which the concentration is high seems to be fairly general. It is useful to speak of the mechanism by which this is brought about as the mechanism of *active transport*, a term which will be used through this address to distinguish the movement of the substances into or out of living cells by a process other than diffusion or ionic exchange. It is useful further to distinguish those instances in which a substance is transferred from the exterior to the interior of the cell against a concentration gradient, referred to here as *accumulation*, from those in which the substance is transferred to a high concentration outside a cell which will be referred to as *secretion*.

There are many examples of secretion and accumulation of a wide variety of compounds, and no attempt is made here to list either the types of secretion or of secretory organs. It is of interest to members of the Society to note that the Presidential Address of Professor W. J. Dakin in 1935 on the subject "The Aquatic Animal and its Environment" was largely concerned with the consequences of the secretory mechanisms responsible for the maintenance of the steady state difference between the body fluids and the external environment. It is interesting, too, that even at that date Professor Dakin acknowledges our almost complete lack of knowledge of the mechanism whereby these differences are maintained. Other familiar examples are to be seen in the kidneys, in the intestine of animals, in the gastric mucosa, in frog skin and in plant cells. Substances undergoing active transport seem to range from water and individual ions to organic molecules such as sugar. The problems relating to this wide variety of fields have been reviewed in books such as those by Hoagland ("Lectures on the Inorganic Nutrition of Plants", 1944) and Höber ("Physical Chemistry of Cells and Tissues", 1946). The mechanisms have been the subject of review articles such as those by Krogh (1946) and Ussing (1949).

In this address no attempt will be made to cover all these fields. It is proposed to review the work on two tissues, widely separated in evolution—the parenchyma cells of plant roots and the oxyntic cells of gastric mucosa in frogs. These two organs are chosen partly because of the detailed work which has been done, and partly because, in their physical similarities, despite evolutionary separation, they illustrate what may be an underlying principle of general application to all secretory and accumulatory organs.

Historical.

Ion Accumulation in Plants.—Apparently it was a long time after the discovery by de Saussure (1804) of the uptake of different substances in varied proportions that it was realized that some complicated mechanism of accumulation was operating in the plant cell. At first it was thought that the uptake of these various substances was dependent entirely upon their power of diffusion into the cells by virtue of being dissolved in water and that the differences in proportion were due to the utilization or destruction of the compounds after absorption. Thus Sachs, in his textbook, states (using A. W. Bennett's translation, 1875): ". . . will be clear that the accumulation of certain substances in the interior of plants depends in the first place on whether the compound of that which is present in the surrounding water is decomposed by the plant; that, moreover, the constituents of the different compounds must accumulate in the plant in definite quantities according to the extent in which they are needed;

and that finally the quantitative composition of the substances in question within the plant usually bears no resemblance to that of the surrounding water." Leunis (1883) quotes examples of selective uptake, such as those of De Saussure, but states that the process is based on the laws of osmosis. Nathansohn (1901) showed that the chloride content of a seaweed *Codium tomentosum*, transferred from sea water to an isotonic solution free of chloride, did not approach physiological equilibrium as long as the material remained turgid. He further showed, in 1903, that there was an unequal absorption of the two ions of any salt. In the early years of this century, it came to be realized that there was something other than diffusion and utilization of ions involved in the absorption by plant cells. From this time on there was a number of examples which showed, first, that ions entered plant cells independently of other ions present in the solution, in quantities which differed widely from the proportion in which the ions were present in the external solution, and, second, that the entry of ions was not governed by the concentration differences between the internal and external medium. Spectacular examples of this kind of ionic movement into cells were obtained. For instance, it was shown that marine organisms frequently had very high concentrations of potassium compared with the concentration in sea water, and the concept of differential permeability was established. It was, however, still thought that the protoplasmic lining of the plant cell constituted a semipermeable membrane through which the dissolved substances diffused to the interior of the cell, but which exercised a certain degree of selective permeability so that some ions were barred where others could enter. During the first twenty years of the century, due largely to the work of Osterhout, who published various papers on this topic, of Pantanelli (1915), of Stiles and Kidd (1919), and of Redfern (1922), it became clear that entry by exchange accounted for the unequal absorption of ions from the external solutions. In exchange phenomena, ions entered in exchange for mobile ions already present in the tissue; these mobile ions passed to the external solution. At the same time, however, it was shown that absorption of both anions and cations from a dilute solution often resulted in much higher concentrations in the cell than in the external solution and that no corresponding ions appeared in the external solution. Hence the phenomenon of accumulation of ions, which could not be accounted for by simple exchange, was clearly established. By 1930, it was possible to distinguish three modes of entry: (1) by diffusion, (2) by exchange, and (3) by the active transport or accumulation mechanism which resulted in relatively high internal concentrations.

During the next few years, two additional discoveries were made. The first was that the accumulation process was connected in some way with the metabolism of the cell and especially with aerobic respiration. It is in connection with this work that the name of Steward is particularly associated. In a series of papers on cut potato tissue beginning in 1932, Steward (vide Steward, 1937) showed the very close dependence of the accumulatory mechanism upon the uptake of oxygen by the tissue. This work was confirmed for other tissues, particularly for accumulation by barley roots, by Hoagland and Broyer (1936). At the same time in a series of researches commencing in 1933, on the relation between the respiration rate and the uptake of salt by wheat roots, Lundegårdh and Burström showed that an increase in the salt accumulation by roots was accompanied by an increase in the oxygen uptake. At that stage Lundegårdh and Burström (1933) described this increase in respiration rate as the anion respiration, a discovery which was to lead subsequently to a useful hypothesis of the mechanism of accumulation.

Up to 1930, in spite of the knowledge of accumulation, there had been few attempts to formulate a possible mechanism. Evidence from the work on large cells like those of *Valonia* and *Nitella*, where the vacuolar sap can be obtained with a minimum of injury to the cytoplasm, suggested very strongly that the accumulation occurred with both ions free in solution in the vacuolar sap. Briggs and Petrie (1928) showed that the observed accumulation could not be accounted for by ionic exchange or Donnan equilibria, unless it were assumed that the cell consisted of two separate phases with

the cation accumulating in one and the anion accumulating in the other; this seemed to be an unlikely explanation of the phenomenon.

S. C. Brooks (1929) suggested that accumulation could result from exchange of cations for hydrogen ions and of anions for bicarbonate ions formed from the respiratory carbon dioxide, the nett result being the entry of both ions of a salt from the external solution. Brooks considered it would be necessary to postulate that the cell maintained a mosaic of cation permeable and anion permeable areas through which the cations and anions respectively might exchange. Briggs (1930) criticized this hypothesis of Brooks and pointed out that a mosaic would be inadequate to account for accumulation by exchange for two ions, such as bicarbonate and hydrogen passing to the exterior of the cell. The same criticism applies to the modified hypothesis by Brooks (1937). Briggs pointed out that it would be possible for an exchange mechanism to bring about accumulation only if there were a phase of cationic permeability alternating in time with a phase of anionic permeability. While this system would be satisfactory on theoretical, thermodynamic grounds, it could not be tested experimentally.

So, by 1935, the dependence of the mechanism on respiration had been clearly illustrated, but a satisfactory hypothesis of the connection between respiration as the energy providing process, and accumulation as a mechanism, had not been formulated.

Hydrochloric Acid Secretion in Animals.—The realization that hydrochloric acid is responsible for the acidity of the stomach of animals was arrived at early by at least some investigators, for in 1824 Prout advanced the view that the acid in the stomach was "free or, at least, unsaturated muriatic acid". In spite of this there seems to have been a long period of uncertainty as to whether the acid so apparent was, in fact, hydrochloric acid, and a good deal of uncertainty as to its origin. Claude Bernard* (1859), for instance, reached the conclusion that the gastric secretion did not acquire its acid properties until it reached the superficial region of the mucosa and had mixed with foods of the stomach, a belief which amounts to the suggestion that the acid is formed externally to the glands and possibly by a fermentation of the mucus. Brücke (1859), on the other hand, reached the opinion that the acid was formed in the glands. Other investigators, after repeated attempts, were unable to associate the acid formation with the glands themselves. It was not until this century that further work, with modified micro-chemical techniques, by Fitzgerald (1910), finally established that the hydrochloric acid was free in the secretion which appears in the canaliculi, but left open the question of whether the acid or its hydrogen ions occurred in the cytoplasm of the cells. It was pointed out that the cytoplasm must constitute a membrane permeable to H^+ and Cl^- ions, but not to Na^+, K^+, PO_4^{---}, or HCO^-_3, and here Fitzgerald makes the suggestion that "if we suppose that the cytoplasm is so constituted as to maintain the ionisation of the hydrogen and the chlorine in the presence of alkalies, we attribute a property to it that could also be responsible for the formation of acid in the first place in the cytoplasm".

As far back as 1845, Bence Jones had suggested the hypothesis that the formation of acid frees alkali, and in 1859 Brücke assumed that if free acid were liberated in the gastric tubules, an alkali of corresponding strength must pass into the blood and lymph. Further extensive work showed that the alkali liberated to the blood by the stomach was equivalent to the hydrochloric acid secreted.

It is not proposed to give a complete historical account of the work in relation to the secretion of HCl. This work has been well summarized by Hollander (1943), and by Babkin (1944). By 1940, it was clear that hydrochloric acid passed from the gastric mucosa into the stomach and simultaneously an approximately equivalent amount of alkali was liberated into the blood. The importance of carbon dioxide in relation to this alkali had been suspected, but the relation was elucidated only by the discovery (Davenport, 1939) of a high concentration of carbonic anhydrase in the parietal or oxyntic cells of the mucosa. Some investigators, notably Ni and Lim (1928), Hanke (1937) and Teorell (1933), had compared the rates of secretion and of respiration without formulating any comprehensive hypothesis as to the mechanism.

* Quoted from Fitzgerald (1910).

Recent Experimental Evidence.

Accumulation in Plant Cells.—Entry of ions by the phenomena of diffusion and exchange may be important under certain circumstances, but, generally speaking, these lend themselves to a comparatively straightforward physical explanation. Diffusion to equality of concentration inside and outside the cells does not seem to be a very common phenomenon. Whereas cations diffuse and exchange readily between the cell wall, cytoplasm and external solution, such movement by anions is restricted because of the large number of indiffusible anions (e.g., proteins) which cannot escape from the cell, particularly in the cytoplasm. This has been illustrated, for instance, in discs of carrot tissue by the electrical diffusion potential which is established when salt is allowed to diffuse along a concentration gradient through the tissue (Briggs and Robertson, 1948). This restriction of entry of anions from the external solution, due to the presence of a high percentage of indiffusible anions in the cytoplasm, may well account for much of the high resistance of the cytoplasm to the entry of salt (vide Frey-Wyssling, 1948). Local concentrations of lipoids may also contribute to this resistance, but the importance of the occurrence of lipoid areas in plant cells has not been fully assessed.

Because of the high percentage of indiffusible anions, many of which are accompanied by diffusible cations in the cell, most of the exchange which occurs is cationic. The nature and quantity of the ions leaving the cell depend both on the previous history of the tissue and on the composition of the external solution. As the ions from the interior of the cell are replaced by ions exchanging from the external solution, the subsequent changes in ionic balance may affect the level of metabolic intermediates, some of which, such as the organic acids, are themselves ions. This has been well illustrated by the work of Ulrich (1941) and of Burström (1945). It can be shown, for instance, that when entry of cations exceeds that of anions, as when potassium bicarbonate is applied, in the external solution, the excess cations are balanced by an increase in organic acid anions and an increase in the total organic acid in the cell. When, as from solutions with a divalent cation, the uptake of anions exceeds that of cations, the organic acid anions and the total organic acid in the cell decrease. These changes in organic acids are related to changes in respiration rate, which are consistent with the acids being intermediates of the respiration process.

Despite the interesting connection between exchange phenomena of this type and the entry of ions, this mechanism alone is not adquate to explain the active transport process which brings about the accumulation of both cations and anions in the cell without the appearance of an equivalent quantity of ions in the external solution. It is necessary here to explain some of the characteristics of the process of salt accumulation before an attempt is made to relate it to the respiration with a view to formulating a hypothetical mechanism.

The ions which are absorbed seem to exist in free solution in the cells and are not absorbed in high concentration because they are destroyed or combined after entry; two kinds of evidence suggest that the ions are free in solution. The ions are liberated readily on rupture of the cells indicating that they are not adsorbed on the cell constituents. In the large cells of *Nitella*, it has been possible to withdraw solution from the vacuole with very little injury to the cytoplasm and to find the accumulated ions free in this solution. Further, the accumulated ions exert the expected osmotic pressure.

The accumulatory power of the cells is considerable. Many figures have been published which illustrate the high ratio of the concentration of a particular salt in the cell to the concentration of the same salt in the external medium. It is common, for instance, to find that tissue is capable of reducing the external concentration almost to zero and simultaneously of building up an internal concentration which is hundreds of times more concentrated than the external solution. The rate of accumulation of salt is of the order of $0{\cdot}4 \times 10^{-5}$ gram molecules per gram of tissue per hour. This rate of accumulation does not remain constant with time, being highest immediately after the salt comes into contact with the surface of each cell, and gradually falling as the

internal concentration rises. During this period it is necessary to distinguish the entry of the salt by the accumulation process, from the entry of the individual ions by exchange; these two processes have been examined in detail by Stiles and Skelding (1940, *a*, *b*) and Stiles and Dent (1946). The accumulation rate proper falls, however, as the internal concentration of salt increases. At no stage is there any suggestion that the entry of both ions of the salt along a concentration gradient is important, for the rate of accumulation does not seem to be altered sharply after the internal concentration becomes equal to that of the external solution.

When the accumulation rates are compared by taking initial rates at different concentrations, it is found that the concentration of the external solution limits the rate at low concentrations, but that the rate approaches a maximum asymptotically as concentration is increased. This shows very clearly that the accumulatory mechanism is basically dependent upon some internal factor in the cells and, except at very low concentrations, is not a function of external concentration. Temperature increases the rate of accumulation and the calculated energy of activation suggests that the active transport is dependent not on a diffusion process, but possibly on a chemical process. The salt that is accumulated is retained by the cell and is not lost to the external solution even when the concentration in the external solution is reduced to a low value by the absorption of salt, or by transferring the tissue back to water. The process of accumulation can be influenced by various inhibitors; the evidence from inhibitor experiments will be discussed later when accumulation and respiration are being considered.

Since the accumulation of salt cannot be brought about (unless one admits that it defies the second law of thermodynamics) without the expenditure of energy on the part of the cell, it is natural that investigations should be designed to determine the relationship between the rate of respiration and salt accumulation. Lundegårdh and Burström (1933) showed that increase in salt accumulation was accompanied by an increase in respiration rate, which they termed the anion respiration. This respiration, which is also known as the salt respiration, was subsequently shown to occur in various tissues (Steward, 1937, Robertson, 1941). It is unfortunate that an early controversy about the validity of this concept of anion or salt respiration obscured the vision of a number of investigators and prevented the ready recognition of the value of this discovery as a basis for a hypothetical mechanism. It can, however, be shown in a variety of tissues that, if cells have been respiring in water, the addition of salt brings about an increase in the rate of respiration which may, under some circumstances, be as great as 100%. This increased rate of respiration is achieved in a very short time (almost as quickly as the salt reaches the surfaces of all the cells), and subsequently continues as long as the salt concentration at the surface of the cell is maintained, despite the change in the rate of accumulation. Salt respiration, as this increase will be termed here, increases with external concentration of salt at low concentrations, but becomes independent at higher concentrations. It is increased by increasing temperature and has a high energy of activation (Robertson, 1944). Transfers of tissue from salt solution to water are followed by a slow decline in the rate of salt respiration (Robertson and Thorn, 1945).

It soon became clear that, at least in those tissues examined in detail, the accumulation mechanism was bound up with the extra respiration which results from the stimulus of the salt applied to the external surface of the cells. The accumulatory mechanism is not dependent merely upon the respiratory level but appears to depend upon some specific stages in the respiration. For instance, most tissues will cease to accumulate and may even lose salt already accumulated if changed from aerobic to anaerobic conditions. Further, the addition of methylene blue, which actually increases the oxygen uptake of respiring tissue, results in a cessation of the accumulatory process (Hoagland and Broyer, 1942). It was, however, the evidence from experiments with inhibitors which established firmly the dependence of the accumulation on the salt respiration. It was found that the salt respiration was inhibited by cyanide, azide and carbon monoxide; these inhibitors also stopped accumulation. These same inhibitors

were either without effect on the basal respiration of tissue respiring in water, or inhibited it only partially (vide Robertson and Turner, 1945). Since it was generally considered that sensitivity of these inhibitors indicated a cytochrome (iron-porphyrin) system as the carrier of hydrogen ions and electrons to the oxygen involved in respiration, this suggested that accumulation was probably linked in some way to the operation of a cytochrome system. This suggestion was first made by Lundegårdh.

The first attempt to obtain a quantitative relation between the amount of salt accumulated and the salt or anion respiration were made by Lundegårdh and Burström (1933). They showed the factors which increase salt respiration also increase the salt accumulation and, taking this together with the inhibitor evidence, Lundegårdh developed a hypothesis for the relation of the accumulatory processes to the cytochrome system. This hypothesis, which was first stated briefly in 1939, has been modified from time to time and is here given in the form which was restated by Robertson and Wilkins (1948). Lundegårdh's hypothesis depends on the fact that when hydrogen atoms are released from respiratory intermediates by the dehydrogenases, the electron of each hydrogen atom passes to the cytochrome carrier, and the hydrogen ion is liberated into the medium. Subsequently, by the intervention of cytochrome oxidase, the electron passes to an atom of oxygen, the hydrogen ion is picked up from the medium and, on the transfer of two electrons and two hydrogen ions to one oxygen atom, a molecule of water is formed.

Lundegårdh suggested that a substance such as cytochrome which carries electrons in one direction because of the change in valency in the iron atom could carry anions in the opposite direction. Simultaneously, the hydrogen ions liberated as the result of the dehydrogenase activity pass to the exterior of the cell, and it was assumed that the cations from the exterior would enter the cell by exchange with those hydrogen ions. Thus the driving force for the accumulation against the concentration gradient would be the continuous production of positive and negative charges which traverse the cell boundary in exchange for the ions of the external solution. Since the hydrogen ions and electrons are ultimately united with oxygen to form water, ions disappear from the external solution.

Lundegårdh's hypothesis suggested the possibility of putting the relation between ion accumulation and salt respiration on a quantitative basis. If the Lundegårdh hypothesis were correct, the maximum possible rate of accumulation would occur when one anion enters as one electron is transferred by the carrier system, and when, simultaneously, one cation enters in exchange for the corresponding hydrogen ion. Since salt respiration has a respiratory quotient of unity and may therefore be considered as a normal carbohydrate respiration, the overall quantitative relation between oxygen uptake and water formation may be represented in the following equation:

$$C_6 H_{12} O_6 + 6O_2 + 6H_2O = 6CO_2 + 12H_2O$$

This allows for the fact that all the oxygen which enters into the respiratory process makes its appearance as water, and each oxygen molecule taken up in respiration is therefore balanced by four hydrogen atoms. This means that for each oxygen molecule, four hydrogen ions are required at the cytochrome oxidase and four electrons have traversed the cytochrome carrier system. Thus the maximum theoretical accumulation rate on the Lundegårdh hypothesis should be four times as much monovalent salt accumulated as oxygen taken up in the salt respiration process when both are expressed in gram molecules. To test this hypothesis quantitatively, we carried out a number of experiments on carrot tissue (Robertson and Wilkins, 1948). These experiments, designed to compare the rate of accumulation and the rate of salt respiration when neither was limited by the external concentration of salt, showed that the ratio of equivalents of salt accumulated to gram molecules of oxygen consumed in salt respiration approached the hypothetical value of four. Thus these quantitative observations which are not in themselves proof of Lundegårdh's hypothesis, are consistent with the possibility that the production of hydrogen ions and electrons in respiration is the driving mechanism for the accumulation of salt in the cells.

Many other factors must be taken into account before this hypothesis can be regarded as complete. Some of these factors have been considered by Lundegårdh (1940, 45, 49). It is necessary to postulate either that there is a specific carrier mechanism for the anions and a separate carrier mechanism for the cations or that, as Briggs has suggested, there is a short period during which the cytoplasm is permeable to anions followed by a period in which the cytoplasm is permeable to cations. If this occurred with a cell in a solution of potassium chloride, for instance, hydrochloric acid would be formed in the cell during the anion permeable phase, with the anion from the external solution balanced by the hydrogen ion liberated from respiration. If then the cell became permeable to cations and temporarily impermeable to anions, the hydrogen ions could be exchanged to the exterior for the potassium ions of the external solution. The significance of this will be discussed subsequently in consideration of the theory of secretory and accumulatory processes.

Whatever the mechanism of active transport of ions across the cell to the vacuole, it is necessary to postulate some degree of resistance to the back diffusion of ions which have been accumulated in the cell in a concentration greater than that of the external solution. It is not yet clear where this resistance to back diffusion occurs. It seems, however, likely that the resistance to ionic movement by free diffusion is quite high since all impedance measurements of plant cells (cf. Cole and Curtis, 1938) indicate a high resistance. Further, it seems unlikely that the mechanism of active transport would be adequate to cope with back diffusion of ions if the resistance of the cytoplasm were as low as that of water. This can be seen in experiments in which inhibitors such as cyanide are used to block the active transport mechanism, when only low rates of leakage from the cells are observed. Lundegårdh considered that most of this resistance to back diffusion is due to a surface layer of lipoid, similar in structure to the Langmuir monolayers. This view is supported by Holm-Jensen, Krogh and Wartiovaara (1944) from their work on *Nitella* and *Tolypellopsis*. The evidence for most of the resistance of the cytoplasm being at or near the tonoplast has been discussed by Arisz (1945). The position of the high resistance zones of the cytoplasm requires further investigation.

Any hypothesis of the mechanism of salt accumulation must also make allowance for the specificity of absorption of different ions. On any simple electrochemical theory, no marked difference would be expected between, for instance, the absorption of sodium and that of potassium, but in many biological systems the cells may absorb one of these ions in much greater quantity than the other. It seems therefore necessary, as pointed out by Ussing (1949), to postulate that an entry of ions by the active transport mechanism occurs as the result of temporary combination with cell substances whose combining power with different ions varies considerably. That such substances exist in biological systems and differ in their combining power, even between similar ions such as sodium and potassium, is a very reasonable possibility (vide Franck and Mayer, 1947), but so far no such substance has been identified as a carrier in active transport mechanisms.

Recent work has suggested the possibility that the accumulatory mechanism may, to some extent, depend upon the phosphate transfer system of the living cell. Since this system is the best understood energy-transfer mechanism linked to the respiratory process, it is not surprising that there have been suggestions for some time that the accumulation mechanism might depend on it (Hoagland, 1944; Nance, 1949). Some evidence for this suggestion has recently been obtained with work on the effect of 2, 4-dinitrophenol which has been shown to inhibit the transfer of energy-rich phosphate from respiration (Loomis and Lipmann, 1948). Robertson and Wilkins (unpublished data) have found that this compound inhibits accumulation without depressing respiration. It is not yet clear whether the phosphorylations are directly concerned in the active transport mechanism or whether this inhibition of accumulation is due to some disorganization of the cytoplasmic structure consequent upon the inhibition of phosphate transfers.

Lundegårdh has tried to correlate the accumulatory mechanism of roots with the electrical potential difference obtained across the root surface and influenced in various ways by the presence of ions in the external solution. These potential differences are difficult to interpret because of the complexity of the system across which the difference is measured. Lundegårdh himself believes that this potential difference and modifications obtained changing from one solution to another, are associated primarily with the outer surface of the epidermal cells of the root, and bases this belief upon the rapid change in potential consequent on a change in the external solution (Lundegårdh, 1941). Ions absorbed by the cut root are concentrated in the sap bleeding from the cut end, which is at a potential different from that of the external solution. The maintenance of this potential difference may assist in the movement of one of the ions of a salt, but can hardly, on our present knowledge, be related directly to the active transport of both ions. Lundegårdh's results are important, however, and suggest a causal relation between the magnitude of this potential difference and the respiration rate.

So far, therefore, evidence suggests that there is a relation of the accumulatory mechanism to the later stages in the respiratory process and that the continued production of positive and negative charges, resulting from the cytochrome system, might provide the energy necessary for the accumulation of ions from the external solution. Any such hypothesis, however, requires to be adequately co-ordinated with observations on the specificity of ion accumulation and on the effect of 2, 4-dinitrophenol which may be assumed to be preventing phosphate transfer.

Hydrochloric Acid Secretion.

The process of secretion by the gastric mucosa results in the production of hydrogen ions in concentrations exceeding (by about three million times) the concentration in the blood. Simultaneously, chloride ions are secreted in approximately equivalent concentrations to the hydrogen ions and in higher concentrations (by about 1·6 times) than the chloride of the blood. Secretion can continue over long periods, working at the rate of 10,000 calories per gram molecule HCl secreted, and is dependent upon the respiration. Following the work of Davenport, it was suggested by Davenport and Fisher (1940) that carbon dioxide, which is changed to carbonic acid by the action of carbonic anhydrase, is associated with the formation of hydrochloric acid and perhaps directly concerned in its production. Subsequently, however, following very careful study of the secretion of hydrochloric acid in isolated pieces of frogs' gastric mucosa, Davies has shown that the activity of carbonic anhydrase is associated with, but is not the direct cause of the acid production. This work by Davies and his collaborators, like that of investigators of accumulation in plant tissue, has attempted to establish the quantitative relationship between the stimulated respiration, which occurs when the mucosa is acted on by histamine to commence secretion, and the hydrochloric acid production. The stimulated respiration is sensitive to cyanide as an inhibitor and secretion ceases when this respiration is inhibited. Anaerobic conditions also bring about a cessation of secretion. Respiration but not secretion is stimulated by brilliant cresyl blue and fluoride, whilst respiration is increased but secretion is inhibited by azide and 2, 4-dinitrophenol. The similarity of the secretory mechanism and the stimulated respiration to accumulation and salt respiration in plant cells, except in behaviour to azide, might therefore suggest an underlying principle common to both mechanisms.

In recent years, Davies and his co-workers have examined the quantitative relation between the amount of oxygen uptake in the stimulated respiration and the hydrochloric acid secreted by the oxyntic cells of the mucosa. Earlier workers had not produced adequate evidence of this quantitative relation, though it can be calculated from the data of some workers that the amount of HCl secreted was in considerable excess of the amount of oxygen absorbed. In 1948 Crane and Davies showed that 11 gram molecules of hydrochloric acid were secreted for each gramme molecule of oxygen taken up. Consequently the simple relationship of ionic secretion to the hydrogen ion production resulting from and electron transport through the cytochrome system, must be modified to apply to the secretion of hydrochloric acid.

The gastric mucosa presents excellent material for study of phenomena of this kind because it is possible to determine the electrical potential between the secretory and non-secretory sides of the mucosa, and even to impose external electrical potentials across the secretory system. In the resting condition the secretory side of the isolated mucosa is about 30 mV negative in the external circuit to the non-secretory side, when both are in a biological saline medium. Experiments with silver chloride and glass electrodes show that the mucosa in the resting condition is apparently impermeable to chloride ions and to hydrogen ions in the range from pH5 to pH8. If connected electrically, the two sides of the mucosa can provide electric power continuously. When secretion begins, the potential difference across the mucosa falls and the resistance rises. When the natural potential difference is enhanced by passing a current through secreting mucosa, the rate of secretion is increased and when the natural potential difference is opposed, the rate of acid secretion is decreased or stopped. Clearly, the secretion of the hydrochloric acid is associated with the activity of an electro-chemical system which succeeds in separating hydrogen ions, not only those from the dehydrogenases of respiration, but also from some other source, from their corresponding hydroxyl ions. The hydroxyl ions have a very short existence because under the action of carbon dioxide and carbonic anhydrase, they are combined to give bicarbonate ions which then pass back from the oxyntic cells into the blood stream in the "alkaline tide" which has been recognized for some time. An exactly equivalent amount of acid and alkali are produced, the acid being secreted to the lumen of the stomach and the bicarbonate passing to the blood stream. Following the early theory enunciated by Crane, Davies and Longmuir (1948), Davies and Ogston (1949, 1950*) have extended the theory to make specific proposals for a way in which metabolic energy is produced and applied to the secretion of this quantity of hydrochloric acid. As this theory is of considerable importance in its possible general application to secretory and accumulatory processes, it will be discussed in some detail.

Davies and Ogston distinguished two mechanisms by which hydrogen ions may be generated, either from the substrates of respiration or from water. The first mechanism is essentially the same as that discussed already, based on Lundergårdh's hypothesis for the accumulation of salts in plant cells. By this mechanism the maximum production of hydrogen ions is four per molecule of oxygen. The second mechanism proposed uses the energy of the unstable phosphate esters to transport the hydrogen ions derived from water. It is suggested that a carrier substance reacts with hydrogen ions and a reduced iron-porphyrin to give reduced carrier and oxidized iron-porphyrin. This reduced carrier then becomes phosphorylated by creatine phosphate and diffuses through a region of the cell which is impermeable to free hydrogen ions. It then liberates hydrogen ions as the result of losing inorganic phosphate and undergoing oxidation by the iron-porphyrin enzyme. The ferrous-ferric system returns to its initial state by transferring electrons, the carrier and the phosphate diffuse back, chlorides diffuse to the hydrogen ions to maintain electrical neutrality and the nett result is the hydrolysis of unstable phosphate and a transport of hydrochloric acid. This mechanism can transfer two hydrogen ions per phosphate, so that if the phosphate/oxygen ratio is two, eight hydrogen ions may be transported with one oxygen molecule taken up.

If the two mechanisms are working together, it is possible for a maximum secretion of twelve hydrogen ions per oxygen molecule absorbed. Thermodynamic considerations showed that the free energy of the hydrolysis of creatine phosphate would be sufficient to account for the secretion of two hydrogen ions per phosphate.

Simultaneously, Davies and Ogston make some suggestions as to the observed potential difference. It is suggested that the outer surface of the resting oxyntic cell is impermeable to H^+ ions and to Cl^- ions so that the surface acts, relative to the interior, as a reversible sodium electrode. Inside the cell, hydrogen ions and sodium ions are maintained at different concentrations by the secretory mechanism. At the onset of secretion the permeability of the outer surface of the cell is changed so that it becomes

* I am indebted to Dr. Davies and Dr. Ogston for permission to see this manuscript before publication.

permeable to hydrogen and to chloride. This tends to cause the observed drop in the potential difference across the cell. The effect of an external source of current on the rate of secretion of active mucosa may be simply electrolytic. This electrolytic effect is not observed in the resting mucosa, probably because of the low permeability to hydrogen ions.

The Davies and Ogston theory, therefore, proposes two mechanisms whereby the high ratio of hydrochloric acid secreted to oxygen absorbed can be explained and the relationship of the mechanism to the phosphate transfers is suggested. These two mechanisms working together in parallel would explain a high ratio of secretion to oxygen uptake. If the two mechanisms were working in series, a low ratio would be obtained, but the system would still depend upon phosphate transport. The possible general significance of these theoretical considerations will be discussed in the next section.

Principles of Accumulation and Secretion of Ions.

We are now in a position to review the principles involved in the ability of living cells to secrete or accumulate ionic substances. Various hypotheses have been made from time to time, but most of these are based either on insufficient information about the chemical entities of the cell or on erroneous assumptions about the physical constitution of the cytoplasm. The most valuable discussion of the theoretical mechanisms involved is that given by Franck and Mayer (1947) in which they discuss, without specific relation to particular secretory processes, the principles involved in the expenditure of chemical energy and its application to performing useful osmotic work. The value of observations on salt accumulation in plants and hydrochloric acid secretion in animals is that they permit the introduction of hypothetical mechanisms dependent upon known biochemical activities of the cell.

The underlying principles of secretion have been dealt with very clearly by Davies and Ogston (1950). Where secretion or accumulation implies nett movement of electrolyte to a higher chemical potential, these authors point out that constraint must be placed on at least one ion. Since a single ion cannot be secreted alone, because of the requirement of maintenance of electrostatic neutrality, a second ion follows that upon which the constraint has been placed. It is useful to distinguish these two ionic behaviours in Davies and Ogston's term of "primary" secretion for the ion undergoing constraint and "secondary" secretion for that which follows to maintain the electrostatic neutrality. Initially the metabolism of the cell must be applied in such a way as to produce and separate electric charges and it is interesting to note in this connection that one of the most important cell mechanisms bringing this about is the cytochrome system in which an electron is removed from a hydrogen ion. Similarly it is possible for the constraint on an ion to be brought about by a carrier which combines so effectively with a "primary" ion that it can remove it from its partner and transfer it elsewhere in the cell. If it were possible for a carrier simultaneously to combine with both ions of a salt and to lose those two ions elsewhere in the cell an accumulation of salt would occur. As Franck and Mayer pointed out, this is possibly an over-simplified picture.

Whatever the mechanism, the primary cause of secretion is the production and separation of positive and negative charges and in many respects the first mechanism suggested above for the secretion of hydrochloric acid, where the hydrogen ion is liberated when an electron is passed through the cytochrome system, represents the simplest expression of the mechanism. After the primary secretion of these hydrogen ions, chloride ions can be regarded as moving toward the hydrogen as the result of the establishment of a potential gradient. Such a mechanism alone, however, could not explain the secretion of both ions of a salt, and now it is necessary to introduce the concept referred to by Davies and Ogston as "tertiary" secretion. Supposing that the hydrogen ion formed initially were transported to the non-secretory surface in exchange for the cation which accompanied the chloride, then salt would have been secreted and the cation is said to have entered by "tertiary" secretion.

A further necessary consideration is that there must be some selective permeability to the ions on which the process operates. Thus, if hydrochloric acid, for instance, is being secreted, the cell must be permeable to the chloride ion, but relatively impermeable to the hydrogen which is the ion of primary secretion. If tertiary secretion is to occur so that the cation accompanying the chloride is to enter, the cell must become permeable to the primary ion (hydrogen) and to the tertiary cation. For the reasons pointed out by Briggs (1930), the secondary entry of chloride following the primary secretion of hydrogen and the tertiary exchange of hydrogen for the cation could not occur simultaneously with ions free in solution. Such a mechanism could operate only if the phase of permeability to chloride were followed, in time, by a phase of permeability to cation. It is not possible to have both ions of a salt moving in solution in the same direction against a concentration gradient, because the potential gradient can favour the movement of only one of the ions. If, however, the ions were not free in solution, but each ion were combined firmly to an appropriate carrier, the secretory mechanism could operate in the manner outlined. The anions would be passed to the secretory surface as a carrier passes anions formed by the cell to the non-secretory surface. Another carrier would pass hydrogen ions formed by the cell to the non-secretory surface and carry cations back to the secretory surface.

As long as the quantity of ions secreted does not exceed that which corresponds to hydrogen ions and electrons coming from the substrates of respiration at the point of action of the cytochrome system, this system will account for the drive to secrete or accumulate. We have seen, however, that the hydrochloric acid secretion, as examined by Davies and his collaborators, appears to exceed that which would be possible on the basis of the operation of the cytochrome system alone. The hypothesis, suggested by Davies and Ogston, accounts for the additional hydrogen ions as being formed in a phosphorylating cycle. It is interesting to note that the passage of electrons through the cytochrome system is still involved in this second mechanism. Schematically these two mechanisms are summarized in Text-fig. 1.

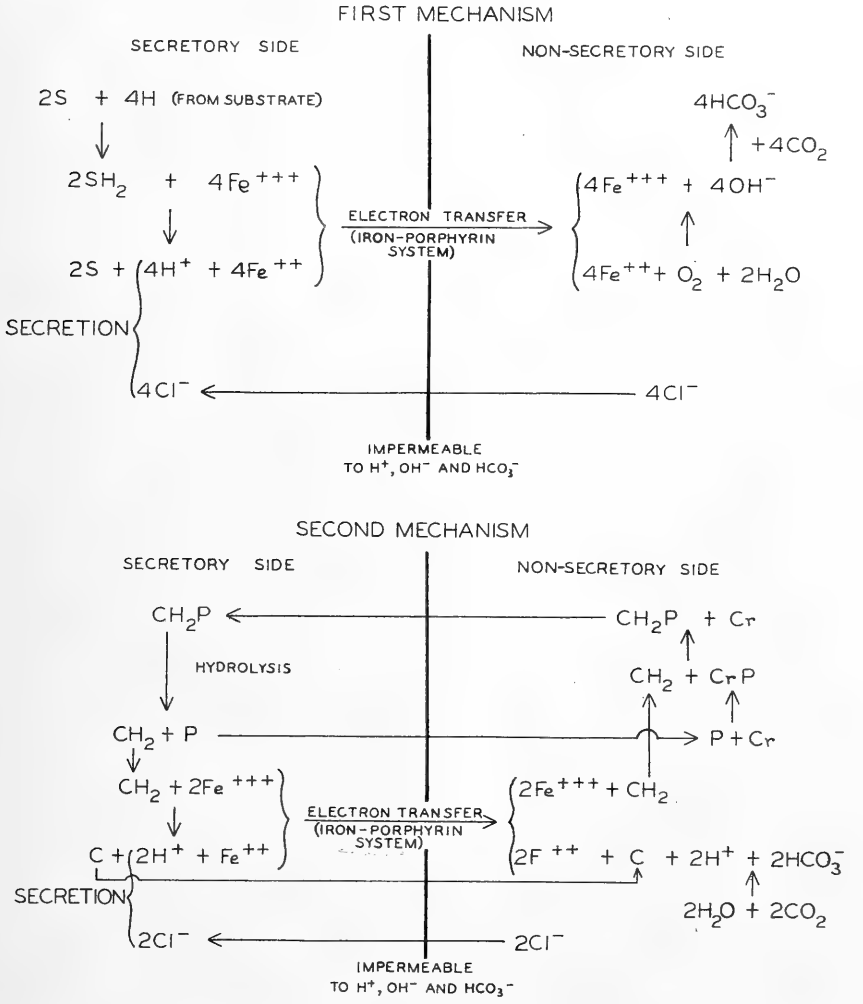
These two mechanisms working in parallel would be adequate to explain the secretion of hydrochloric acid. Perhaps in the accumulation by plants, the two systems operate in series so that the amount accumulated never exceeds that which is possible from the liberation of charges by hydrogen from substrate molecules. If this were so, it would account for the inhibitory effect of a substance like 2, 4-dinitrophenol which can prevent phosphate transfers.

Davies and Ogston have discussed the thermodynamic relationships of the mechanism of secretion and have reached the conclusion that the energy liberation in the two mechanisms suggested would be adequate to bring about the concentration of hydrochloric acid observed on the secretory side. A detailed thermodynamic examination of the suggested mechanism applied to plant cell secretion has not yet been carried out. It has previously been pointed out (Robertson, 1941) that the energy necessary to bring about accumulation represents a very small fraction of the total energy liberated simultaneously in the process of salt respiration.

The mechanisms suggested by Davies and Ogston are shown to be compatible with the potential differences observed in the resting and in the secreting mucosae. The application of similar hypotheses to plant cytoplasm in the accumulating state and further consideration of Lundegårdh's work, may lead to a satisfactory explanation of the potential differences observed, and reveal the connection between these potentials and respiration on the one hand and salt accumulation on the other. Davies and Ogston conclude with some suggestions as to how variations of these two mechanisms could be applicable to other types of secretion, such as secretion of acids by the tubules of the kidney, absorption of bicarbonate from the tubular fluid, secretion of bicarbonate in the pancreas and secretion of neutral salts. Thus we have the opportunity of formulating hypotheses which can be tested by suitable experimental observations on some of these tissues. We have reached a stage in the investigation of secretion and accumulation when reasonable explanations, using biochemical entities of the cell, can be

suggested for the general principles of these two processes. There is still much to do, much we do not know, and there are many gaps to be filled.

Perhaps the largest gap in our knowledge is that which arises from our ignorance of the submicroscopic organization of the cytoplasm and of the distribution of the active constituents of the cell. It is perhaps necessary here to warn against hypotheses which



Text-fig. 1.

The two mechanisms suggested by Davies and Ogston (1950) for the secretion of hydrochloric acid. Symbols: S, a carrier (e.g., flavoprotein); C, a carrier; Cr, creatine; P, phosphate. It is assumed that the separating structure in the second mechanism is less permeable to CH_2 than to CH_2P and C or that CH_2P is decomposed only on the secretory side.

suggest the orientation of the functional constituents in relation to hypothetical cell membranes whose existence is difficult to demonstrate and whose positional relation to the active constituents of the cell is practically unknown. That there is an external membrane consisting of a few layers of oriented molecules in a lipo-protein complex, seems a very likely hypothesis for some cells. There is, however, no evidence that such an external membrane is of universal occurrence and it may be that the active constituents of the cytoplasm operate in some mesh not protected from an external solution by a neatly arranged lipoid membrane. The lipoid-like properties which

explain so many observations on permeability of the cytoplasm need not necessarily be on the surface, but may be at some depth in the cytoplasm. Possibly it is here that the specific substances which act as carriers of ions, pick up the ions, and differentiate in their powers of combination between different ionic substances. These carriers themselves may depend for their existence upon the high degree of organization of the cytoplasm and might, if they depended upon a lipo-protein complex, for instance, lose their identity with the destruction of the cytoplasm in any way. This could explain why it is difficult to find ions associated with any particular substances when secretory or accumulatory cells are broken up for analysis. In this connection, it is interesting to note the very marked differences obtained by Jacobson and Overstreet (1947), using radio-active ions, between the absorptive capacity of living and killed plant cells. Furthermore, if active transport is intimately concerned with the late stages of respiration and perhaps with the phosphorylations which accompany them, we shall have to examine the possibility of this mechanism being included in the organized regions of the cells in which these respiratory actions occur. Recent work points to the particulate nature of the cyclophorase system and the probable association of the dehydrogenases and phosphorylases with the cytochrome enzymes in the mitochondria. The possible significance of these observations in relation to the suggested secretory mechanisms has still to be explored.

Epilogue.

We have reached a partial explanation of the accumulatory and secretory mechanisms. However incomplete and however much detail remains to be filled in, we can see that there are reasonable possibilities, depending only on the kind of biochemical changes which we know to be going on continuously in the cell, for explaining the once mysterious mechanisms of secretion and accumulation. We see that the living cell does not disobey the second law of thermodynamics and that the demons have been exorcized.

The living cell has the characteristic property of harnessing the energy flux to perform useful work. Here, there is no incompatability with physical law, but, as Schrödinger (1948) points out, the living cell "feeds on negative entropy" or, unlike the physical universe, "produces order from order". The secretory mechanism is just a special example of the living organism's ability to use energy derived in abundance, fundamentally from carbohydrate, itself formed in accordance with physical laws, to do useful work. In doing so it is using matter which is obeying the laws of matter so that to return to the words of J. T. Wilson with which I began, "in so far as a strictly scientific representation of this phenomenon is the object, this can only be given in terms of physical cause or mechanism". On this, perhaps, the modern biologist has resolved the old controversy between the vitalists and the mechanists. The organism exhibits properties which though not apparent from a study of non-living matter, are consistent with all the facts derived from such a study; such properties do not merit the terms "vital force". The demons of the vitalists were never more than the flight of fancy which Clerk-Maxwell intended his demon to be.

I wish to thank Professor C. W. Emmens, Dr. H. S. McKee and Miss M. J. Wilkins for their helpful criticism of the manuscript on which this address is based.

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The Honorary Treasurer, Dr. A. B. Walkom, presented the Balance Sheets for the year ended 28th February, 1950, duly signed by the Auditor, Mr. S. J. Rayment, F.C.A. (Aust.), and he moved that they be received and adopted, which was carried unanimously. A vote of appreciation for Dr. Walkom's care of the Society's finances was passed.

No nominations of other candidates having been received, the President declared the following elections for the ensuing year to be duly made:

President: D. J. Lee, B.Sc.

Members of Council: R. H. Anderson, B.Sc.Agr.; W. R. Browne, D.Sc.; N. A. Burges, M.Sc., Ph.D.; A. N. Colefax, B.Sc.; S. J. Copland, M.Sc.; D. J. Lee, B.Sc.; J. M. Vincent, B.Sc.Agr

Auditor: S. J. Rayment, F.C.A. (Aust.).

A cordial vote of thanks to the retiring President was carried by acclamation.

GENERAL ACCOUNT. Balance Sheet at 28th February, 1950.

LIABILITIES.

	£ s. d.	£ s. d.
Capital—		
Amount received from Sir William Macleay during his lifetime ..	14,000 0 0	
Further sum bequeathed by his Will	6,000 0 0	
		20,000 0 0
Contingencies Reserve		10,323 0 0
Accumulated Funds		30,323 0 0
Suspense	1 13 0	
Bookbinding Account	404 14 1	
Commercial Banking Company of Sydney Ltd.	156 9 2	
Income Account	2 5 7	
Current Liabilities		565 1 10
		£30,888 1 10

ASSETS.

	£ s. d.	£ s. d.
Fixed Assets—		
Commonwealth Loans, at cost	15,048 10 0	
Debentures:		
Metropolitan Water, Sewerage and Drainage Board, at cost	994 7 6	
Science House (one-third share), at cost	14,835 4 4	
		30,878 1 10
Current Assets—		
Cash in hand		10 0 0
		£30,888 1 10

INCOME ACCOUNT. Year Ended 28th February, 1950.

	£ s. d.	£ s. d.
To Balance from 1948–49		222 18 4
„ Salaries		894 11 7
„ Printing Proceedings	737 17 6	
„ Printing Reprints	127 9 7	
„ Blocks	25 1 2	
		890 8 3
„ Insurance		16 5 1
„ Postage	63 1 2	
„ Petty Cash	36 1 9	
		99 2 11
„ Audit	13 2 6	
„ Printing and Stationery	70 19 1	
„ Expenses	25 13 6	
„ Cleaning	39 15 0	
„ Library	23 13 5	
„ Legal Expenses	123 9 9	
„ Bank Expenses	3 0 6	
		299 13 9
„ Transfer to Bookbinding Account ..		43 18 1
„ Balance to 1950–51		2 5 7
		£2,469 3 7

	£ s. d.	£ s. d.
By Subscriptions: 1949–50	198 9 0	
Arrears	13 13 0	
In advance	18 18 0	
		231 0 0
„ Entrance Fees		27 6 0
„ Interest		494 1 6
„ Science House		547 0 0
„ Sales		203 18 1
„ N.S.W. Government Grant		100 0 0
„ Fellowships Account (surplus income at 28th February, 1950, transferred)		260 4 3
„ Bank Expenses		2 5 8
„ Transfer from Contingencies Reserve Account		603 8 1
		£2,469 3 7

AUDITOR'S REPORT TO MEMBERS.

I have examined the books of account and vouchers of the Linnean Society of New South Wales for the year ended 28th February, 1950, and certify that the above Balance Sheet and accompanying Income Account are correct and in accordance therewith, and in my opinion present the true state of the Society's affairs at 28th February, 1950, as shown by the books. Certificates of the investments have been inspected.

S. J. Rayment, Chartered Accountant (Aust.),
Auditor.

Sydney, 10th March, 1950.

A. B. Walkom,
Hon. Treasurer

6th March, 1950.

LINNEAN MACLEAY FELLOWSHIPS ACCOUNT.

BALANCE SHEET at 28th February, 1950.

LIABILITIES.	£ s. d.	ASSETS.	£ s. d.	£ s. d.
Accumulated Funds—		Fixed Assets—		
Amount bequeathed by Sir William Macleay ..	35,000 0 0	Commonwealth Loans, at cost	30,450 0 0	
Surplus Income Capitalized	17,926 15 4	Debentures:		
		Metropolitan Water, Sewerage and Drainage Board, at cost	11,125 19 9	
		Rural Bank of N.S.W., at cost ..	2,172 15 0	
		Inscribed Stock:		
		Metropolitan Water, Sewerage and Drainage Board, at cost	1,005 0 0	
		Loans on Mortgage	7,950 0 0	
				52,703 14 9
		Current Assets—		
		Commonwealth Savings Bank	38 1 6	
		Commercial Banking Company of Sydney Ltd.	184 19 1	
				223 0 7
	£52,926 15 4			£52,926 15 4

INCOME ACCOUNT. Year Ended 28th February, 1950.

	£ s. d.		£ s. d.
To Salaries of Linnean Macleay Fellows	1,416 13 4	By Interest	1,869 17 6
„ Payroll Tax	9 13 3		
„ Balance, being Surplus Income transferred to General Account	260 4 3		
„ Capital Account	183 6 8		
	£1,869 17 6		£1,869 17 6

AUDITOR'S REPORT TO MEMBERS.

I have examined the books of account and vouchers of the Linnean Society of New South Wales for the year ended 28th February, 1950, and certify that the above Balance Sheet and accompanying Income Account are correct and in accordance therewith, and in my opinion present the true state of the Society's affairs at 28th February, 1950, as shown by the books. Certificates of the investments have been inspected.

S. J. Rayment, Chartered Accountant (Aust.),
Auditor.

Sydney, 10th March, 1950.

A. B. Walkom,
Hon. Treasurer.

6th March, 1950.

BACTERIOLOGY ACCOUNT.

BALANCE SHEET at 28th February, 1950.

LIABILITIES.	£ s. d.	ASSETS.	£ s. d.	£ s. d.
Accumulated Funds—		Fixed Assets—		
Amount bequeathed by Sir William Macleay ..	12,000 0 0	Commonwealth Loans, at cost	17,320 0 0	
Accumulated Income Capitalized	3,820 0 0	Debentures: Metropolitan Water, Sewerage and Drainage Board, at cost	800 0 0	
	15,820 0 0			18,120 0 0
Income Account at 28th February, 1950	3,077 14 11	Current Assets—		
		Commercial Banking Company of Sydney Ltd.	723 5 5	
		Commonwealth Savings Bank	54 9 6	
				777 14 11
	£18,897 14 11			£18,897 14 11

INCOME ACCOUNT. Year Ended 28th February, 1950.

	£ s. d.		£ s. d.
To Expenses	25 17 0	By Balance from 1948–49	2,334 17 2
„ Balance to 1950–51	3,077 14 11	„ Interest	568 14 9
		„ Donation	200 0 0
	£3,103 11 11		£3,103 11 11

AUDITOR'S REPORT TO MEMBERS.

I have examined the books of account and vouchers of the Linnean Society of New South Wales for the year ended 28th February, 1950, and certify that the above Balance Sheet and accompanying Income Account are correct and in accordance therewith, and in my opinion present the true state of the Society's affairs at 28th February, 1950, as shown by the books. Certificates of the investments have been inspected.

S. J. Rayment, Chartered Accountant (Aust.),
Auditor.

Sydney, 10th March, 1950.

A. B. Walkom,
Hon. Treasurer.

6th March, 1950.

ABSTRACT OF PROCEEDINGS.

ORDINARY MONTHLY MEETING.

29th March, 1950.

Mr. D. J. Lee, President, in the Chair.

Library accessions amounting to 56 volumes, 250 parts or numbers, 18 bulletins, 16 reports, and 1 pamphlet, total 341, had been received since the last meeting.

PAPERS READ (by title only).

1. Reduvioidea from New South Wales. Notes and Descriptions (Hemiptera). By Petr Wygodzinsky. (*Communicated by J. W. T. Armstrong.*)

2. Australian Ephemeroptera. Part I. Taxonomy of New South Wales Species and Evaluation of Taxonomic Characters. By Janet E. Harker.

ORDINARY MONTHLY MEETING.

26th April, 1950.

Mr. D. J. Lee, President, occupied the Chair.

The President announced that the Council had elected Dr. A. B. Walkom to be Honorary Treasurer and Dr. Lilian Fraser, Dr. G. D. Osborne, Dr. R. N. Robertson and Mr. A. R. Woodhill to be Vice-Presidents for the 1950–51 session.

The President congratulated Dr. I. M. Mackerras on being awarded the Clarke Memorial Medal of the Royal Society of New South Wales for 1950 for his work on Diptera, and Dr. T. B. Kiely for obtaining the degree of D.Sc.Agric., and the award of the Royal Society's Medal for his work on the Black Spot of Citrus. Miss Muriel Morris, Linnean Macleay Fellow in Zoology, was congratulated on obtaining the M.Sc. degree of the University of Sydney.

The President referred to the death on 2nd April, 1950, of Professor W. J. Dakin, who had held the Chair of Zoology in the University of Sydney from 1929 to 1948 and who had been a member of the Society since 1929. He was a member of Council from 1930 to 1943 and President during the 1934–35 session. He was a noted authority on marine biology.

Messrs. K. G. Brown, Randwick; L. D. Crawford, B.Sc., Newtown; Dr. K. V. Krishnamurthy, M.A., Sydney University; and Mr. A. K. O'Gower, B.Sc., Harbord, were elected Ordinary Members of the Society.

Library accessions amounting to 7 volumes, 47 parts or numbers, 2 bulletins, and 4 reports, total 60, had been received since the last meeting.

PAPERS READ.

1. A Study of the Alkaline Phosphatase Reaction in Tissue Sections. Parts I and II. By K. W. Cleland.

2. Dilation of the Foot in *Uber* (*Polinices*) *strangei* (Mollusca, Class Gastropoda). By Muriel C. Morris, Linnean Macleay Fellow in Zoology.

3. The Hair Tracts in Marsupials. Part IV. Direction Characteristics of Whorls and Meristic Repetition of Radial Fields. By W. Boardman.

4. A Hybrid Eucalyptus. By L. D. Pryor.

AUSTRALIAN EPHEMEROPTERA.

PART I. TAXONOMY OF NEW SOUTH WALES SPECIES AND EVALUATION OF TAXONOMIC CHARACTERS.

By JANET E. HARKER, B.Sc., Department of Biology, New England University College, Armidale, N.S.W.

(One hundred and one Text-figures.)

[Read 29th March, 1950.]

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INTRODUCTION.

The Ephemeroptera or mayflies have attracted the attention of entomologists in Europe and America far more than in Australia, perhaps the main reason being the lack in Australia of the large swarms which have compelled notice elsewhere.

Linné first divided the Ephemeroptera into two groups within the genus *Ephemera*, basing the division on the presence or absence of the appendix dorsalis. Further subdivision was based on the presence or absence of hindwings (*Baëtis* and *Cloëon* Leach, 1815) and then on the combination of this and the character used by Linné (*Brachycercus* Curtis and *Caënis* Stephens). Later entomologists divided the order on the basis of cross veins (Burmeister, 1839) and the number of joints in the tarsi, and in 1843 Pictet based a scheme on venation and the condition of the eyes.

Eaton published the first monograph in 1871, upon which all later classification has been based; it is in this monograph that the first Australian species are described apart from two isolated descriptions—*Atalophlebia costalis* Burm. and *Atalophlebia australasica* Pict.

In his second monograph, "Monograph of Recent Ephemeridae" (1883-1888), Eaton described seven Australian species. Seventeen species are mentioned by Ulmer (1916) in his results of Dr. Mjöberg's Scientific Expedition, which collected mainly in Queensland.

No further work was published until Tillyard (1933) described the mayflies of the Mount Kosciusko region, which he followed with a redescription of the genotype of *Atalophlebia* and later listed thirteen Australian species from Tasmania (1935).

Phillips (1930) in describing the New Zealand Ephemeroptera has clarified the position of several genera which are represented in both Australia and New Zealand.

Taxonomy.

Evaluation of taxonomic characters.

Many of the characters on which the classification of Australian Ephemeroptera has been based seem unsatisfactory. An evaluation of these characters is therefore made, being based on long series of specimens. Definition of terms used is given where it is thought necessary, otherwise all terminology is taken from Snodgrass (1935).

Head. The head is of the usual insect form, but the extraordinary reduction of the mouth parts and their accompanying musculature leaves a shelf-like projection above the space where the mouth parts would normally be. The nasal carina (a longitudinal ridge on the shelf in front of the middle ocellus) varies in size, as does the shelf itself, but it varies to a certain extent within a species.

The antennae are reduced, with an almost vestigial flagellum; the scape is usually short and stout, and the pedicel varies in length and form, being reduced in *Atalophlebia* and very stout in *Caënis*.

The compound eyes show a great range in size and form, those of the male being larger than the female in all described Australian species. The males of some species show subdivision of the eyes, the upper region being larger and lighter in colour than the lower. The extreme development is seen in *Baëtidae*, where "turban eyes" are present, the upper division being pedunculate on the lower. This development may have evolved from the habit of mating in the air, and the consequent necessity for the male to see the female above (Needham, Traver and Hsu, 1935).

Three ocelli are present, varying in size with sex to a certain extent. The middle ocellus tends to atrophy, while the spatial relations of all three vary generically. For example, they lie far apart in *Caënis*, close together in *Baëtis*, and become ascalophoid in *Atalophlebia*.

Thorax. The mesothorax is the largest division of the thorax, being itself subdivided into the antecosta, prescutum, scutum, scutellum and postscutellum (Needham, Traver and Hsu, 1935).

The morphology of the thorax has been little used as a taxonomic character, except for occasional reference to location of colour markings. But, although the colour and marking of the thorax are a useful guide in most cases, a good deal of variation occurs and the colour marking for any species described must not be followed as a rigid limit.

Abdomen. This consists of ten segments with paired genital openings and specialized genitalia pertaining to the ninth segment of the male. In the male the sternum bears the articulated styliger plate (forceps base), which in turn bears the forceps or appendicular clasping organs, each forcep consisting of two parts, the distal stylus (usually this alone is known as the forcep), and the basal coxopodite. There is evidence that the styliger plate is formed by the union of the median part of the sternum with the coxopodite, and the separation of this from the rest of the sternum (Snodgrass, 1935).

The styli themselves are commonly jointed, usually into three segments, but some show two or four, and those of *Caënis* are unjointed.

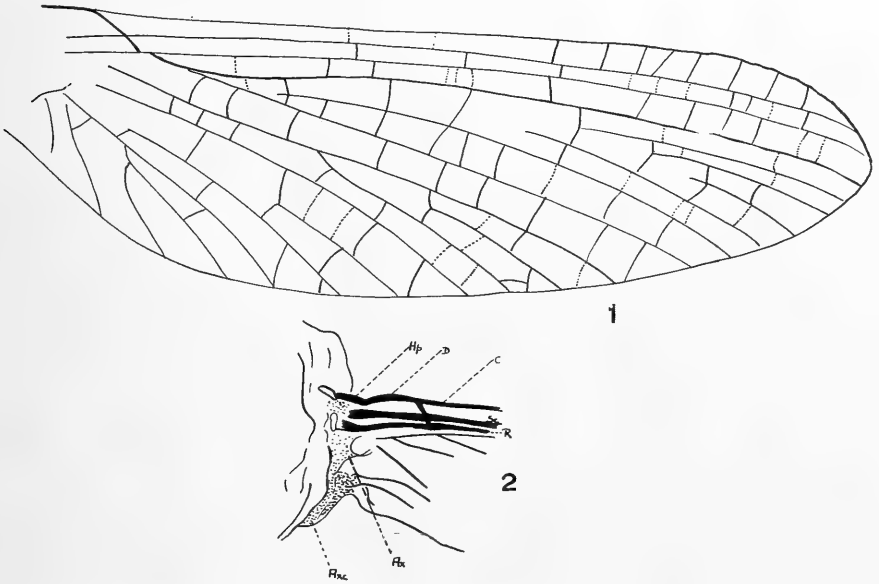
The basal part of the stylus can be easily confused with the coxopodite in some cases—the only way of determining the stylus is to trace the origin of the stylus muscles, which always arise in the coxopodite (Snodgrass, 1935). Many authors have not taken this into account, but it is urged that where the segmentation of the forceps is taken as a taxonomic character the correct evaluation of the styli should be recognized.

The penes are primitively two tubular processes, but usually are found to be united basally to some degree, and on the penes appendages are common and are chitinous and usually backwardly directed.

The genitalia are probably one of the most constant of characters, and therefore one of the most important; owing to the large number of descriptions from either pinned specimens or specimens in which the genitalia could not be adequately examined there has been insufficient use of the genitalia as a taxonomic character.

In the female the sternum of the ninth segment is extended backward, and although it varies in shape considerably it is fairly constant within a species.

The Caudal Filaments. These are two, or three, in number. The two lateral cerci are always present, but the appendix dorsalis may be present and equal in length to the cerci or be in various stages of abortion to complete absence. The presence or absence of the appendix dorsalis has been deemed more important than is warranted, as there is one case at least where it is absent in some specimens and present in others (*Atalophlebia australis*), yet it is a useful guide to species as long as the fact that it is only a guide is kept in mind.



Text-figures 1-2. *Atalophlebia parva.*

1, right and left forewings superimposed, cross-veins which appear in only one wing are dotted; 2, wing base, *Ax* axillary region, *AxC* axillary cord, *D* intermediary plate, *HP* humeral plate.

The lateral margins of the abdomen may show remarkable lateral extensions which are usually directed posteriorly; this is of most constant occurrence in Siphlonuridae, but the shape of the lateral margins varies considerably in the Leptophlebiidae, and is important taxonomically.

Legs. All the legs are weak and in many genera are completely useless for walking, but they are nevertheless highly differentiated. The forelegs are most specialized, differing from the middle and hind pair, and in the male they are usually considerably lengthened and hold the female in copulation—the tarsi being turned backward for this purpose as the male approaches the female from underneath.

The relative proportions of the tibia and tarsus have been used by some authors and seem to be a constant character if kept to a reasonable comparison, that is a relative length comparison; this has been used as a generic characteristic by Tillyard in the Siphlonuridae.

The tendency for fusion of the first tarsal joint with the tibia is of importance taxonomically, the fusion taking place most frequently in the hind leg, so that it is usually the hind leg which is used in comparisons. The relative proportions of the tarsal joints is another character used by Tillyard, but the variation found within a series of specimens was such that it is not used in this paper. The claws of the pair on each tarsus may be similar in form, both being hooked at the tip, or one may be blunt and flattened, or even both may take this latter form.

Specimens have been captured, and also bred in the laboratory, in which legs have been regenerated in the late nymphal stages and show varying degrees of reduction in the imago. When both legs have been damaged there is no indication that this has occurred. Thus, as damage to the nymphal appendages is not infrequent, discretion should be used in the comparison of measurements.

Wings. The terms applied to venation are those interpreted by Tillyard (1932). Table 1 shows systems used by other authors in specific descriptions.

TABLE I.

	TILLYARD 1932 AND THIS PAPER	NEEDHAM AND TRAVER 1935	TILLYARD 1926	TILLYARD 1923	MORGAN 1912	COMSTOCK AND NEEDHAM 1912	EATON 1883 (NUMBERS)	EATON 1883 (NAMES)	NEEDHAM AND MURPHY 1924
	C+	C	C	C	C	C	1	COSTA	C
	Sc−	Sc	Sc	Sc	Sc	Sc	2	SUBCOSTA	S_c
	R_1+	R_1	R_1	R_1	R_1	R_1	3	RADIUS	R
	R_2−	R_2	R_2	IR_{2a}	M_1	R_2	–	BRANCHES OF SECTOR	M_1
	IR_2+	–	IR_2	$2R_{2a}$	RS_2	–	–		
	$R_3{}^a$		$R_3{}^a$	–	–	–			
	IR_{3a}+		IR_{3b}	–	–	–	–		
	R_{3b}−		R_{3b}	$5R_{2a}$	RS	R_3	–		
	IR_{3b}+		IR_{3b}	R_{2b}	INTERPOLATED VEIN 1.	R_4	4		M_2
	R_4+5	R_3	R_4+5	R_3	M_2	R_5	5	CUBITUS	
	MA_1+	R_4	MA_1	$R_4{}^a$	M_3	M_1	6	PRAEBRACHIAL	M_3
	IMA−	Or	IMA	$R_4{}^b$	–	M_2	–		
	MA_2+	R_5	MA−	R_5	M_4	M_3	6	POBRACHIAL	M_4
	MP_1−	M_1	MP_1	M_1	Cu_1	Cu_1	7		Cu_1
	IMP+		IMP	M_2	INTERPOLATED VEIN 2				
	MP_2−	M_2	MP_2	M_{3+4}	Cu_2	Cu_2	7		
	CuA_1+	Cu_1	Cu_{1a}	Cu_{1a}	1A	1A	8	ANAL	A_1
	ICuA−		ICu_1	Cu_{1b}	1A	1A	–		A_2
	CuA_2+	Cu_2	Cu_{1b}	Cu_{1c}	1A	1A	8		
	CuP−	1A	Cu_2	Cu_2	2A	2A	D_1	AXILLARIES	
	A_1−	1A	1A	1A	3A	3A	9_2		
	1A+		2A	2A	3A	3A	9_2		
	A_2+		3A	3A	3A	3A	9_2		
	A_3A_4+ (AXILLARIES)	–	–	–	–	–	–		
AUTHORS FOLLOWING THIS SYSTEM						NEEDHAM (BEFORE 1932) McDUNNOUGH LESTAGE ULMER PHILLIPS			

The cross vein system has frequently, and incorrectly, been referred to as constant. Text-fig. 1 shows the right and left wings of one specimen superimposed, those cross veins which appear on only one wing being dotted; this reveals such a variation even in the same specimen that the use of cross veins as a diagnostic character is rejected.

Marginal veinlets between the longitudinal veins may be present, and the number of these is a distinct generic character. The wing base (Text-fig. 2) is unusual, the Ephemeroptera holding the wings vertical at rest and not flexing them as do most insects. At the base of each wing is a humeral plate between the costal vein and the tergite, the posterior part of the wing base is continuous with the posterior margin of the tergum, but the weak sclerites in the axillary region seem to have some homology with those of other insects.

The shading of the wing and its coloration are of value and are usually considered constant, at least in the subimago; however, the effect of physiological variation within the animal, particularly in the larva, alters the relative amount of pigmentation of the cuticle, and this may result in an effect on the wing shading.

The hindwing is of more importance in some respects than the forewing, particularly in reference to the shape of the anterior margin, which appears to be constant. The presence or absence of the hindwing is in some families used for generic segregations.

NYMPH.

The nymphal characters can be used with possible advantage over those of the adult, as it is probably in the former stage that specific divergence occurs.

Head. The shape of the head varies considerably, and in its extremes is used to characterize families. The shape of the head is associated with the type of habitat and the habits of the larva. The position of the eyes varies with the shape of the head, being dorsal in *Atalophlebia*, which have flattened heads, and lateral in Siphlonuridae, with hypognathous heads. The colour of the eyes is fairly constant and has at times a taxonomic significance; in the male the eyes become divided in the late instars. The antennae vary in diameter and in length, but they are so easily broken that little significance should be attached to them.

Mouth Parts. The labrum is present in all Australian forms so far described; it takes the form of a transverse, more or less rectangular plate with spines and hairs variously disposed over the surface, and the anterior edge varies in shape considerably and is often of specific importance.

The mandibles are divided into two distinct areas: the canine and molar areas. The canine area in particular has often been credited with taxonomic significance, but the variation in a series is extreme, and the development also varies considerably with the stage of development of the nymph. Between the canine and molar areas arises the prostheca (otherwise known as lacinia mobilis, mandibular palp, or mandibular endopodite); this varies between a large structure bearing a brush of hairs to a minute hairless spine.

The maxillae have a separate cardo and stipes, but the galea and lacinia are fused lengthwise and their fused "plate" often ends in a tooth or hook, or more often in a thick brush of hairs below which is a row of pectinate "rakes". The maxillary palp is modified in various ways; it is probable that the primitive form is three-segmented and covered with hairs, but these segments may be reduced to two or may be greater than three (fifteen in *Ameletopsis*), and any of the segments may lose their sensory hairs.

The labium is of primitive form and the palpal segments may differ in relative length and the form of the paraglossa is often a specific variant.

Thorax. The variation in the thorax appears to depend largely on the habitat; probably the variation is a function of the muscular development. The colour and markings are useful characters, but the same precautions must be taken as with the use of colour in the adult.

Legs. The legs vary greatly with habit. In forms which live in swift-flowing streams the front femora are often greatly thickened, and in other forms which flatten themselves against rocks the legs are flattened with the femora broad and the tibiae slender. All the legs bear a one-jointed tarsus with a single claw, which varies greatly in form.

Gills. Abdominal gills occur only on segments one to seven, being present on the dorsal side of the lateral margin, and their number and disposition are of considerable importance. The two principal types are lamelliform or plate-like and filiform or threadlike; one pair of gills may become enlarged to cover the others—this is a modification found in forms frequenting silty waters and creeks which partially dry up in summer.

From this discussion of the various parts of the insect it appears that very few characters are stable enough to be used as primary taxonomic characters; however, it is not by single characters that species are separated, but rather by combinations which only become recognizable when a long series of specimens is examined.

Types.

All type specimens have been deposited in the Australian Museum, Sydney, and paratypes are being deposited in the British Museum. Wherever possible, for each species a holotype (the male imago), allotype (female imago), morphotype (male sub-imago), allomorphotype (female sub-imago), and a nymphal morphotype have been selected. The terminology used is that defined by Davis and Lee (1944).

Tillyard has described several species for which the type specimens have not been able to be traced in any Australian Museum, the Commonwealth Scientific and Industrial Research Organization Entomological Branch, or the British Museum. It has not been found possible to recognize these species from the descriptions.

Method of Description.

As text-figures are a clearer guide than verbal descriptions, the latter have been abbreviated.

Measurements. All measurements have been made from a series of a minimum of twenty specimens, unless otherwise stated. The measurement given is the average of these figures, and the range of the measurements is given in brackets.

General Colour. This has been ascertained by naked eye, being the general colour impression given by the mayfly.

Wings. Where the cross venation varies the text-figure shows the maximum number.

Life Cycle. Wherever possible, the imagines, subimagines and nymphs have been connected by breeding them from the nymphal stage; as a further check, it having been found that on no occasion has the egg differed in the late nymphal stage from that of the imago or subimago, the relationship of these stages has been shown by the eggs.

Specimens Examined. These have all been collected by the author unless otherwise stated.

Text-figures. These have all been drawn with the aid of a camera lucida.

Females, Subimagines. Where the male imago has been described in detail the following descriptions of other forms only give details in which they differ from the male.

Key to the Ephemeroptera of New South Wales.

Families suspected or known to be present, but not actually described, are included in this key.

IMAGINES.

Superfamilies.

1. In forewing veins MP and CuA strongly divergent at base. MP_2 strongly bent towards CuA basally. Hind tarsi with four movable joints or less; if a fifth is present it is immovably united to tibia ..... S.F. EPHEMEROIDEA.
   In forewing veins MP and CuA parallel at base or weakly divergent. Fork of MP nearly symmetrical ..... 2.
2. Hind tarsi with four movable joints, if a fifth is present it is immovably united to tibia ..... S.F. BAETOIDEA.
   Hind tarsi with five freely movable joints ..... S.F. HEPTAGENOIDEA.
   [*Atopopus spadix*, sp. nov.]

Superfamily Baetoidea.

1. Forewing with tornus from two-fifths to half the length of the wing from the base, CuA nearly straight, with a descending series of pectinate branches. CuP curved concavely to CuA ..... SIPHLONURIDAE.
   Forewing with tornus at not more than one-quarter of wing length from base. CuP sigmoidly curved ..... 2.
2. In the forewing MA clearly forked ..... 3.
   In the forewing MA not forked, although behind it are two free veins which are not attached at base. Usually few cross veins. Hindwing small and narrow, sometimes absent, with at most 2 or 3 longitudinal veins ..... BAETIDAE.

3. Wings milky or infuscated, ciliate on hind margin. Hind wings absent, but may be occasionally present in subimago. No unattached intercalaries, frequently only few cross veins ..... CAENIDAE.
Wings hyaline, hind wings nearly always present, wings with numerous cross veins .... 4.
4. In forewing CuP usually widely separated at base from CuA, but lying close to A_1. No unattached intercalated veins between media and cubitus ..... LEPTOPHLEBIIDAE.
CuP in forewing approximating at base to CuA, but widely separated from A_1. Several, usually two, unattached intercalated veins between media and cubitus, and also in front of the posterior branch of the media ..... EPHEMERELLIDAE.

Family BAETIDAE.

1. Hind wing present, forewing small with marginal veinlets in sets of two or more ....*Baëtis.*
2. Hind wing absent, forewing with marginal veinlets single ..... *Cloëon.*
[*Cloëon fluviatile* Ulm.]

Family CAENIDAE (BRACHYCERCIDAE Lestage).

1. Wings not exceptionally broad, ratio of length to breadth approximately 3 : 1 ..... *Tasmanocoenis.*
2. Wings exceptionally broad near base, ratio of length to breadth 2 : 1 or less ..... *Caenis.*
[*Caenis scotti* Till.]

Genus *Baetis.*

1. Costal angulation of hindwing acute ..... *B. baddamsae,* sp. nov.
Costal angulation of hindwing not acute ..... *B. confluens,* sp. nov.

Family LEPTOPHLEBIIDAE.

1. Tarsal claws all narrow and uncinate ..... 2.
Of every pair of tarsal claws one broad and obtuse, the other narrow and uncinate .... 3.
2. Hindwing more or less obtusely subovate ..... *Atalophlebia.*
Hindwing oblong, oblique; its marginal area abbreviated and relatively very broad ..... *Adenophlebia.*
3. Hindwing obtusely ovate or oval; its marginal area narrow throughout and extended .. 4.
Hindwing strongly angulated in front; its marginal area narrow throughout and far extended ..... *Thraulus.*
4. Appendix dorsalis much shorter than caudal filaments ..... *Blasturus.*
Appendix dorsalis equal to the caudal filaments ..... 5.
5. Penes separated almost to base; a long flap-like appendage, narrowed distally, is attached near apex of each and extends inwards between lobes of penes ..... *Leptophlebia.*
[*L. crassa,* sp. nov.]
Penes without flap-like appendage ..... *Deleatidium.*
[*D. annulatum,* sp. nov.]

Genus ATALOPHLEBIA.

IMAGINES.

1. Forewing less than 8·0 mm. in length ..... 2.
Forewing more than 8·0 mm. in length ..... 3.
2. No cross veins present in basal region of C–Sc area ..... *A. parva,* sp. nov.
Cross veins present in basal region of C–Sc ..... *A. marowana,* sp. nov.
3. Sculpturing on the egg reticulate without any other marking being present ..... *A. albiterminata* Till.
Not as above ..... 4.
4. Sculpturing on egg reticulate with circular markings at the angles of the "cellular lines" ..... *A. longicaudata,* sp. nov.
Sculpturing reticulate with raised circular areas also present ..... *A. incerta,* sp. nov.

Female imago *A. maculosa* unknown.

The male imagines cannot be satisfactorily differentiated except on the genitalia (Text-figs. 19-27).

SUBIMAGINES.

1. Forewing mottled ..... 3.
Forewing uniformly grey ..... 2.
2. Cross veins absent in basal region C-Sc area ..... *A. parva,* sp. nov.
Cross veins present in basal region C–Sc area ..... *A. marowana,* sp. nov.
3. Lambda mark almost complete ..... *A. incerta,* sp. nov.
Lambda mark incomplete ..... 4.
4. Forewing darkly blotched, whole wing darkly shaded ..... *A. longicaudata,* sp. nov.
Forewing not so heavily shaded, little shading of cells ..... *A. albiterminata* Till.

Subimago of *A. maculosa* unknown.

Family SIPHLONURIDAE.

1. MP_2 and IMP attached basally to CuA. Tarsal claws alike. Costal angulation of hindwing near base, slight ..... *Ameletoides* Till.
MP_2 and IMP normal in forewing of every pair of tarsal claws, one blunt and one acute ..... 2.

2. Tarsi four segmented ..... *Tasmanophlebia* Till.
   Tarsi five segmented ..... *Coloburiscus* Eat.

NYMPHS.

Superfamilies.

1. Mandible with an external tusk projecting forward ..... S.F. EPHEMEROIDEA.
   Mandibles without such a tusk ..... 2.
2. Head strongly depressed. Eyes dorsal. Upper member of each gill pair plate-like .....
   ..... S.F. HEPTAGENOIDEA.
   Head not strongly depressed. Eyes lateral ..... S.F. BAETOIDEA.

Superfamily BAETOIDEA.

1. Caudal filaments fringed on both sides ..... 2.
   Caudal filaments fringed on inner border only ..... 3.
2. Seven pairs of gills inserted laterally at sides of abdomen, sometimes all filamentous or first pair reduced and others leaf-like ..... LEPTOPHLEBIIDAE.
   Five or six pairs of gills, first pair very small, second enlarged, covering the following pairs, which bear a long fringe ..... CAENIDAE.
3. Body cylindrical, head bent downwards, hind corners of abdominal segments not produced
   ..... BAETIDAE.
   Body more or less flattened, head held horizontally or nearly so, hind corners of abdominal segments produced backwards ..... SIPHLONURIDAE.

Family BAËTIDAE.

1. Gills—lamellae double on abdominal segments 1-6 ..... *Cloëon.*
2. Gills—lamellae single on all abdominal segments ..... *Baëtis.*

Family LEPTOPHLEBIIDAE.

1. Gills single ..... *Deliatidium.*
   Gills double ..... 2.
2. Gills on abdominal segment 1 deeply forked with slender linear divisions, on other segments oval, lamelliform and fringed around the entire margin ..... *Thraulus.*
   Not as above ..... 3.
3. Gills lanceolate with narrowed tail-like tip. Lateral spines on abdominal segments 8 and 9
   ..... *Leptophlebia.*
   Gills either lanceolate or digitate, spines usually on abdominal segments 2 and 9 .....
   ..... *Atalophlebia.*

Genus *Atalophlebia.*

1. Gills digitate ..... 2.
   Gills lanceolate ..... *A. parva,* sp. nov.
2. Gills multidigitate; 7-15 filaments ..... *A. albiterminata* Till.
   Gills trifurcate ..... *A. incerta,* sp. nov.

Family SIPHLONURIDAE.

1. Thorax humped, very broad. Gills deeply bifid ..... *Coloburiscus* Eat.
   Thorax not humped. Gills lamellate ..... 2.
2. Dorso-ventrally flattened. Gills carried dorsally on abdominal segments .....
   ..... *Tasmanophlebia* Till.
   Nymphs slightly laterally flattened. Gills carried laterally ..... *Ameletoides* Till.

DESCRIPTIONS OF SPECIES.

Genus ATALOPHLEBIA Eaton.

Synonymy: *Atalonella** Needham and Murphy, Bull. Lloyd Libr., 24, 4, 1924.
Genotype: *A. australis* Walk.

Imago.

"Hindwing in front somewhat arched, the summit of the arch obtusely sub-angular, situated usually before the middle of the curve; sub-costa strongly arched, meeting the margin very obliquely; radius usually nearly straight, constituting as it were the chord of the arc described jointly by the sub-costa and the portion of the margin included between its extremity and the radius; hence while the narrow marginal area is broadest at its base and acuminate at its termination, the sub-marginal area is broadest at the middle or a little before the middle, and tapers gradually to its oblique apex. Cross veins abundant in the forewing, those in the marginal area before the bulla well defined. At the terminal margin the longitudinal nervures are provided with curved simple branchlets and there are no isolated veinlets. The two intercalar nerves

* See Appendix I, p. 30.

of anal-axillar interspace of the forewing have simple branchlets, and usually the hinder one, close to its proximal extremity, curves forward to unite with the other, which simply curves forwards to join the anal nervure . . . . Tarsal ungues all nearly alike, small, narrow and hooked at the tip . . . . Forcep limbs of male 3 jointed." (Eaton, 1881.)

Nymph.

Body flattened dorso-ventrally.

Head: The eyes are lateral and antennae long.

Mouth Parts: Both the maxillary and labial palps are three-segmented. The labium is notched medio-anteriorly and the maxillae bear a broad brush of terminal hairs.

Legs: The claws are all toothed.

Caudal Filaments: These bear short hairs at the intersegmental regions.

Gills: Paired and lanceolate or lamelliform gills are borne on the first seven abdominal segments.

ATALOPHLEBIA LONGICAUDATA, sp. nov.

Holotype ♂, Tenterfield, 2,831′, 10 : 1948.
Allotype ♀, Pine Forest, Armidale, 3,300′, 9 : 1948.
Morphotype Subimago, Marowan Cr., Glen Innes, 3,520′, 10 : 1948.
Paratype, Alloparatype, Morphoparatype, Marowan Cr., Glen Innes, 3,520′, 10 : 1948.

DESCRIPTION.

Male Imago.

Measurements: Body length, 17 mm. (15–18 mm.). Cerci, 30 mm. (28–31 mm.). Appendix dorsalis, 20 mm. (16–32 mm.). Forewing, 16 mm. (14–17 mm.). Hindwing, 3·5 mm. (3·0–4·0 mm.).

General Colour: Black with lighter markings and with a distinct white line running beside the prescutoscutal suture.

Head: The head is black with ill-defined lighter markings, and bears antennae with black pedicels and dark brown flagellae. The lower lobes of the eyes are slightly darker than the dull orange-brown upper lobes and the ocelli are white with black bases.

Thorax: This is black with light markings and white outlines to the prescutoscutal sutures.

Abdomen: The abdomen is brown-yellow with dark brown markings, and in some specimens there is a white posterior edge to the last three segments.

Wings: In the forewing the pterostigma is shaded brown, all the veins are black, and the cross veins in the C and Sc area are shaded (Text-fig. 39). In the hindwing all the veins are opaque and colourless except those in the C–Sc region, which are brown.

Legs: The legs are yellow-brown with dark brown markings. The fore-femora bear one or two longitudinal brown markings, but the hind-femora darkens only at its proximal end, as do all the tibiae. The tarsal claws are all narrow and uncinate.

Genitalia (Text-fig. 20): The forceps are brown with lighter distal joints and the penes are uniformly brown.

Caudal Filaments: These are black, or in some specimens lighter towards the distal end, the tip ranging from white to a colour not appreciably different from that of the base. The cerci are longer and stouter than the appendix dorsalis.

Female Imago.

Measurements: Body length, 15 mm. (11·0–18·0 mm.). Cerci, 20 mm. (18–22 mm.). Appendix dorsalis, 13 mm. (11·0–15·0 mm.). Forewing, 14 mm. (11·0–17·0 mm.). Hindwing, 3·8 mm. (3·0–5·0 mm.).

Egg (Text-fig. 36): These shows a sculpturing of pentagonal figures with circular markings at the angles of each pentagon.

Male Subimago.

Measurements: Body length, 15·5 mm. (10·5–18·5 mm.). Cerci, 19·0 mm. (17·0–21·0 mm.). Appendix dorsalis, 13·0 mm. (12·0–18·0 mm.). Forewing, 15·0 mm. (11·0–18·0 mm.). Hindwing, 4·0 mm. (3·5–5·0 mm.).

General Colour: Yellow-brown with dark brown markings.

Head: The head is yellow-brown with dark brown markings, and bears yellow-brown antennae which are slightly lighter at the tips. The upper lobes of the eyes are yellow-brown and the lower dark brown to black. The ocelli are yellowish, and the lateral ones in particular are very prominent.

Thorax: The thorax is dark brown with light brown markings and a white or purplish tinge at the wing base.

Abdomen: This is yellow-brown with dark markings on either side of the median line, and the posterior edge of each segment is outlined with brown. The lateral margins of each segment come to a point at about two-thirds of their length, which gives the appearance of a serrated edge to the whole abdomen.

Wings (Text-fig. 38): The wings are creamy with dull brown irregular shadings and shading on all the cross veins, although the longitudinal veins are opaque and nearly colourless.

Female subimago (Text-fig. 7).

Nymph unknown.

Specimens Examined.

Imago. Serpentine R., Point Lookout, 4,000′, 10 : 1948; Dumaresque Cr., Armidale, 3,000′, 11 : 1948, 9 : 1948, 10 : 1948; Pine Forest Cr., Armidale, 3,000′, 9 : 1948; Marowan Cr., Glen Innes, 3,520′, 10 : 1948; Tenterfield, 2,831′, 10 : 1948; Badga, 3,000′, 12 : 1939, H. M. Stephens; Solitary Cr., Tarana, 4, : 1941, E. Garret; Mienna, Tas., 3,300′, 12 : 1928; Shoalhaven R., 2,500′, 12 : 1929, H. M. Stephens; Bolaro, 3,400′, 12 : 1928, H. M. Stephens.

Biology.

This species has only been found in the vicinity of flowing streams, over which it flies in a characteristic manner. This flight covers about two hundred yards and consists of a gliding motion in the downstream direction and a rapid dart upstream. In the downstream flight both males and females trail their caudal filaments in the water and are made conspicuous by the habit of holding the filaments at right angles to the body.

ATALOPHLEBIA ALBITERMINATA Till.

Proc. Roy. Soc. Tasmania, 1935.

Holotype ♂, Lake Echo, Tasmania, 2 : 1933, Tillyard.

Allotype ♀, *Morphotypes* Subimagines and Nymphs, Lake Echo, Tasmania, 2 : 1933, Tillyard.

Note.—It has not been possible to trace any of these types, so a neotype has been selected. The neotype is not, however, topotypical, as no further specimens have been collected from Lake Echo to my knowledge.

Neotype ♀, Marowan Cr., Glen Innes, 3,520′, 9 : 1947.

DESCRIPTION.

To the original description by Tillyard the following features are added.

Male Imago.

No male imagines have been collected by the author, but pinned specimens are present in the Australian Museum collection. As these pinned specimens are useless for an adequate description only measurements are given.

Measurements: Body length, 16·5 mm. Cerci, 39 mm. Appendix dorsalis, absent. Forewing, 15 mm. Hindwing, 3 mm. Foreleg, 3·0, 3·6, 6·0 mm. Hindleg, 3·6, 7·5, 3·0 mm.

Female Imago (Text-fig. 3).

Measurements: Body length, 15 mm. Cerci, 11 mm. (10–15 mm.). Appendix dorsalis, 11 mm. (10–15 mm.). Forewing, 15 mm. (13–17 mm.). Hindwing, 4·0 mm. (3·5–4·5 mm.). Foreleg, 2·5, 2·4, 2·0 mm. Hindleg, 3·0, 2·0, 2·0 mm.

General Colour: Dark brown.

Head: Dark brown with yellow markings, and grey-brown eyes and grey ocelli.

Thorax: This is yellow-brown with dark brown markings.

Abdomen: The abdomen is yellow-brown with very distinct dark brown markings.

Wings (Text-fig. 40): The wings are hyaline except in the costal and subcostal area of the forewing, which is brown with darkly shaded cross veins; all the venation is black.

Legs: The hindlegs are lighter than the dark brown forelegs, and their tarsi are almost white.

Cerci: These are dark coloured, but in some specimens may end in a white tip.

Egg: The eggs show a hexagonal sculpturing and are laid in a gelatinous substance which dissolves as it passes through water.

Female Subimago.

General Colour: Yellow with distinct brown markings.

Head: The head is yellow with brown markings, black eyes, and white ocelli borne on black bases.

Thorax: This is yellow with dark brown to black markings.

Wings (Text-fig. 41): The wings are yellowish with black veins and have all cross veins shaded dull grey. In the hindwings Sc reaches almost to the apex, Rs is forked and arises about half-way along MA, and MP is forked and an intercalary is present.

Caudal Filaments: These are dark brown with black annulations and a broad black band at the end of each fourth segment.

Nymph.

Measurements: Body length, 10–16 mm.

General Colour: This varies considerably with the environment, more so than in any other nymph known to the author; generally it is dark brown, grey, greenish brown or pale cream.

Head: This is cream or light brown with brown markings, and the antennae are approximately one and a half times as long as the head and thorax, with the basal segment light brown, and the following segments transparent. The eyes are brown or black.

Mouth Parts (Text-figs. 64–66): The labrum has a median incision on its anterior border which bears five to seven flat, dentate processes; recurving spines are also present on this border and bend in towards the central line; posterior to these is another row without recurving tips. On the lateral angles spines are present and directed at an angle following the curve of the labrum. In the mandibles the outer canines have three or fourth teeth and the inner two, the prosthecas are sigmoid in shape with a brush of well-developed, inwardly directed hairs. The molar surfaces are well developed with 10–13 parallel serrated ridges, and a few scattered hairs are present on the outer surface of the mandible. The maxillae bear three-segmented palps, of which the middle segments bear spines on the inner surface and the apical segments are covered with hairs on the outer surface. A row of pectinate "rakes" is present below the brush of terminal hairs on the plate. The labium bears three-segmented yellow palps of which the first segments carry hairs, but the second are practically bare. The paraglossae have hairs present on the anterior edge and the glossae, which are rectangular with rounded corners, bear a dark patch lengthwise in a central position and very few hairs, those which are present being mainly on the basal surface. A single row of spines is present close to the distal end of the glossae.

Legs: Creamish-yellow, and on the inner surface of the tibia there are rows of branched hairs.

Gills: These are double and present on the first seven abdominal segments, decreasing in size posteriorly.

Biology.

The nymphs live among debris and clinging to the bottom of rocks in running streams, frequently in the more sluggish parts. The early instar nymphs often lie partially buried among the sand or debris of the bottom, while the older nymphs run backwards and sideways with great agility seeking shelter in crevices rather than swimming.

Specimens Examined.

Imagines. Marowan Cr., Glen Innes, 3,520′, 9 : 1947.

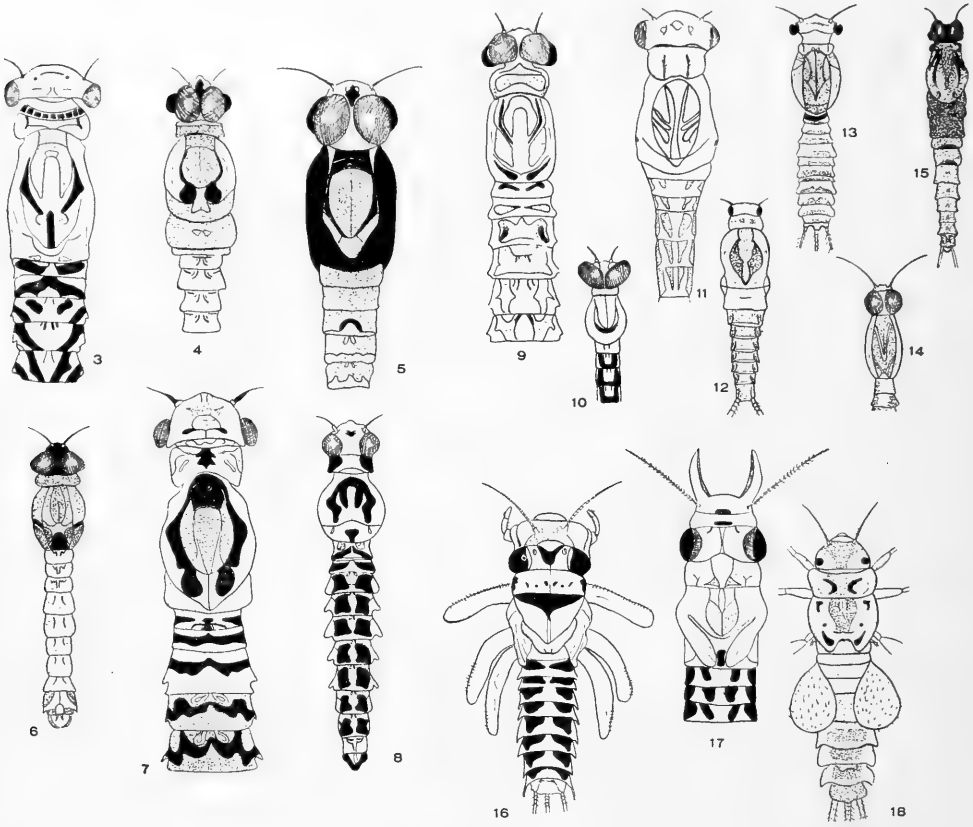
Nymphs. Dumaresque Cr., Armidale, 3,300′, 9 : 1948, every month 1947 ; Pine Forest Cr., Armidale, 1947, Commissioners Waters, Armidale.

ATALOPHLEBIA MAROWANA, sp. nov.

Holotype Subimago ♂, Marowan Cr., Glen Innes, 3,520′, 10 : 1948.

Allotype Subimago ♀, as above.

Paratypes Serpentine R., Point Lookout, 4,000′, 10 : 1948.



Text-figures 3-18. Imagines.

3, *Atalophlebia albiterminata*, female ; 4, *Atalophlebia incerta*, male ; 5, *Atalophlebia maculosa*, male ; 6, *Atalophlebia parva*, male ; 7, *Atalophlebia longicaudata*, female subimago ; 8, *Atalophlebia marowana*, female subimago ; 9, *Leptophlebia crassa*, male subimago ; 10, *Deleatidium annulatum*, male imago ; 11, species described in Appendix II, female imago ; 12, *Baëtis baddamsae*, female subimago ; 13, *Caënis scotti*, female subimago ; 14, *Baëtis confluens*, male imago ; 15, *Baëtis baddamsae*, male imago. Nymphs. 16, *Leptophlebia crassa* ; 17, species described in Appendix II ; 18, *Caënis scotti*. (Various magnifications.)

DESCRIPTION.

Male Subimago.

Measurements: Body length, 9·2 mm. (7·0–10·0 mm.). Cerci, 15·0 mm. Appendix dorsalis, 15·0 mm. Forewing, 6·8 mm. (4·0–8·0 mm.). Hindwing, 1·3 (1·0–1·8 mm.). Foreleg, 2·0, 3·0, 1·0 mm. Hindleg, 2·0, 2·0, 1·0 mm.

General Colour: This is yellow-brown with dark brown markings and almost black annulations on the abdomen.

Head: The head is yellow-brown with black antennae, and eyes of which the upper lobes are grey-brown and the lower dark brown. The ocelli are large, bulging, and dark brown.

Thorax: This is yellow-brown with dark brown markings.

Wings (Text-figs. 51, 61): The wings are grey with clear longitudinal veins and brown or black cross veins.

Legs: The legs are yellow-brown with dark brown areas surrounding the femoro-tibial articulation. The tarsi are four-jointed and the tarsal claws all narrow and uncinate.

Caudal Filaments: These are yellow-brown with dark annulations on the first proximal segment.

Female Subimago (Text-fig. 8).

Head: The eyes are black and the ocelli are white on a black base and widely separated.

Eggs: The egg is spindle-shaped and sculptured with irregular star-shaped markings with a central pit (Text-fig. 30).

Imago, nymph unknown.

Specimens Examined.

Subimagines. Tillbuster Cr., Armidale, 3,300′, 11 : 1947; Serpentine R., Point Lookout, 4,000′, 10 : 1948; Marowan Cr., Glen Innes, 3,520′, 10 : 1948; The Creel, Thredbo R., 3,000′, 11 : 1928, H. M. Stephens.

ATALOPHLEBIA MACULOSA, sp. nov.

Holotype ♂, Serpentine R., Point Lookout, 4,000′, 10 : 1948.
Allotype ♀, *Paratypes,* Serpentine R., Point Lokout, 4,000′, 10 : 1948.

DESCRIPTION.

Male Imago (Text-fig. 5).

Measurements: Body length, 11·5 mm. (10·0–13·0 mm.). Cerci, 24·0 mm. Appendix dorsalis, 24·0 mm. Forewing, 11·4 mm. (10·0–15·0 mm.). Hindleg, 3·0, 3·0, 1·0 mm.

General Colour: This is black with yellow or yellow-red stripes running transversely on the abdomen.

Head: The head is black with eyes of which the upper lobes are orange and the lower black, and the ocelli are white on black bases.

Thorax: The thorax is black with white bands beside the prescutoscutal suture.

Abdomen: This is dark brown with a yellow or yellowish-red band on the posterior margin of each segment, except the last three, which often bear a white margin. The lateral margins are smooth and rounded.

Wings (Text-fig. 43): The wings are clear with very dark venation, and about half-way along Sc, usually in the region of the bulla, a striking black spot occurs which covers two or three cells.

Legs: The forelegs are dark brown, and some specimens show a slightly lighter line marking the tarsal joints. The mid- and hindlegs are yellow to orange, with one black mark about half-way along the femur and another on the femoro-tibial joint. In all the legs the tarsi are darker than the other segments.

Genitalia (Text-fig. 22): The forceps are light yellow, and the penes, which are dark brown, are large and distinctive.

Caudal Filaments: These are uniformly black, and the appendix dorsalis may be similar to the cerci or may be slightly thinner.

Female Imago.

The body is often stouter than that of the male.

Subimago and nymph unknown.

Specimens Examined.

Imagines. Serpentine R., Point Lookout, 4,000′, 10 : 1948; Marowan Cr., Glen Innes, 3,520′, 10 : 1948.

ATALOPHLEBIA INCERTA, sp. nov.

Holotype ♂, Gara R., Armidale, 3,330′, 4 : 1947.
Allotype ♀, *Morphotype* subimago, *Paratypes,* Gara R., Armidale, 4 : 1947.

DESCRIPTION.

Male Imago (Text-fig. 4).

Measurements: Body length, 10·0 mm. (7·5–12·0 mm.). Cerci, 30 mm. (17–45 mm.). Appendix dorsalis, 25 mm. (17–30 mm.). Forewing, 10 mm. (8·0–12·5 mm.). Hindwing, 2·5 mm. Foreleg, 8·0 mm. (6·5–10·0 mm.). Hindleg, 5·5 mm. (4·5–6·0 mm.).

General Colour: This is dark brown.

Head: The eyes are black and the ocelli white.

Thorax: This is dark brown with black markings and a white outline to the prescutoscutal suture.

Abdomen: The abdomen is of slightly lighter colour than the thorax and has black markings.

Legs: The legs are yellow-brown with dark brown markings on the femora, four-segmented tarsi, and tarsal claws which are all narrow and uncinate.

Wings: These have brown venation and are clear except in the C–Sc region of the forewing, which is opaque, particularly towards the apex, in which the cross veins are all shaded.

Genitalia: The genitalia are similar to *A. australis*, but the penes are more separated than in this species.

Caudal Filaments: These are dark brown.

Female Imago.

Egg (Text-fig. 29): On the egg are hexagonal markings separated by circular areas.

Male Subimago.

Wings: The forewing is heavily shaded with dull brown, giving in some cases the complete lambda pattern described for this genus by Tillyard (1933), but in others the shading is much less noticeable so that it gives a superficially complete brown colour to the wing.

Nymph.

Measurements: Body length, 9·0 mm.

General Colour: This is light brown to greenish-brown.

Mouth Parts (Text-figs. 68–71): The labrum bears a median concavity with five dentate incisions on the anterior border, and sparse hairs on the outer anterior angles. The mandibles show a variable number of teeth in both the right and left canines, the minimum being five in the outer canine; and the molar area of the left mandible is larger than that of other *Atalophlebia.* The labium bears a two-segmented palp and the paraglossae are large, with two distinct rows of spines, while the glossae are small and ovate with three encircling rows of spines.

Abdomen: In the abdomen the lateral margins are produced into striking recurving spines.

Gills (Text-fig. 67): The gills are trifurcate, the three finger-like processes on each gill of a pair arising from a broad lamellate process.

Specimens Examined.

Imagines. Lake Leake, Tas., 2,000′, 1 : 1929, H. M. Stephens; Dumaresque Cr., Armidale, 3,300′, 2 : 1947; Gara R., Armidale, 3,000′, 3 : 1948.

Nymphs. Dumaresque Cr., Armidale, 3,300′, 4 : 1947.

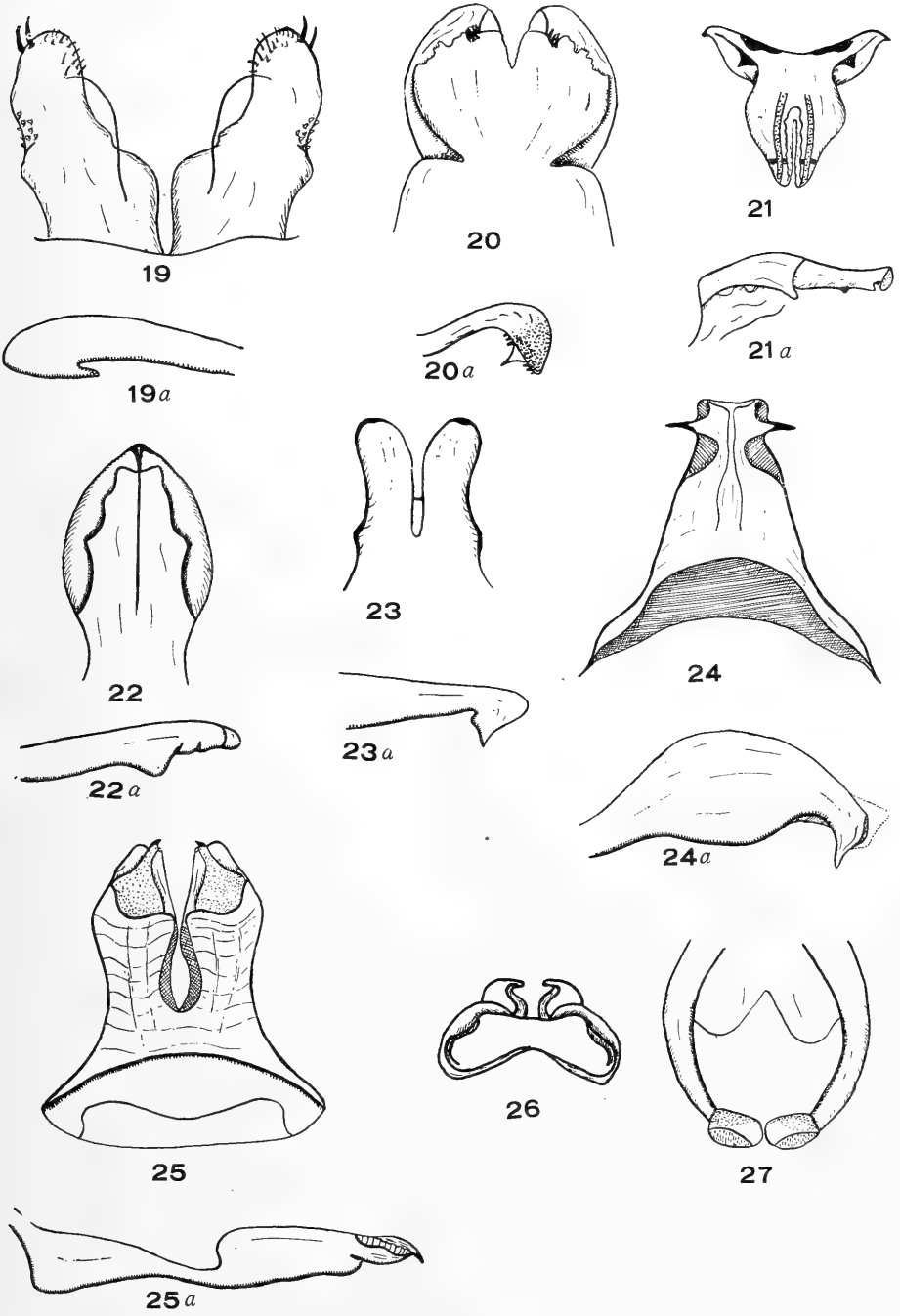
ATALOPHLEBIA PARVA, sp. nov.

Holotype ♂, Gara R., Armidale, 3,333′, 3 : 1948.
Allotype ♀, and *Morphotype*, Gara R., Armidale, 3,333′, 3 : 1948.
Paratypes, Dumaresque Cr., Armidale, 3 : 1948.

DESCRIPTION.

Male Imago (Text-fig. 6).

Measurements: Body length, 7·0 mm. (6·5–8·5 mm.). Cerci, 7·0 mm. Appendix dorsalis, absent. Forewing, 7·0 mm. (6·5–8·0 mm.). Hindwing, 1·2 mm. Foreleg, 1·5 2·3, 2·5 mm. (Tarsi, 0·8, 0·09, 0·09, 0·15 mm.). Hindleg, 1·4, 2·0, 0·5 mm. (Tarsi, 0·09, 0·09, 0·09, 0·15 mm.).



Text-figures 19-27. Penes. ("*a*" after a number indicates lateral view.)

19, *Atalophlebia marqwana*; 20, *Atalophlebia longicaudata*; 21, *Atalophlebia parva*; 22, *Atalophlebia maculosa*; 23, *Atopopus spadix*; 24, *Deleatidium annulatum*; 25, *Leptophlebia crassa*; 26, *Baëtis confluens*; 27, *Baëtis baddamsae*. (All figures × 25.)

General Colour: This is a yellowish-brown, except for the thoracic region, which is black.

Head: The head is black with short colourless antennae and white ocelli. The eyes are divided into an upper yellow to orange-brown region and a lower dove-grey or black.

Thorax: This is dark brown with black markings and a distinct white line which runs down beside the prescutoscutal suture.

Abdomen: The abdomen is dull brown with lighter brown markings and lighter intersegmental areas.

Wings: These are transparent with creamy venation, except in the costal area of the forewing, which is opaque, particularly in the pterostigmatic region (Text-figs. 45, 57).

Legs: The forelegs have reddish-brown femora with dark brown markings and yellow-brown tibiae and tarsi. The mid- and hindlegs are yellow-brown with dark brown markings. The tarsal claws are all narrow and uncinate.

Genitalia (Text-fig. 21): The forceps are three-jointed; the basal joint is dull brown changing to white distally, and the two distal joints are white.

Caudal Filaments: The appendix dorsalis is absent. The cerci are yellow-brown with dark brown annulations.

Female Imago.

Measurements: Body length, 6·5 mm. (5·5–9·0 mm.). Cerci, 7·0 mm. Appendix dorsalis, absent. Forewing, 6·2 (5·5–6·5 mm.). Foreleg, 1·0, 1·0, 0·5 mm.

General Colour: This is dark reddish-brown with the thorax showing distinctly darker brown.

Head: The eyes are greyish-black.

Abdomen: This is more uniform in colour than that of the male imago, being dark reddish-brown with a lighter coloured mid-dorsal line.

Eggs: The eggs are very distinctive. They bear two rows of appendages at either pole and the immature eggs show a sculpturing of hexagonal figures each with a central pit, while the mature eggs show radiating lines from the pit to the angles of the hexagon (Text-fig. 35).

Male Subimago.

The presence of uniformly grey wings distinguishes this stage from the imago.

Female Subimago.

In the majority of female subimagines the appendix dorsalis is present. Measurement, 7·0 mm. (6·5–8·5 mm.).

Nymph.

Measurements: Body length, 8·0 mm.

General Colour: This is chocolate-brown.

Head: The head is rectangular in shape when viewed from the dorsal surface. The eyes are black and the ocelli black with white bases.

Mouth Parts: The labrum (Text-fig. 75) is slightly concave on its anterior margin, the concavity being dentate. The mandibles (Text-figs. 73, 74) bear outer and inner canines with a variable number of teeth. The molar surface of the left mandible tapers to a sharp point on the inner edge and a chitinized angular projection is present on the inside edge below the molar surface. The prostheca is present in both mandibles, but is both more acuminate and more heavily chitinized apically in the right. The maxillae (Text-fig. 77) bear three-segmented palps, of which the basal two segments are about equal in size while the distal segment is much shorter. The anterior edge of each maxilla is fringed with a thick brush of brown hairs, below which is a row of about twenty parallel pectinate "rakes". The labium (Text-fig. 76) bears two three-segmented palps, the distal two segments being much narrower than the proximal segment. Spines occur on the basal segment, particularly on the outer margin, and

also on the distal portion of the middle segment. The paraglossae bear a group of spines on the anterior margin, while the glossae are densely covered with hairs.

Thorax: This is yellow-brown with curving, dark-brown markings.

Abdomen: The abdomen is dull brown with yellowish-brown markings.

Legs: The femur and tarsus each bear one dark band, and the claws are prominent and hooked at the tips.

Caudal Filaments: These are yellow-brown with dark annulations.

Gills (Text-fig. 72): These are paired and lanceolate in shape. The outer member of each pair narrows at about two-thirds of its length and then expands to a lamelliform tip.

Biology.

The subimago of this species is very sluggish and apparently attracted to light colours, as it will settle on the clothing of the collector and shows no tendency to fly away.

Specimens Examined.

Imago. Queanbeyan R., 1,901′, 2 : 1948; Gara R., Armidale, 3,300′, 4 : 1948, G. Davis; Dumaresque Cr., Armidale, 3,000′, 3 : 1948; Commissioners Waters, Armidale, 3,000′, 4 : 1948; Serpentine R., Point Lookout, 4,000′, 10 : 1948; Mienna, Tasmania, 3,000′, 12 : 1928, H. M. Stephens.

Nymphs. Queanbeyan R., 1,901′, 2 : 1948; Gara R., Armidale, 3,000′, 4 : 1948, G. Davis; Dumaresque Cr., Armidale, 3,000′, 3 : 1948.

Genus LEPTOPHLEBIA Westwood.

Intro. Mod. Classif. Ins., 2: 31, 1840.

Synonymy: *Euphyurus* Bengtsson, Ent. Tidskr., 38 : 177, 1914.

Genotype: *L. marginata* Linn.

Male Imago.

Wings: In the forewing the posterior branch of Rs sags posteriorly; in the hindwing the costal margin is very flatly arcuate with a shallow depression in the middle region. Cross veins are abundant in both wings.

Genitalia: The forceps are three-segmented, but a fourth may be present. The penes usually each bear an acuminate spine at the tip, and a reflex spur reaching down to the base of the central notch.

Legs: One of each pair of tarsal claws on a tarsi is blunt and the other hooked.

Nymph.

The gills are lanceolate and double, and there are well-developed lateral spines on the abdominal segments.

LEPTOPHLEBIA CRASSA, sp. nov.

Holotype Subimago ♂, Dumaresque Cr., Armidale, 3,300′, 3 : 1947.
Allotype Subimago ♀, Dumaresque Cr., Armidale, 3,300′, 8 : 1947.
Morphotype Nymph, Barrington Tops, 4,750′, 3 : 1948, B. McMillan.
Paratypes, as above.

DESCRIPTION.

Male Subimago (Text-fig. 9).

Measurements: Body length, 11·5 mm. Cerci, 20·0 mm. Appendix dorsalis, 19·0 mm. Forewing, 13·0 mm. Hindwing, 3·0 mm. Foreleg, 3·0, 2·5, 1·5 mm. Hindleg, 3·5, 2·0, 0·8 mm.

General Colour: This is yellow with brown markings.

Head: The head is yellow with chocolate-brown markings and brown antennae. Both regions of the eyes are dark brown and the ocelli are white.

Thorax: The thorax is yellow with definite brown markings.

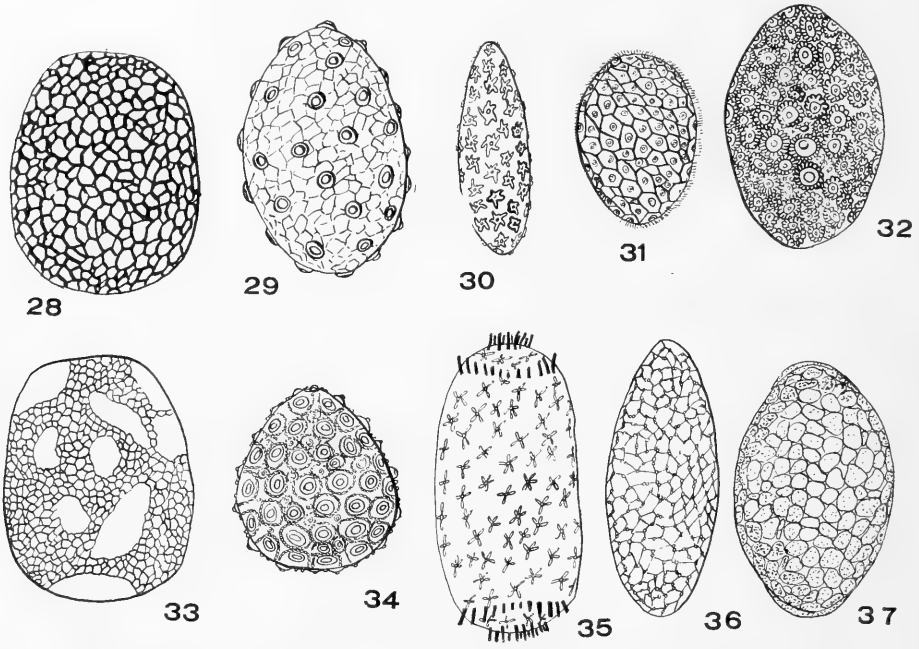
Abdomen: This is yellow with dark brown to black markings; on the lateral margin, which is slightly acute, there is a distinct black spot.

Wings (Text-fig. 60): The wings are grey with slightly darker veins, and in the hindwing the costal margin shows a slight concavity near the middle.

Legs: These are yellow with brown markings, the tarsi being four-segmented and bearing tarsal claws, of which one of each pair is narrow and acuminate and the other blunt.

Genitalia: The forceps are three-segmented, and from the tip of each penis projects a weak acuminate spine.

Caudal Filaments: These are yellow with a single brown band at the distal end of the basal segment. The appendix dorsalis is slightly shorter than the cerci.



Text-figures 28-37. Eggs.

28, *Atalophlebia albiterminata*; 29, *Atalophlebia incerta*; 30, *Atalophlebia marowana*; 31, species referred to in Appendix II; 32, *Deleatidium annulatum*; 33, *Cloëon fluviatile*; 34, *Leptophlebia crassa*; 35, *Atalophlebia parva*; 36, *Atalophlebia longicaudata*; 37, *Caënis scotti*. (All figures × 60.)

Female Subimago.

Measurements: Body length, 12·5 mm. (9·0–14·0 mm.). Cerci, 20·0 mm. Appendix dorsalis, 19·0 mm. Forewing, 11·0 mm. (9·0–12·0 mm.). Hindwing, 2·5 mm. (2·0–4·0 mm.). Foreleg, 3·0, 2·0, 0·8 mm. Hindleg, 3·0, 2·5, 0·8 mm.

Head: The head is darker than that of the male.

Egg: The egg is almost spherical and bears raised knobs, each one of which is surrounded by a circle of minute knobs.

Nymph.

Measurements: Body length, 9·0 mm. Cerci, 9·0 mm. Appendix dorsalis, 10·0 mm.

General Colour: Yellow with dark markings on the abdomen.

Head: The head is dark brown to black with white ocelli and short antennae.

Mouth Parts (Text-figs. 79–83): The labrum has no median concavity but bears a row of minute hairs along the anterior border; the lateral margins are extended and incurved. The left mandible has both the outer and inner canines bearing only one tooth, which is sharply pointed apically, and a prostheca which is acuminate and chitinized apically; the right mandible has serrated canines and no chitinized process in the prostheca. The maxillae bear three-segmented palps, of which the two distal segments bear hairs. The "plate" region is flattened and disc-shaped, and bears a tuft of brown hairs intermingled with which are recurving pectinate "rakes". The labium

bears three-segmented palps, the segments of which decrease in size distally. The paraglossae are egg-shaped, with the narrow end facing inwards towards the glossae, and bear spines along the anterior borders and the latero-anterior angles. The glossae are narrow, with almost parallel sides, and a bear a few hairs at the tips.

Thorax: The thorax is light brown with darker markings, and the wing buds are generally black.

Abdomen: The lateral margins of the abdomen are produced into spines, of which those of the ninth segment are the longest.

Legs: The legs are almost colourless, but may be tinged with brown along the ventral portion of the femora and tarsi. The tarsal claws bear large denticles.

Gills: The gills are paired, lanceolate, and seven in number. The gills on the third abdominal segment are largest and the following pairs decrease in size with order (Text-fig. 78).

Specimens Examined.

Subimagines. Serpentine R., Point Lookout, 4,000′, 10 : 1948; Marowan Cr., Glen Innes, 3,520′, 10 : 1948; Mienna, Tas., 3,300′, 12 : 1928, H. M. Stephens.

Nymphs. Dumaresque Cr., Armidale, 3,300′, 7 : 1948; Tumut R., Talbigo, 1,200′ (under rocks), 3 : 1948, B. McMillan; Barrington Tops, 4,750′, 3 : 1948, B. McMillan.

Genus DELEATIDIUM Eaton.

Trans. Ent. Soc. London, 1899.

Genotype: *D. lilli* Eat.

Imago.

"Distinguished as a genus from *Leptophlebia* by the male imago having genitalia conformable in pattern to those of an *Atalophlebia*" (Eaton).

Nymph.

Body flattened dorso-ventrally.

Head: Square in shape, with eyes borne laterally. Mouth parts similar to *Atalophlebia.*

Gills: Single.

DELEATIDIUM ANNULATUM, sp. nov.

Holotype ♂, Serpentine R., Point Lookout, 4,000′, 10 : 1948.

Allotype ♀, *Morphotype* Subimago, *Paratypes,* Talbigo, 1,200′, "at dusk", 12 : 1947, B. McMillan.

DESCRIPTION.

Male Imago (Text-fig. 10).

Measurements: Body length, 10·0 mm. Cerci, 11·0 mm. Appendix dorsalis, 11·0 mm. Forewing, 9·0 mm. Hindwing, 1·5 mm. Foreleg, 1·5, 3·0, 0·5 mm. Hindleg, 2·0, 1·0, 0·5 mm.

General Colour: This is tan.

Head: The head is orange with brown markings, while the upper region of the eyes is pale reddish-brown, and the lower and the ocelli are grey.

Thorax: Creamish-orange with light brown markings.

Abdomen: The abdomen is creamish-orange with definite markings which change in the posterior segments from dark brown to red-brown.

Wings: The forewing is faintly opalescent in the C-Sc region, in which the cross veins are unevenly shaded; elsewhere the wings are clear with brown venation.

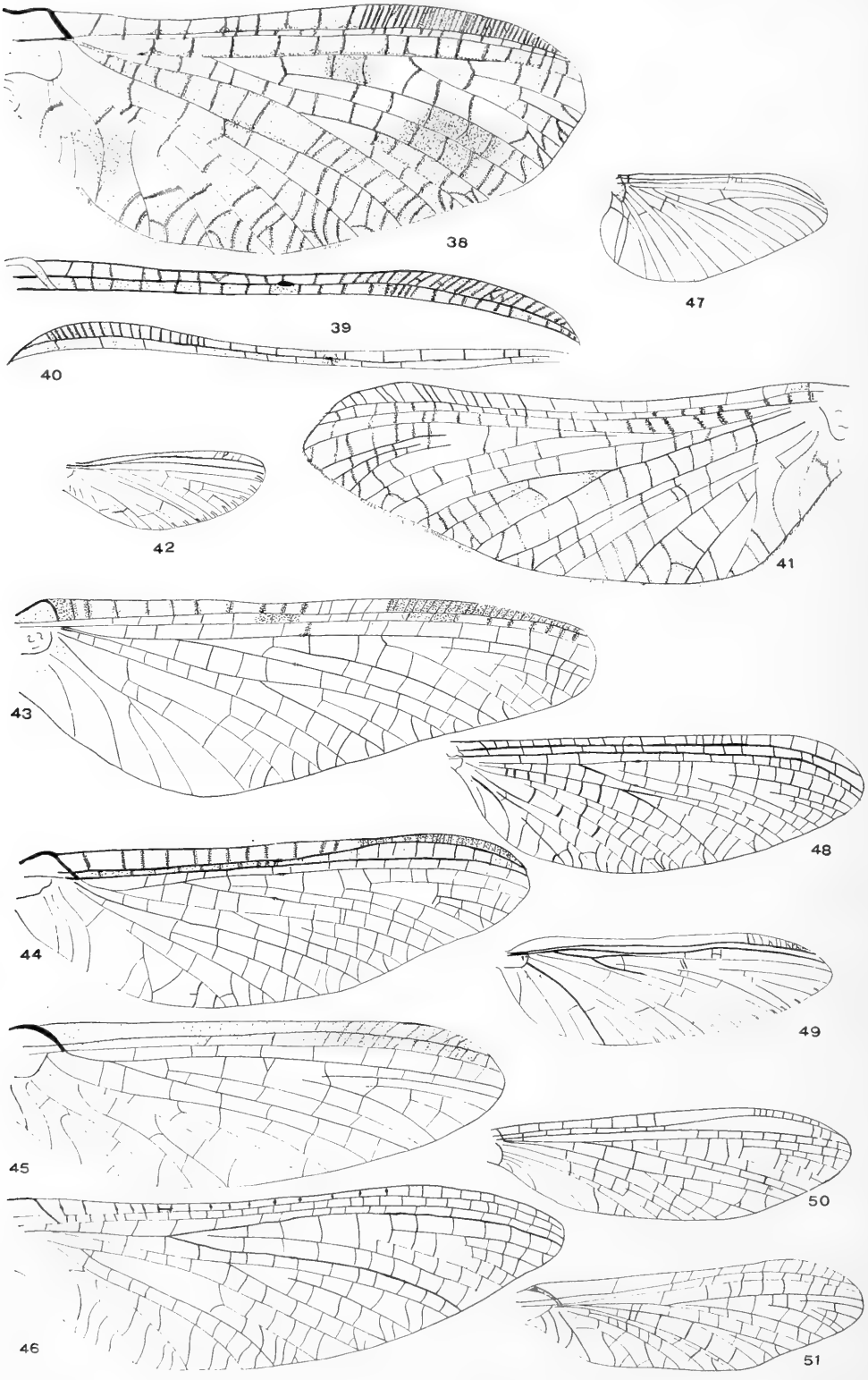
Legs: The legs are cream with two red-brown bands on the femora, and on each tarsus one claw of the pair is narrow and acuminate and the other is blunt.

Genitalia: The forceps are yellow and the penes red-brown.

Caudal Filaments: These are cream with definite brown annulations.

Subimago.

Wings: The wings are grey-brown in colour.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Specimens Examined.

Imagines. Juanama Cr., Talbigo, 1,200′ (at dusk), 12 : 1947, B. McMillan; Tumut-Talbigo, 900′-1,200′ (in rain), 12 : 1947, B. McMillan; Marowan Cr., Glen Innes, 3,500′, 10 : 1948; Serpentine R., 4,000′, 10 : 1948.

Family Baetidae.

Imago.

Head: In the male the development of the compound eye is outstanding and is known as a "turban eye"; the upper part is raised on a broad pedestal and is lighter in colour than the lower, normally rounded, portion.

Thorax: The pronotum of the female is narrow, closely connected to the mesonotum and receding behind.

Wings: In the forewing the cross veins are usually greatly reduced, and along the margin is developed a series of short marginal veinlets between the branches of the main veins. The middle branches of all the treads are disconnected, except occasionally IR_3b and ICuA, and the anal area is greatly enlarged, with a reduction of anal venation. The hindwings are greatly reduced or absent.

Caudal Filaments: The appendix dorsalis is aborted.

Nymph.

Mouth Parts: The maxillary palps are three-segmented.

Gills: Seven pairs are present, all being exposed and lamelliform.

Genus baëtis Leach.

Brewst. Edinb. Encycl., 9: 137, 1815.

Synonymy: *Brachyphlebia* Westwood; Introd. Mod. Classif. Ins., 2 : 25, 1840. *Cloë* Pictet; Hist. Nat. 2 Ephem. Neurop., 1843 *Acentrella* Bengtsson; Ent. Tidskr., 1912.

Genotype: *B. binoculatus* Linn.

Imago.

Wings: The forewing is 2–9 mm. and has marginal intercalaries occurring in pairs and basal costal cross veins are entirely wanting. In the hindwing there are only two or three longitudinal veins.

Genitalia: The forceps are four-segmented, the basal segment being the stoutest but contracting at its distal end, and the third segment being the longest.

Nymph.

Head: The head is hypognathous.

Mouth Parts: The labrum bears a notch in a median position on its anterior margin. The mandibular canines are large with blunt teeth. The maxillary palp may be three- or two-segmented.

Gills: Single, obtusely ovate or obovate gills are present on abdominal segments 1–7.

Baëtis baddamsae, sp. nov.

Holotype ♂, Guyra, 4,300′, 9 : 1948, G. Baddams.

Allotype ♀, Guyra, 9 : 1948.

Morphotypes Subimagines, nymphs, Billy's Cr., Grafton-Armidale Rd., 3 : 1948.

Paratypes, Marowan Cr., Glen Innes, 3,520′, 10 : 1948.

DESCRIPTION.

Male Imago (Text-fig. 15).

Measurements: Body length, 6·5 (6·0–7·0 mm.) *or* 11·6 mm. (11·0–12·0 mm.). Cerci, 10·2 mm. *or* 16·0 mm. Appendix dorsalis, absent. Forewing, 6·3 mm. (5·5–7·0 mm.) *or* 9·9 mm. (9·0–11·0 mm.). Hindwing, 2·0 mm. *or* 3·0 mm. Foreleg, 1·5, 2·0, 2·5 mm. Hindleg, 4·0, 4·0, 2·0 mm.

Text-figures 38-51. Forewings.

38, *Atalophlebia longicaudata,* subimago; 39, costal area imago, *Atalophlebia longicaudata*; 40, *Atalophlebia albiterminata,* costal area imago; 41, *Atalophlebia albiterminata* subimago; 42, *Baëtis confluens*; 43, *Atalophlebia maculosa*; 44, *Atalophlebia incerta*; 45, *Atalophlebia parva*; 46, species referred to in Appendix II; 47, *Caënis scotti*; 48, *Deleatidium annulatum*; 49, *Baëtis baddamsae*; 50, *Atopopus spadix*; 51, *Atalophlebia marowana.* (All figures × 4.)

General Colour: Black with conspicuous orange eyes.

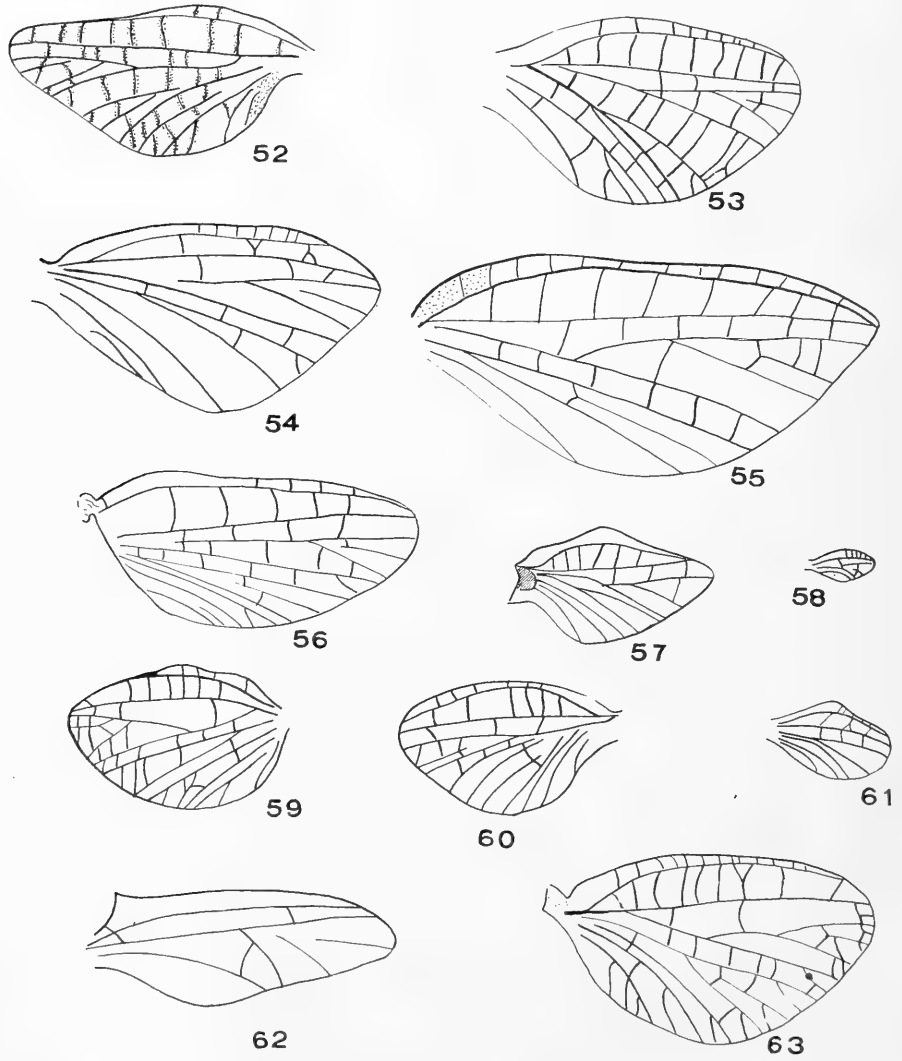
Head: This is black with very short antennae and eyes of which the upper lobes are bright orange and the lower dark brown or black.

Thorax: The thorax is black with very narrow yellow markings.

Abdomen: In the abdomen the anterior two segments are black with yellow markings and the following segments are brown with slightly darker markings in small areas; a distinct white line runs along the lateral margins.

Wings: These are colourless with yellow-brown venation, and in the hindwing the anterior margin rises to an acute peak close to the base.

Legs: The legs are grey-brown and the fore-tarsi have a distinctly crenulate outline. The tarsal claws of each pair are dissimilar, one being narrow and acuminate and one blunt and ovate.



Text-figures 52-63. Hindwings.

52, *Atalophlebia albiterminata*, subimago; 53, *Atalophlebia maculosa*; 54, *Atopopus spadix*; 55, species referred to in Appendix II; 56, *Deleatidium annulatum*; 57, *Atalophlebia parva*; 58, *Baëtis confluens*; 59, *Atalophlebia incerta*; 60, *Leptophlebia crassa*; 61, *Atalophlebia marowana*; 62, *Baëtis baddamsae*; 63, *Atalophlebia longicaudata*. (All figures × 16.)

Genitalia: The forceps are four-jointed.

Caudal Filaments: Basally these are brown with lighter annulations, but distally they are cream with dark annulations.

Female Imago.

General Colour: This is brown with a black head and thorax.

Head: The eyes are grey.

Thorax: This is dark brown with a distinctive yellow pattern.

Eggs: The eggs are of a distinctly greenish colour when first laid, but darken after the first day; there is no sculpturing on the egg at all. The eggs are laid in masses which stick together by means of a gelatinous substance which adheres closely to the substratum.

Subimago (Text-fig. 12).

General Colour: Creamy-yellow with darker thorax.

Wings: The wings are faintly grey with yellow venation.

Legs: These are yellow with segments outlined with dark brown.

Nymph.

Measurements: Body length, 10·0 mm. *or* 6·0 mm.

General Colour: Grey-brown.

Head: The head is cream with dull brown markings, black eyes and grey ocelli.

Mouth Parts (Text-figs. 84–88): The labium is deeply notched in the middle region of the anterior margin, which also bears a row of spines.

The mandibles bear blunt canines, the inner one bearing three teeth and the outer not being toothed, and a prostheca being present only in the right mandible. The maxillae bear two-segmented palps, of which the distal segments are the longest. The galea-lacinia narrows anteriorly, the galea region terminating in an acuminate point while the lacinia region is much broader and ends in a row of spines. On the labium the palps are two-segmented, the basal segments being the longest and broadest and the distal segments bearing spines on the outer surface; the distal segment also appears to have an inner lobe, which is deeply incised from the outer lobe. The paraglossae and glossae are of similar dimensions and are both pointed, the glossae bearing a row of spines on the inner surface.

Biology.

These Baëtids are seldom found flying over water; they appear to emerge and fly almost perpendicularly upwards out of sight. The nymphs are present among algae, where they can be seen gently raising and lowering their caudal filaments as they feed.

There appear to be two distinct physiological races present in this species. From a large series which was collected from one location in a stream all the imagines, subimagines and nymphs could be divided into two distinct groups on size, the groups not overlapping.

Specimens Examined.

Imagines. Gara R., Armidale, 3,300′, 12 : 1947; Guyra, 4,300′, 9 : 1948, G. Baddams; Tillbuster Cr., Armidale, 3,300′, 9 : 1948; Marowan Cr., Glen Innes, 3,520′, 10 : 1948; Mienna Cr., Tasmania, 3,300′, 12 : 1928, H. M. Stephens.

Nymphs as above and Billy's Cr., Armidale-Grafton Rd., 1,800′, 3 : 1948.

BAËTIS CONFLUENS, sp. nov.

Holotype ♂, Dumaresque Cr., Armidale, 3,300′, 9 : 1947.

Allotype ♀, *Paratypes,* Dumaresque Cr., Armidale, 3 : 1948.

DESCRIPTION.

Male Imago (Text-fig. 14).

Measurements: Body length, 4·5 mm. Cerci, 9·0 mm. Forewing, 4·0 mm. Hindwing, 0·5 mm. Foreleg, 1:1:2 (2·9 mm.). Hindleg, 3:2:2 (1·5 mm.).

General Colour: Light brown to orange-brown.

Head: The head is light brown with "turban" eyes, of which the surfaces are orange and the pedestal yellow, and the ocelli are slightly stalked.

Thorax: This is light brown with darker brown and yellow markings.

Abdomen: This is light brown with sparse markings and the lateral margins are slightly concave, especially in the posterior segments.

Wings (Text-fig. 42): In the forewing the costal and apical area of the subcostal region is slightly milky, Rs forks close to the base and IR_3b commences close to the fork, MA is unfolded. In the hindwing very few cross veins are present and the anterior margin is convex.

Legs: The legs are light brown and the tarsal claws of each pair on a tarsus are dissimilar, one being narrow and acuminate and one blunt and ovate.

Subimago and nymph unknown.

Specimens Examined.

Dumaresque Cr., Armidale, 3,300′ (rain), 9 : 1947.

CLOËON FLUVIATILE Ulm.

Archiv. für Naturgeschichte, 11, 1919.

To the original description all that need be added is a description of the egg.

Egg: Slightly ovate, cream in colour. Sculpturing reticulate; the markings surround almost circular smooth areas (Text-fig. 33).

Specimens Examined.

Armidale, 3,300′, 9 : 1948.

Family CAËNIDAE.

(*Brachycercidae* Lestage.)

The type genus of this family, genus *Caënis*, was suppressed by Lestage as a synonym of *Brachycercus* Curtis, which changed the family name to Brachycercidae. Since then no other authors have followed this alteration, and there seems some doubt as to the true synonymy of these two genera. Therefore until further evidence can be put forward it is proposed to use the name in general usage, *Caënis*.

Imago.

Head: The compound eyes are button-shaped and widely spaced in both sexes, the antennal second joint is at least twice as long as the first, usually longer, and the posterior margin of the head is nearly straight.

Wings: The anal area is well rounded and usually has only one anal vein. The cross vein system is greatly reduced and no free marginal veinlets are present. The forewing is 2–5 mm. in length and the hindwing is nearly always absent.

Nymph.

The gills of the second abdominal segment are enlarged and form a gill cover for the succeeding gills.

The only genus recorded from Australia is the Type genus.

Genus CAËNIS Stephen.

Ill. Brit. Ent., 6:61, 1835.

Synonymy: *Ordella* Campion, Ann. Mag. Nat. Hist., 11, s. 9, 64 : 515. *Ordella* Lestage, Ann. Soc. Ent. Belg., 65 : 61.

Genotype: *C. halterata* Fab.

Imago.

Wings: Exceptionally broad near the base (a feature which distinguishes *Caënis* from the Tasmanian genus *Tasmanocaënis* Lestage). The cross veins usually occur singly in each intercalary region.

Antennae: The second joint of the antennae is not more than twice the length of the basal joint.

Appendix dorsalis: Always present and may be slightly longer than the caudal filaments.

Nymph.

The nymph is important in distinguishing this genus from the closely related *Brachycercus* and *Tricorythodes*, so that although these two genera have not been found in Australia they are mentioned in comparison with *Caënis*, as it is very likely both of the former genera are present in Australia.

Mouth Parts: These are similar in all three genera, but the labrum is less broad in *Caënis* than *Brachycercus* and its apical margin is slightly concave. The glossae and paraglossae are fused in *Tricorythodes* but differentiated in the other genera.

Gills: The gills on segments 3–6 are single in *Caënis* and *Brachycercus*, but double in *Tricorythodes*.

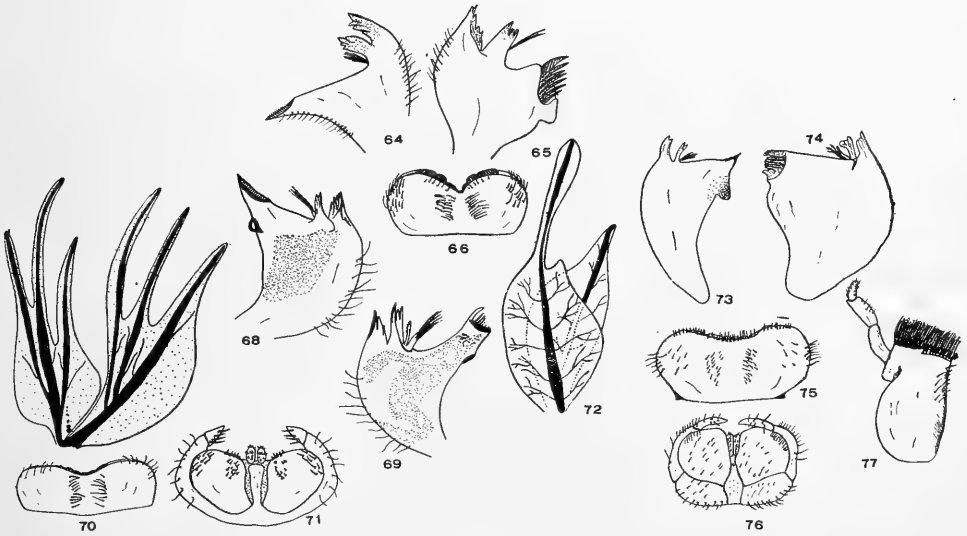
The postero-lateral spines of the abdominal segments are upcurved in *Brachycercus* but not in the other genera.

CAËNIS SCOTTI Till.

Proc. Roy. Soc. Tasmania, 1935 (published 1936).

Holotype Imago, Esk River, Clarendon, Tasmania, 3 : 1933.

Tillyard has only described the male imago of this species. The imago has not been found at all in Australia, and the five specimens taken were unfortunately placed in spirit and not allowed to undergo their final ecdysis.



Text-figures 64-77.

Atalophlebia albiterminata nymphal mouth parts. 64, right mandible; 65, left mandible; 66, labrum. *Atalophlebia incerta* nymph. 67, gill; 68, right mandible; 69, left mandible; 70, labrum; 71, labium. *Atalophlebia parva* nymph. 72, gill; 73, right mandible; 74, left mandible; 75, labrum; 76, labium; 77, maxilla. (All figures × 4.)

Female Imago.

Egg: (Text-fig. 37.)

Female Subimago (Text-fig. 13).

Five specimens only in the series.

Measurements: Body length, 6·4 mm. Cerci, 2·5 mm. Appendix dorsalis, 3·5 mm. Forewing, 5·0 mm. Hindwing, absent.

General Colour: Cream with dark brown markings, grey-brown antennae and dark brown eyes and ocelli.

Thorax: This is cream with dark brown markings and a dull brown mesonotum.

Abdomen: The abdomen is cream with brown stippled markings, a light cream venter with black markings, and the lateral margins are gently curved.

Wings (Text-fig. 47): The wings are opaque with dark brown venation. The hindwings are absent.

Legs: In the foreleg the femur is white with a brown outline and the tibia and tarsus light grey; the mid- and hindlegs are white with black markings. The tarsal joints are four-segmented, but the divisions are very indistinct. Of each pair of tarsal claws one is blunt and rounded and the other curved and pointed.

Nymph (Text-fig. 18).

Measurements: Body length, 5·5 mm.

General Colour: Cream with dull brown markings giving an impression of light dull brown.

Head: This is cream with brown markings, black eyes and short antennae.

Mouth Parts (Text-figs. 92–96): The labium is slightly concave on its anterior margin, the lateral margins of the concavity being slightly dentate. The mandibles bear two groups of canines with a varying number of teeth in each group; the molar surface is strongly chitinized and a prostheca is present. In each maxilla the plate (fused galea and lacinea) is almost pointed, being strongly convex on its inner surface and concave on its outer, with just the distal portion convex; a few spines are present at the distal extremity and the distal end of the outer surface is hairy. The palp is three-jointed, the proximal segment being the longest, and spines are present on the inner edge of the distal segment. The labium bears three-jointed palps, the basal joints of which are stout and short and the third joints are very short; all three joints bear stout spines. Both glossae and paraglossae are almost oblong and also bear stout spines.

Thorax: This is cream with brown markings, there being two large semi-circular markings on the pronotum and scattered markings on the mesothorax; the pronotum is narrow and collar-like.

Abdomen: The abdomen is cream with brown markings, the venter is creamy white with a dark line following the alimentary canal, and the lateral margins are convex with slightly incurving tips.

Legs: The legs bear black markings and rows of spines are present around the tibio-tarsal joint. The tarsal claws bear fine denticles.

Gills: There are only six gills present. The first (Text-fig. 89) is extremely small and of remarkable form, being apparently jointed into three segments, or sometimes four, and bearing hairs. The second gill (Text-fig. 90) is modified to form a gill cover for the remaining pairs and is heavily chitinized all over. The last four pairs are filamentous and very flexible (Text-fig. 91).

Biology.

The nymph is distributed widely, occurring in every district from which mayflies have been collected. It has been found below the surface of gravel bottoms of the running streams, but more often is found in the muddy banks or the silted edges of a stream. It is often present in streams which are drying up, usually either in the mud bottom or under and amongst any algae which may be present. In the laboratory it has been kept alive under a small clump of *Spirogyra*, which retained some moisture, for a period of five weeks; it has also been found under the caked surface of the muddy bottom of dishes which have dried out.

The adult appears not to be as prevalent as the abundance of larvae would lead one to expect, but this may be a result of collecting at the wrong times; however, it is the only nymph which has not been able to be bred through in the laboratory.

Specimens Examined.

Imago. Dumaresque Cr., Armidale, 3,000′, 8 : 1947.

Nymphs. Umberumberka Dam, Silverton Shire, 983′, 8 : 1947, A. Stokes; Gara R., Armidale, 3,000′, 2 : 1948; Queanbeyan R., 1,901′, 2 : 1948: Pine Forest, Armidale, 3,000′, 10 : 1947.

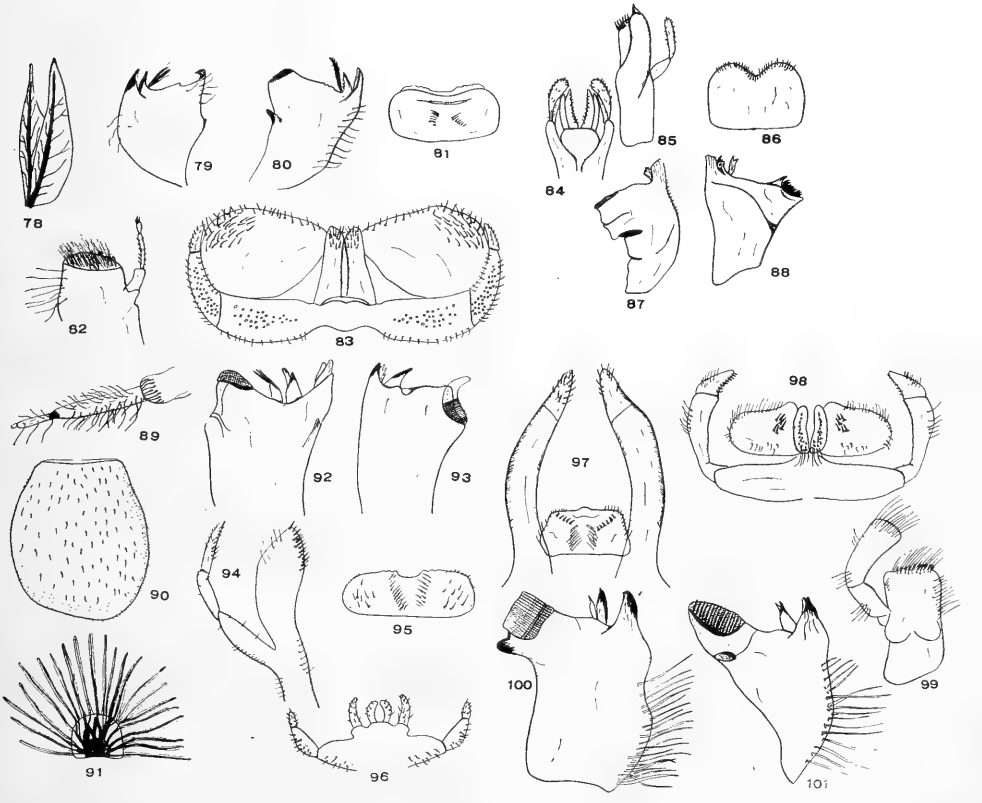
Genus ATOPOPUS Eaton.

Ent. Mo. Mag., 17–18:191–197, 1881.

Genotype: *A. tarsalis* Ent.

Imago Male.

"Foreleg about as long as body; tarsus about 1·4 as long as tibia, and this nearly 1·2 as long as femur. The tarsal joints in order of shortening rank 1, 2, 3, 4, 5, and the first about 1·3 as long as the second, and nearly half as long as the tibia. Hind-tarsus as long as second, and nearly 0·6 as long as femur. The first joint about 3·4 as long as the second, and upward of 1·1 as long as the tibia. Ungues unlike each other in every tarsus. Forceps limbs 3 jointed . . . ."



Text-figures 78-101.

Leptophlebia crassa nymph. 78, gill; 79, right mandible; 80, left mandible; 81, labrum; 82, maxilla; 83, labium. *Baëtis baddamsae* nymph. 84, labium; 85, maxilla; 86, labrum; 87, right mandible; 88, left mandible. *Caënis scotti* nymph. 89, gill from 1st abdominal segment; 90, gill from 2nd abdominal segment; 91, gill from 3rd abdominal segment; 92, right mandible; 93, left mandible; 94, maxilla; 95, labrum; 96, labium. Species referred to in Appendix II, nymph. 97, frontal process of head; 98, labium; 99, maxilla; 100, right mandible; 101, left mandible. (Various magnifications.)

ATOPOPUS SPADIX, sp. nov.

Holotype ♂, Armidale, 3,300′, 10:1948.

Allotype ♀, *Paratypes*, Badja, 3,000′, 11:1929, H. M. Stephens.

DESCRIPTION.

Male Imago.

Measurements: Body length, 9·5 mm. Cerci, 12·0 mm. Appendix dorsalis, absent. Forewing, 9·0 mm. Hindwing, 2·0 mm. Foreleg, 1·8, 3·2, 4·0 mm. Hindleg, 2·0, 1·0 2·5 mm.

General Colour: Red-brown with clear black markings.

Head: This is red-brown with dark brown markings, eyes which are dark brown and undivided, and white ocelli.

Thorax: This is dark brown with black margins to the sutures except that of the prescutoscutal, which is white.

Abdomen: The abdomen is yellowish-red to red-brown with clear-cut brown marking.

Wings: The wings are clear with yellow venation, and the cross veins in the C–Sc area of the forewing are tinged with reddish-brown.

Legs: The legs are yellow with two dark markings on the femora. The hind- and midlegs, as is usual in this genus, are remarkable for their proportions, and the tarsal claws on each tarsus are dissimilar.

Genitalia: The forceps are three-segmented and yellow in colour.

Female Imago.

As only pinned specimens were examined no eggs could be described.

Nymph unknown.

Specimens Examined.

Armidale, 3,300′, 10:1948; Badja, 3,000′, 11:1929, H. M. Stephens.

Although the practice of describing species from pinned specimens is not followed in general, in the case of this species, where a sufficient number of specimens in spirit were not obtainable for a good series, the series has been completed with pinned specimens from the H. M. Stephens collection, which are in very good condition.

Check List of Australian Species.

(Including Tasmania.)

Superfamily Baetoidea.

Family Leptophlebiidae.

Atalophlebia Etn., 1881; Tillyard, 1933. Type *A. australis* Walk.

albiterminata Till., 1935. Proc. Roy. Soc. Tasmania. Type location unknown.

australasica Pict., 1843, in *Baëtis*; *Leptophlebia* Eaton, 1871; Eaton, 1888; Ulmer, 1917. Synonym: *A. costalis* Burm. Type location: British Museum.

australis Walk., 1853, in *Ephemera*; *Leptophlebia* Etn., 1871; Etn., 1888; Till., 1933. Type location: British Museum.

brunnea Till., 1935. Type location: British Museum.

**costalis* Burm., 1893, in *Baëtis*; *Potomanthus* Pict., 1853; *Leptophlebia* Etn., 1871; Etn., 1888; Hutton, 1898, Trans. N.Z. Inst., Vol. 31; McLachlan, 1894, Ent. Mo. Mag.; Ulmer, 1920. Synonym of *A. australasica* Pict.

delicatula Till., 1935. Type location unknown.

**furcifera* Etn., 1871, in *Leptophlebia*; Etn., 1888. Synonym of *Deleatidium furcifera*. Type location: National Museum of Victoria.

fuscula Till., 1935. Type location unknown.

hudsoni Till., 1935. Type location unknown.

ida Till., 1935. Type location: British Museum.

inconspicua Etn., 1871, in *Leptophlebia;* Etn., 1888. Type location: Hope Museum, Oxford.

lucida Ulm., 1917. Type location: Stockholm Museum.

sexfasciata Ulm., 1917. Type location: Stockholm Museum.

simillima Ulm., 1919. Type location: Stockholm Museum.

superba Till., 1935. Type location: British Museum. Var. *pallida* Till., 1935.

**strigata* Etn., 1871, in *Leptophlebia*; Etn., 1888; Ulmer, 1920, in *Delatidium*.

uncinata Ulm., 1917. Type location: Stockholm Museum.

Leptophlebia Westwood, 1840. Genotype: *L. marginata* Linn., in *Ephemera*.

**australis* Etn., 1871; Etn., 1888, in *Atalophlebia*.

**furcifera* Etn., 1871; Etn., 1888, in *Atalophlebia*.

**inconspicua* Etn., 1871; Etn., 1888, in *Atalophlebia*.

**strigata* Etn., 1871; Etn., 1888, in *Atalophlebia*.

* Denotes synonymy.

DELEATIDIUM Etn., 1899. Genotype: *D. lilli* Etn.
strigatum Etn., 1899. Synonym: *Euphyurus bicornis* Ulm.
furcifera Etn., 1871, in *Leptophlebia*; Etn., 1888, in *Atalophlebia*.

Family SIPHLONURIDAE.

TASMANOPHLEBIA Till., 1921. Genotype: *T. lacustris* Till.
lacustris Till., 1921. Type location: British Museum.
lacus-coerulei Till., 1933. Type location unknown.
nigriscens Till., 1933. Type location unknown.

AMELETOIDES Till., 1933. Genotype: *A. lacus-albinae* Till., 1933.

COLOBURISCUS Eat., 1864, Ent. Mo. Mag. Synonym: *Coloburis*. Genotype: *C. humeralis* Walk., in *Ephemera*.
giganticus Till., 1933. Type location: C.S.I.R.O., Canberra.
haleuticus Eat., 1871. Type location: National Museum of Victoria.
munionga Till., 1933. Type location: C.S.I.R.O., Canberra.
tonnoiri Lest., 1935. Type location unknown.

Family BAËTIDAE.

BAËTIS Leach., 1815, Brewst. Edinb. Encycl. Genotype: *B. binoculatis* Linn.
**australasica* Pict. Synonym of *Atalophlebia costalis* Burm.
frater Till., 1935. Type location: British Museum.

CLOËON Leach, 1815. Genotype: *C. dipterum* Linn., in Ephemera. Synonyms: *Cloë* Pictet, 1843; *Cloëopsis* Vayssière, 1882.
fluviatile Ulm., 1919. Type location: Stockholm Museum.
tasmaniae Till., 1935. Type location: British Museum.
virens Klap., 1905. Synonym: *C. viridis* Till., 1935; Ulm., 1916.

Family CAËNIDAE.

CAËNIS Steph., 1935, Ill. Brit. Ent. Genotype: *C. halterata* Fab. (in *Ephemera*).
scotti Till., 1936. Type location: British Museum.

NEW SPECIES DESCRIBED IN THIS PAPER.

ATALOPHLEBIA.
incerta.
longicaudata.
maculosa.
marowana.
parva.

ATOPOPUS.
spadix.

BAËTIS.
baddamsae.
confluens.

DELEATIDIUM.
annulatum.

LEPTOPHLEBIA.
crassa.

GEOGRAPHICAL DISTRIBUTION OF GENERA OCCURRING IN AUSTRALIA.

Atalophlebia: New Zealand, Chile, Ceylon, Cape of Good Hope, Japan, South Africa.
Leptophlebia: Europe, North America, Chile.
Deleatidium: New Zealand, Chile.
Tasmanophlebia:
Ameletoides:
Coloburiscus: New Zealand, North America.

* Denotes synonymy.

Baëtis: Europe, Canada, Greenland, Egypt, North America, Central and South America, Indo-Malayan Region.
Cloëon: Europe, Indo-Malayan region, Japan, Chile, China, North America.
Caënis: Europe, Egypt, Morocco, Cape Colony, Ceylon, North America.
Atopopus: Borneo.

Techniques.

Preservation.

All stages are fixed in Carnoy's fluid and preserved in 70% alcohol. Pinned specimens are extremely unsatisfactory, as the colour darkens and body markings usually become indistinguishable, due to the shrivelling of the specimen. By fixing the specimen it is ensured that if new taxonomic techniques are evolved entailing histological examination the paratype will be in a suitable condition.

Preparation.

Genitalia. The terminal segments of the male are dissected away and the forceps and penis dissected apart prior to any preparation—this was found to be the surest way of preventing the parting of the two halves of the penis. The parts are then boiled in KOH until the flesh dissolves, rinsed in water, allowed to stand for a minute in glacial acetic acid, and washed again in water. If they are not perfectly cleared the process can be repeated. It was found that if slides were prepared of the genitalia some detail was lost in that only one view of the penis could be observed; therefore the parts were mounted on a glass slip in glycerine jelly and placed in the same jar as the insect from which they were taken. The advantage of this method is that the preparation can be moved into any position with a warm needle, and there can be no confusion as to which insect they refer.

Wings. When necessary wings were mounted by removing and placing in a drop of alcohol on a glass slide, the alcohol allowed to evaporate and a cover slip sealed on. Care must be taken to prevent the mounting medium from covering the wing itself, as some of the veins become invisible.

Mouth Parts. These were prepared in a similar fashion to the genitalia, but mounted in Canada balsam.

Eggs. These were dissected out and stained with neutral red or Orsein, and examined with an oil-immersion lens.

Appendix I.

Genus Atalonella Needham and Murphy.

Bull. Lloyd Libr. 24, Ent. Ser., 4:1–79, 1924.

The erection of this genus has been criticized by Lestage (1931); to his arguments the following points are added and cases cited.

Forewing.

1. Costal cross veins in basal half of Sc space supposed *numerous in Atalophlebia* and wanting in *Atalonella*. It has been found in several series of forewings that the cross veins in some species may be present or absent in this area, e.g., *Atalophlebia fuscula* Till.

2. Costal veins of stigmal region erect in *Atalophlebia*, aslant in *Atalonella*; this varies within a series, but not very noticeably. Intermediate forms occur and it would be difficult to differentiate between erect and slanting.

3. Bisector of MA, MP fork at its proximal end nearer MP_2 in *Atalophlebia*, in the middle of the fork in *Atalonella*. This has been found to vary so much that the two types have even been found on the right and left wings respectively of the same specimen.

4. CuA_1 straight in its apical third in *Atalophlebia*, curved in *Atalonella*. This, too, has been found to vary in a series.

Hindwing.

1. Tip of subcostal vein at nine-tenths of wing length in *Atalophlebia*, three-quarters in *Atalonella*. This is a constant character in all the specimens examined.

2. Upper lobe of median vein normal in *Atalophlebia*, disconnected at base in *Atalonella*. This again has been found to occur in both forms, one in each wing of a single specimen. However, the apparently missing piece can be distinguished if the wing is stained so that care is needed in the use of such a character.

3. Cross veins between the anal veins present in *Atalophlebia*, absent in *Atalonella*. These may vary within a series.

Table 2 gives species showing combinations of *Atalonella* and *Atalophlebia* characters.

TABLE 2.

Species.	*Atalonella* Characters.	*Atalophlebia* Characters.
Atalophlebia brunnea Till.	Number cross veins in costal area. Cross veins in stigmal region aslant. CuA_1 straight apically. Tip Sc at three-quarters wing length. Bisector lower fork MP absent. Cross veins between anal veins absent.	Bisector MA, MP closer to MP_2. Upper fork of median vein normal.
Atalophlebia delicatula Till.	Cross veins in stigmal area aslant. Bisector MA, MP in middle. Tip Sc at three-quarters wing length. Upper fork median vein detached. Bisector of lower fork MP absent.	Cross veins in basal half of Sc-C area present. CuA_1 vein straight at tip.
Atalophlebia longicaudata, sp. nov.	Bisector of MA, MP fork medianly placed. CuA_1 curved all the way to the tip.	Cross veins in the basal half Sc region numerous. Stigmal cross veins erect. Tip Sc at nine-tenths wing length.

Nymph.

In distinguishing between the nymph of *Atalophlebia* and *Atalonella* Needham and Murphy apply characters which not only appear in combination within each genera, but these characters are also applied in two conflicting descriptions of *Atalophlebia* characters.

To *Atalophlebia* nymphs are assigned the character "Posterior lateral angles of rear abdominal segments not tipped with thin flat lateral spines" (page 11), whereas on page 36, in comparing *Atalophlebia* and *Atalonella*, lateral spines are said to be present on abdominal segments 5–9.

Again on page 11, "Femora not dilated"; page 36, "Femora dilated, *Atalonella* femora slender"; and in reference to *Atalonella*, page 11, "Femora dilated".

APPENDIX II.

A specimen has been collected from Dumaresque Cr., Armidale, with unusual characters. As only one adult has been bred out the author cannot describe a new species, but it seems likely that this specimen cannot be included in any of the known genera.

DESCRIPTION.

Female Imago.

Measurements: Body length, 13·0 mm. Cerci, broken. Forewing, 13·0 mm. Hindwing, 3·0 mm. Foreleg, 3·0, 3·0, 1·5 mm. Hindleg, 3·0, 2·5, 1·0 mm.

General Colour: Cream with red-brown marks on the abdomen.

Head: The head is cream with brown markings, black eyes, short antennae (0·3 mm.), and cream ocelli with brown bases.

Thorax: The thorax is cream with very faint light brown outlines to the thoracic sclerites.

Abdomen: This is cream with red-brown markings, and the lateral margins are rounded.

Wings: The wings have dark brown or red-brown veins which in the C-Sc region of the forewing are slightly shadowed, the wing itself in this region being opaque.

Legs: These are cream or white without any markings, and the claws of each pair on a tarsus are dissimilar, one being hooked and the other blunt.

Nymph.

Measurements: Body length, 14 mm.

General Colour: Yellow with darker markings.

Head (Text-fig. 97): This is triangular with two small projections arising from the head on either side of the labrum (*not* from the mandibles) 1·4 mm. long and each bearing hairs at the tip. The head is light golden-brown with chocolate-brown markings. The eyes are brown and the antennae are quite stout and bear whorls of longish hairs.

Mouth Parts (Text-figs. 98–101): The labrum bears a small convex protuberance in the middle region of the anterior margin, and hairs and small spines are present on the antero-lateral angles together with a row of stout spines which slant inwards towards the mid-line. The mandibles are stout with long hairs on the outer margin. The outer canines bear two teeth, and the inner, one; a prostheca is present and the molar region is very broad. The maxillae bear two-segmented palps, of which the distal joints are almost rectangular with broad anterior edges bearing hairs; only a few hairs are present at the distal end of the plates, below which are single rows of pectinate spines. The labium bears two-segmented palps, of which the distal segments are short and conical, and each bears a row of stout spines on the inner surface. The paraglossae are broad and bear a clump of stout spines in the centre of the ventral surface, the glossae are slightly kidney-shaped with a mid-longitudinal line of spines.

Thorax: The prothorax is oblong and golden-brown with chocolate-brown markings.

Abdomen: This is similar to the thorax, but with black markings; the venter is white with a single median black line following the alimentary canal.

Legs: The legs bear long hairs.

Gills: These are double and lanceolate with a long filamentous distal end, and hairs all over the surface of the gill. They are white with black tracheae.

Caudal Filaments: These are colourless with whorls of hair at the end of each segment. The appendix dorsalis is slightly longer than the cerci.

Biology.

The nymph lives under the surface of the stream bed and can only be found by dredging.

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A STUDY OF THE ALKALINE PHOSPHATASE REACTION IN TISSUE SECTIONS.

PART I. THE POSSIBILITY OF ITS QUANTITATIVE USE.

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(Nine Text-figures.)

[Read 26th April, 1950.]

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Introduction.

Despite the enormous body of data comprising formal genetics, the mechanism of gene reproduction and the method by which the genes exert their effect in the cytoplasm are still only matters of speculation.

Before the problem can be rationally approached at the biochemical level much further data on the chemical composition, and especially the enzyme constitution, of the nucleus will be necessary.

As a contribution to this problem a study of the variations in amount of a number of phosphatases in the meiotic and spermateleotic cycles of the testis cells of guinea-pigs was projected, making use of the reaction in tissue sections.

The first histochemical demonstration of phosphatase appears to have been made by Robison and Soames (1930), who incubated thin slices of limb bones in solutions of pH 7·4 containing calcium, inorganic phosphate and hexosephosphate. After prolonged incubation the calcium phosphate in the slices was demonstrated by the von Kossa method. Rosenheim and Robison (1934) applied the same methods to kidney, aorta and lung. The pictures obtained by these methods have little resemblance to those resulting in the modern phosphatase technique, because in the former the pH was sub-optimal and wandering of calcium phosphate and enzyme occurred in the unfixed

tissue. Although Martland and Robison (1929) had observed that bone phosphatase survived treatment with alcohol, this fact was not applied to the histochemical study of the enzyme for another ten years.

In 1939 Gomori and Takamatsu greatly improved the method by introducing fixation, replacing freehand sections with microtome sections, and by using an optimal pH and a greater substrate concentration. Gomori (1941) replaced the generally unsatisfactory von Kossa method of demonstrating the calcium phosphate formed by enzyme action with the current cobalt conversion method. Kabat and Furth (1941) suggested the routine addition of magnesium to the incubating solution.

Despite the enormous body of literature which has accumulated on the distribution of and physiological changes undergone by the enzyme in various tissues and cells, there have been few attempts to examine the reaction critically. The most important study of the validity of the reactions has been made by Danielli (1946), who concluded that the reaction was capable of giving a valid intracellular localization of the enzyme. Lison (1948) in a review of the reaction is of the opinion that diffusion and adsorption of the enzyme may, when the tissue is poorly fixed, result in false intracellular localizations. Various aspects of the method have been examined by Emmel (1946*a*), Stafford and Atkinson (1948), and Doyle (1948).

Histochemical results are usually expressed in subjectively determined grades of colour. While the human eye is well able to compare the depths of two colours and to arrange a series of colours in order of their depth, it cannot determine the quantitative relationships in such series.

The first serious attempt to give a quantitative expression to cytochemical and histochemical results appears to be that of Marza and Chiosa (1935). By means of a comparison ocular the object in the microscope field was compared with a series of colours produced by known concentrations of the substance being studied. The method was applied by Marza et al. (1937) in a study of the histochemistry of the ovary of *Fundulus*.

Apart from the methods of Caspersson (1936, 1941), which are specialized and require apparatus beyond the reach of most laboratories, the first use of photoelectric methods in histochemistry appears to have been made by Stowell (1942) in a study of the variations in intensity of the Feulgen reaction during experimental carcinogenesis. Stowell did not, however, demonstrate that the intensity of the Feulgen reaction was a valid estimate of the desoxyribonucleic acid content of tissue sections. This fact has been very elegantly established by Di Stefano (1948).

The difficulty of applying ordinary photoelectric methods to cytochemical problems is the difficulty in getting enough output to work the usual type of measuring instrument. This problem has been resolved in two ways. Pollister and Ris (1947) and Pollister and Moses (1949) have made use of electron multiplier photocells which give a large output and a low noise signal ratio, while Ely and Ross (1948) and Stowell (1948) have used densitometry of photomicrographs.

In the present investigation use has been made of an instrument purchased locally for use as a photomicrographic exposure meter. The instrument is rather crude but is adequate for the present study, since all the points at issue may be decided by photometry of microscope fields rather than of cells and parts of cells.

The aim of this investigation has been to establish the optimal conditions for demonstrating alkaline phosphatase in tissue sections and to decide whether it is possible to obtain a valid estimate of the enzyme in tissues and cells. Such an investigation forms a necessary preliminary to the projected study noted above. Because it has a number of technical advantages over other tissues, renal cortex has been the main tissue studied. The temperature of incubation has been 40°C. throughout.

Methods.

(*a*) *Photometric.*

The photometric unit consisted of a vacuum photocell with a caesium cathode, and a two-stage D.C. amplifier, the output of which was read on a microampmeter.

The photocell was mounted in a light-tight cylinder. Opposite the cathode, and of the same diameter, was an aperture which could be closed by a sleeve inside the cell mounting. From this aperture projected a cylinder one inch long.

Coupling with the microscope was effected by means of a Reichert demonstration ocular. A fitting was made which was placed over the end of the horizontal arm of the demonstration ocular, and into it the spout of the photocell mounting was inserted. All junctions were light-tight. This method of coupling allows simultaneous visualization of the field and measurement of its light absorption.

Illumination of the microscope field was by critical illumination of the Kohler type, the light source being a low-voltage concentrated filament lamp, run on heavy-duty accumulators. The lamp input was regulated by a variable resistance.

The response of the apparatus over the light intensities used was tested and slight deviations from logarithmic response noted. These were shown to be due to the amplifier, not the photocell.

The mean percentage transmission readings of the experimental sections were expressed as a percentage of the transmission of the control sections incubated in the absence of substrate. The resulting transmission was expressed in "density units" by reference to a standard semi-logarithmic graph, the ordinate being log.% transmission and the abscissa being so divided that 100 density units occur between 100 and 30% transmission, the curve being taken from the standard response calibration. One density unit thus corresponds to an optical density (2-log.G) of 0·00523.

(*b*) *Histological.*

The organs, removed from male guinea-pigs, killed by a blow on the head, were cut into fairly large pieces, about 7 mm. thick. The use of these large pieces of tissue is justified later. After fixation at 2°C. for 18–24 hours, dehydration was completed by cold absolute alcohol (8–24 hours). Clearing was in two changes of benzol (6–24 hours) and embedding in paraffin was accomplished in the shortest feasible time (2–5 hours). The acetone, alcohol and benzol volumes were at least 50 times the volume of the tissue. Sections were cut at 8 microns, the microtome being adjusted to minimize paraffin compression (Dempster, 1943).

The sections were flattened in a beaker of distilled water immersed in a thermostatically controlled water bath at 39–40°C. The time needed for complete flattening was determined and a time about half as much again chosen (usually 20–30 seconds). All sections in the series were flattened for exactly this time and were mounted serially in groups of two on numbered slides.

After drying in the incubator for minimal times, the slides were stored in specimen jars with ground-glass greased lids. In the bottom of the jar was a layer of silica gel (with indicator), and the jar was stored in a refrigerator.

Under these conditions of storage no detectable change in the phosphatase content of sections could be demonstrated photometrically over periods of up to four months after cutting of the sections. Sections which had remained in sealed jars over spent silica gel at room temperature for a period of six months still gave a typical reaction with little or no diminution in intensity.

All authors who have studied the stability of the enzyme in sections—e.g., Danielli (1946), Lison (1948), Doyle (1948)—seem agreed that much of the enzyme is lost in a period of about two weeks after cutting. The discrepancy between these reports and the present experience is very difficult to understand.

Tissues embedded in paraffin blocks were also found to be stable for periods of at least nine months. This is again at variance with the reports of Lison and Doyle.

Surface areas were determined by planimetry of microprojection drawings; in kidney sections only the cortical area was measured.

(*c*) *Chemical.*

All inorganic chemicals used in this study were of analytical grade. The glycerophosphate and phenylphosphate were B.D.H. laboratory reagents, the former being a mixture of α and β ester.

Calcium phosphate was extracted from tissue sections by means of N/10 HCl and the inorganic phosphorus determined by the molybdenum blue method, using stannous chloride as the reducing agent. The absorption was measured with the same photometric unit as was used on the microscope, the range being 1–10μg. P. The precision (2 × C.V.) was 3% at the 4μg. level and wherever possible aliquots containing approximately 4μg. were taken.

All pH measurements were made with a Jones pH electrometer and measured to the nearest 0·1 unit.

The phosphate solubilities were determined by addition of an excess of inorganic phosphate to the solution under test, centrifuging the precipitate and analysis of the supernatant fluid.

Accuracy of the Photometric Estimate.

The following are the main sources of variability:

(*a*) Errors in setting zero and full-scale deflection. These seldom reach half a unit and can be ignored, since they are frequently checked.

(*b*) Histological variability in different fields. The coefficient of variation of fields of renal cortex in any one section was 5%. Since 10 readings are made on each section the coefficient of variation of the section mean due to this source is 1·5%.

(*c*) Variation in section thickness. This source of error is difficult to assess accurately. By using sections as thick as possible without obscuring the cytological picture it can be minimized, and the use of section pairs eliminates the effects of any tendency of the microtome, due to mechanical instability, to cut alternate thick and thin sections. Richards (1944), using an interference method of estimating section thickness, quotes a standard deviation of 0·3μ for sections cut with a microtome setting of 10μ. This gives a coefficient of variation at the 5% level of significance of 6%. The method of flattening (if any) is not stated. Linderstrom-Lang, Holter and Ohlsen (1934) have reported an error of 3% (in weight) in paraffin sections, due mainly to variations in the microtome.

(*d*) Variation due to section flattening. This appears to be the largest potential source of variability, and for this reason stringently controlled flattening technique was used. In four groups of ten serial sections, flattened in the way described, the coefficient of variation of the surface area was found to be 1·5–2%.

(*e*) Variability due to the histochemical procedures was presumably the largest source in the present case; an idea of its magnitude can be obtained from a study of the total variability.

The total variability of density was studied in four groups of ten serial sections which had been subjected to the phosphatase reactions and the C.V. found to vary between 3% and 6%. By the use of four sections in any density determination the standard error is reduced to about 2½%, and the 5% probability limits are thus about 5% of the mean. In all the experiments of the present paper four sections were used in the computation of the density units quoted.

The Inorganic Basis of the Reaction and Histochemical Technique.

Since in the present case it was intended to use the histochemical method in a quantitative manner, some knowledge of the inorganic basis of the technique seemed desirable, both as a general background and as a means of devising suitable methods.

(*a*) *The Demonstration of Calcium Phosphate.*

(i) *Washing* after incubation should remove any particles of calcium phosphate adhering to the section and neutralize the buffer used for incubation, since alkaline solutions can cause an increased blank reaction by interaction with cobalt. These aims were met in the present work by using M/20 $CaCl_2$ dissolved in M/50 veronal buffer of pH 7·5. The solution is saturated with inorganic phosphate before use. Addition of phenyl mercuric nitrate prevents bacterial growth. Four dips in the solution is sufficient.

(ii) *Conversion to cobalt phosphate.* Some conditions affecting the solubility of cobalt phosphate are shown in Table 1.

TABLE 1.
Solubility of Cobalt Phosphate at Room Temperature.

Precipitant (at M/20).	Vehicle.	pH.	µg. P/10 ml.
$Co(NO_3)_2$	Distilled water.	6·2	154
,, $+CaCl_2$	,, ,,	,,	138
$CaCl_2$	,, ,,	6·7	,,
$Co(NO_3)_2$	Veronal (M/50).	7·2	8
,, ...	,, ,,	7·4	4
,, $+CaCl_2$	,, ,,	,,	8·5
$CaCl_2$	,, ,,	7·4	115

It will be seen that cobalt phosphate has a considerable solubility when precipitated in distilled water, and that a great reduction in solubility results when veronal buffer is used as a vehicle for the cobalt. When the cobalt phosphates prepared from distilled water and veronal buffer were washed twice with distilled water and re-suspended in distilled water considerable differences in solubility were noted. After leaving overnight the former was soluble to an extent of 5mg. P/10 ml., while the latter showed a solubility of 140µg. P/10 ml.

Large quantities of cobalt phosphate may be dissolved in glycine buffers.

For these reasons the conversion of calcium to cobalt phosphate was made by the use of cobalt nitrate (M/20) dissolved in veronal buffer of pH 7·3; in tissue sections the reaction was complete in 10 minutes. The solution is saturated with inorganic phosphate before use.

(iii) *Washing.* Danielli (1946) has indicated that this step is a critical one. By the use of the veronal-cobalt solution its latitude is increased, as indicated above. In this investigation, washing after the cobalt solution was performed in three jars of distilled water, the slide being dipped four times in each jar.

(iv) *Conversion to cobalt sulphide.* Because of the variability of yellow ammonium sulphide both between batches and in the same batch with time from manufacture, it is very difficult to standardize this step.

Since the importance of this factor was not appreciated until late in this investigation, the densities quoted in different experiments are not strictly comparable.

With the stock ammonium sulphide used in the present study no difference in maximum depth of colour produced was detected in dilutions down to 1 in 200, but below this the colour developed was reduced and the cytological picture was changed.

In the work here reported a dilution of 1 in 100 was used; the reaction was complete in five minutes.

(v) Danielli has indicated that the *dehydration in alcohol* is a critical step. In order to standardize this step better the slides were dipped six times in two lots of tap-water, blotted gently, dipped four times in two lots of absolute alcohol and then in two lots of xylene. The mountant used was Gurr's Euparal. The alcohols were frequently changed.

(*b*) *Validity of Demonstration Method.*

As a test of the validity of the histochemical technique for visualization of the inorganic phosphate, and to obtain some idea of the relation of the density to the absolute amounts of inorganic phosphate, experiments with known amounts of phosphate incorporated in cigarette papers were performed.

Pieces of rice cigarette paper were impregnated with known amounts of sodium phosphate and dried. A control piece received only distilled water.

When these papers are placed in an M/50 $CaCl_2$ solution of pH 9·5 considerable losses of phosphorus occur, presumably because of non-absorption of the resulting calcium phosphate. When placed directly in buffered cobalt nitrate, put through ammonium sulphide in the usual way, blotted, dried for minimal times in the incubator and mounted, a linear relation between the density and the amount of phosphorus present was found over a wide range, as shown in Text-fig. 1.

This linearity being established, it was then necessary to show that no loss of phosphorus, like that found in cigarette papers, occurs in sections incubated in buffer substrate. As indicated by Table 2, no significant loss of phosphate from tissue sections occurs in the course of the histochemical incubation.

TABLE 2.
Loss of Phosphate from Kidney Sections During Incubation.

Time	1 hr.	15 hr.	21 hr.	40 hr.
Increase in phosphorus calcium in fluid, µg.	1	5	8	12
Phosphorus present in sections, µg. ..	40	120	130	150

Gomori (1949) has suggested that one of the complicating factors of the histochemical reaction is the balance between formation of calcium phosphate and its solution or diffusion from the section. The present results do not favour such an interpretation, provided the solution is saturated with calcium phosphate before incubation.

It may then be concluded that the techniques used for the demonstration of the calcium phosphate formed by enzyme action are suitable for quantitative use.

(c) *The Formation of Calcium Phosphate.*

(i) *Choice of Buffer.* Of the three common buffers available in the pH range of alkaline phosphase borate has the greatest acid-buffering capacity.

In the histochemical reaction borate buffer was found to give higher final densities than veronal. (Cf. Table 7.)

In fresh tissue homogenates or tissue sections studied non-histochemically (Ca absent from incubating fluid) it was found that the enzyme activity in borate and veronal buffers was the same in testis but less with borate in kidney.

Borate buffers, usually with glycine added (p. 43) were mainly used in this study. Since buffer capacity is probably important (p. 44), equimolar mixtures of borate and veronal, which give linear buffering over this range, were used in the study of the pH optimum.

(ii) *The Solubility of Calcium Phosphate.* The solubility of calcium phosphate was unaffected by calcium concentration, buffer composition or concentration, but was very dependent on pH, as shown in Table 3.

TABLE 3.
Effect of pH on Solubility of Calcium Phosphate.

pH.	µg. P/10 ml. Solution.
10·0	1
9·5	2
9·0	4
8·5	10
8·0	30
7·5	105

M/50 borate buffer, M/100 calcium chloride. Temperature 40° C.

That this increasing solubility is important in the histochemical method is shown in Table 4, where the density produced in the presence and absence of excess inorganic phosphate at several pH values is shown.

TABLE 4.

Effects of Excess Phosphate on Density Resulting after Incubation at Various pH Values.

pH.	Excess Phosphorus.	Density.
9·5	Present.	39
9·5	Absent.	40
8·7	Present.	35
8·7	Absent.	34
8·2	Present.	24
8·2	Absent.	15
7·7	Present.	5
7·7	Absent.	0
7·0	Present.	1
7·0	Absent.	0

Incubation times vary for the different pH values. M/100 glycerophosphate, M/100 mg., M/50 borate buffer pH 9·4. Kidney sections.

At temperatures below 40°C. an increase in the solubility of calcium phosphate was observed.

(iii) *Choice of Calcium Compound and its Concentration.* Calcium chloride and nitrate were compared for their effect on enzyme activity at several concentrations over the range M/1000 to M/10 and no differences found. Since the former was available in A.R. grade it was preferred, the concentration used being M/100.

The Behaviour of the Enzyme in Sections.

(*a*) *Comparison of Fixatives.*

The preservation of enzyme is difficult to compare in differently treated sections because of a number of histological variables, namely:

(i) Shrinkage of material. A comparison of the shrinkage produced by different fixatives may be based on the cube of the mean proximal tubule radius.

(ii) The degree of flattening. Flattening not only corrects the paraffin compression caused by the microtome but may also cause an expansion of the section by increasing the intertubular area. This latter factor will depend on the degree of hardening of the intertubular connective tissue by the fixative. The time taken for flattening of the sections forms a basis for comparing different fixatives.

TABLE 5.

Apparent Enzyme Activity after Various Fixatives.

Fixative.	Time for Flattening.	Shrinkage Factor.	Enzyme Density.	Blank Density.
Absolute acetone	1 min.	100	31	8·5
80% alcohol	2 min.	105	29	10
25% pyridine in 80% alcohol	5 min.	90	35	12
20% pyridine 70% alcohol 10% formalin	More than 10 min.	80	16	24

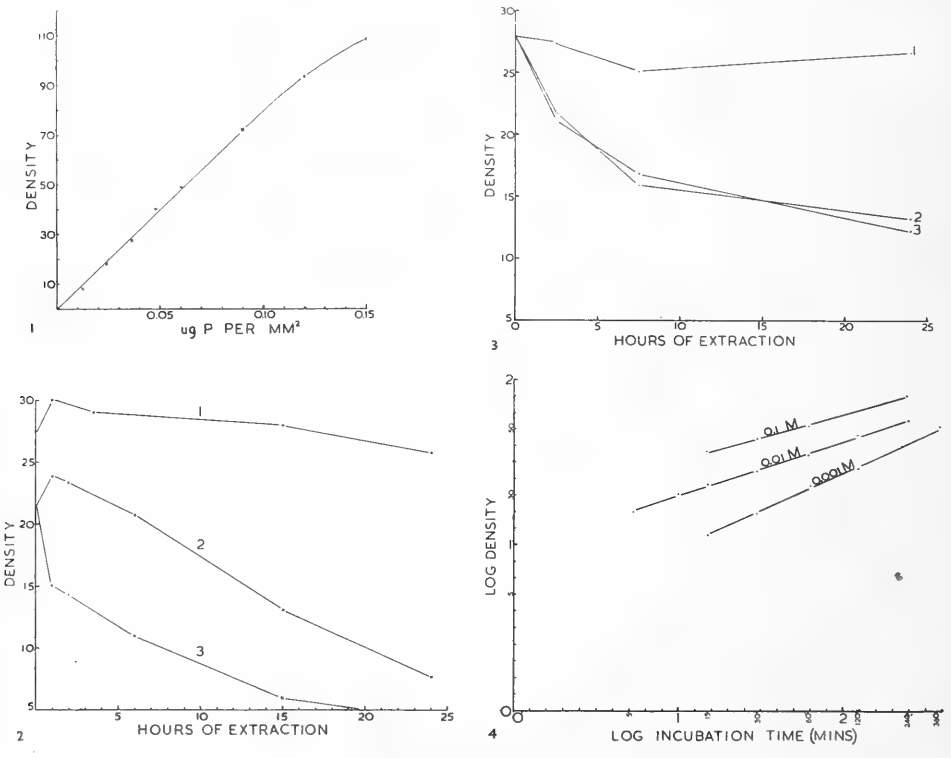
M/50 borate buffer pH 9·4, M/100 Mg and substrate. Incubation 30 mins. Kidney sections. The values quoted are the means of four separate experiments.

A further factor to be considered in the choice of fixative is the intensity of the blank reaction, which was found to vary with different fixatives.

Table 5 shows the effect of different fixatives on kidney enzyme. It will be seen that when the histological factors are considered there is little difference in enzyme preservation between the first three fixatives; the fourth causes much enzyme destruction and is associated with a high blank density. There was little difference in the quality of fixation with the first three fixatives and acetone was preferred in the present study.

Slight apparent increases in the enzyme activity of blocks fixed at room temperature or of blocks smaller than usual were shown to be due to differences in shrinkage.

In only two blocks of about 80 used in the present study was there any evidence of a gradient of enzyme from surface to interior; these two cases were evidently due to incomplete clearing in benzol.



Text-figures 1-4.

Figure 1.—The relation between known concentrations of inorganic phosphate incorporated in cigarette papers and the density (in units defined on p. 37) resulting from the histochemical procedure adopted.

Figure 2.—Loss of enzyme in kidney sections incubated for varying times in buffer with Ca and Mg ions (M/100) but without substrate. The density is that resulting after a subsequent incubation with substrate. Curve 1 is typical of the majority of kidney sections tested. Curves 2 and 3 are from sections showing much loss of activity, curve 2 demonstrating the protective action of glycine (0·006M). The stimulation of enzyme activity after short periods of incubation is due to the presence of magnesium ions.

Figure 3.—The effect of the presence of calcium phosphate in the section on the loss of enzyme activity. Curve 1 is from sections incubated with substrate, the calcium phosphate extracted and the section reincubated with substrate. Curve 2 is from slides in which the primary incubation was conducted in the absence of substrate, and curve 3 from slides where Ca was omitted in the primary incubation. All sections are of kidney.

Figure 4.—The time course of the phosphatase reaction in kidney sections at various substrate concentrations.

(*b*) *Stability of the Enzyme in Sections.*

In a study of the enzyme surviving after various times of incubation in a number of different substrate free solutions the following points were noted:

(i) In the majority of kidney blocks studied the loss of activity after 24 hours' incubation in borate buffer of pH 9·5 was less than 15%; some blocks, especially testis, may show almost complete loss. This rapid inactivation could not be related to any known factor in their past history.

(ii) The loss of activity was relatively uninfluenced by the presence of magnesium in the incubating solution, suggesting that the loss is not due to loss of coenzyme.

(iii) Veronal, borate and glycine buffers (M/50) gave essentially the same inactivation rates when tested at pH 9·4.

(iv) The inactivation was not influenced by borate buffer concentration over the range M/50–M/10.

(v) The losses were greater the higher the pH, but the differences are not marked.

(vi) A decision between inactivation or solution of enzyme could not be made.

The stability of a considerable number of enzyme solutions is known to be increased by the presence of amino-acids (cf. Weil-Malherbe, 1948). Alkaline phosphatase solutions are stabilized (or activated) by the presence of glycine at 0·006M concentrations (Bodansky, 1936).

Text-fig. 2 indicates that the addition of glycine stabilizes the enzyme in sections also; the effect was maximal at 0·006M concentration in tissue sections.

The enzyme changes occurring under the conditions of the histochemical reaction were investigated by removing with acetate buffer (M/10, pH 5) the calcium phosphate in sections incubated for various periods, reincubating the sections with substrate for 30 minutes, and determining the resulting density. In one control series the first incubation is performed without substrate and in another with substrate but without Ca. The latter series permits a study of the effect of substrate *per se*.

The results, which are shown in Text-fig. 3, indicated that the enzyme was considerably stabilized by the presence of calcium phosphate in the sections.

The experiments reported above indicate that there will be little loss of enzyme during incubation in the histochemical reaction in sections with initially high enzyme activity. In sections with low initial activity considerable losses of activity would be possible.

Conditions of Optimal Enzyme Activity.

(*a*) *Effect of Substrate Concentration.*

With glycerophosphate as substrate the section density increased with increasing substrate concentration up to the highest concentration tested (M/10), the effect being the same for both testis and kidney, as shown in Table 6.

Table 6.

Effect of Substrate Concentration on Enzyme Activity.

Substrate Concentration.	M/10.	M/50.	M/100.	M/1000.
Kidney	70	51	32	20
Testis	81	63	46	21

M/50 borate glycine buffer pH 9·4, M/100 Mg and Ca. Incubation 30 mins. for kidney and 60 mins. for testis. The figures quoted are density units.

Phenylphosphate and glycerophosphate at M/100 gave similar densitities, but little change in enzyme activity occurred with the former over the range M/10–M/1000. The same difference in substrate enzyme affinities has been noted in test-tube experiments by Schmidt and Thannhauser (1943). The time course of the reaction with M/100

glycero- and phenylphosphate are the same over the range of incubation times—fifteen minutes to four hours. This indicated that diffusion of the substrate through the calcium phosphate shell around the enzyme sites was unlikely to be a limiting factor at this glycerophosphate concentration.

When the time course of the reaction at three glycerophosphate concentrations is followed it is found that the slopes of the three lines are different, as shown in Text-fig. 4. The causes are threefold: (*a*) the normal relation between enzyme activity and resulting density, (*b*) the changing relation between calcium phosphate content of the section and density, (*c*) movement of calcium phosphate in the section. These three factors will be discussed later.

When sections are incubated to a given density at different substrate concentrations by varying the incubation time, important changes in apparent cytological localization result, a phenomenon which will be discussed in Part II of this paper.

(*b*) *Effect of pH.*

Text-fig. 6 indicates that the pH optimum of kidney and testis for short-term experiments was 9·4, a value similar to that found for the test-tube enzyme.

An apparent change of pH optimum with time was noted with kidney sections, as may be inferred from Text-fig. 6. When sections are incubated to a given density at different pH values by varying the incubation time, differences in apparent cytological localization occur. The main difference is a greater density in the nuclei at the lower pH values due to factors which will be discussed in another paper. It is to these factors that the apparent change in pH optimum with time is due. The mechanism is outlined on page 46.

(*c*) *Effect of Buffer Concentration.*

An unexpected considerable increase in histochemical density was found with high borate buffer concentrations, as shown by Table 7, veronal buffer showing little change with different concentrations.

TABLE 7.
Effect of Buffer Concentration.

Concentration.	M/10.	M/20.	M/50.	M/200.
Kidney:				
Veronal	62	61	58	—
Borate	89	72	64	62
Testis:				
Veronal ..	34	—	32	—
Borate	52	—	38	—

pH 9·4. M/40 glycerophosphate, M/100 Mg and Ca. Incubated 45 mins. for kidney and 60 mins. for testis. The figures quoted are density units.

The activity at the high borate concentrations appears to be increased at all intracellular sites. The interpretation of the phenomenon is difficult, the following seeming the most likely.

At the sites of enzyme activity localized shifts to a more acid pH occur, due to phosphate both free and in combination with calcium, and to the local excess cation caused by removal of calcium. High buffer concentration is more able to neutralize this acid shift.

The apparent stimulating effect of high borate concentration on enzyme activity is not demonstrable in homogenates or in tissue sections incubated in the absence of calcium.

(*d*) *Effect of Activators.*

Test-tube experiments have implicated the following metal ions as possible coenzymes for phosphatases: Mg, Mn, Co, Zn.

When tested at pH 8·2, in the presence of an excess of inorganic phosphate, and an ion concentration of M/100, Mg gave a greater stimulation than Mn, while Co gave about twice the density of Mg. By placing the section incubated in the cobalt solution directly into ammonium sulphide it could be demonstrated that the primary precipitate was all cobalt phosphate. The main cytological difference in the Co incubated sections was that the brush border reaction was very wide.

At pH 9·4, at which pH calcium phosphate was the least soluble phosphate, Mg was found to be the best activator, as indicated by Table 8.

TABLE 8.

Effect of Ions at pH 9·4.

Buffer.	Ion.	Concentration (M).	Section Density.
Borate	Co	10^{-4}	23
Borate glycine	Co	10^{-3}	24
Borate	Mn	10^{-4}	22
Borate	Mg	10^{-3}	25
Borate glycine	Mg	10^{-3}	25
Borate	Mg	10^{-2}	29
Borate glycine	—	—	21
Borate	—	—	21·5

M/100 glycerophosphate and Ca. Incubation 30 mins. Kidney sections.

By the following experiment it was possible to infer that the apparent activation by Co at the lower pH was due to differences in the properties of calcium and cobalt phosphates. Cobalt can be dissolved in M/50 glycine buffer to M/100 concentration at pH 9·0, at which pH cobalt phosphate is about five times more soluble than calcium phosphate. Three series of slides were incubated for the same time in the following solutions: (*a*) M/100 Co in glycine buffer with added substrate; (*b*) solution (*a*) with M/100 Ca added; (*c*) glycine buffer of the same pH as the previous with substrate and Ca added. Control sections were incubated in these three solutions in the absence of substrate.

The resulting densities of the sections were 65, 34 and 32 units respectively.

It is demonstrated in the next section that the presence of calcium phosphate at the enzyme site causes an inhibition of the enzyme. The greater width of the brush border reaction in sections incubated with cobalt in the absence of calcium suggests that cobalt phosphate causes less enzyme inhibition than calcium phosphate.

It is unfortunate that these cobalt solutions cause such a high blank impregnation of the tissue, a factor which makes visual interpretation difficult, for the relations in enzyme content at the various cellular sites are more truly represented than by the classical technique.

The enzyme activation by Mg was found to be maximal at a Mg concentration of M/100.

Zinc ion was found to have an apparent inhibitory action on the section density produced, varying from 70% to 5% at M/600 and M/60,000 Zn concentration respectively. This was apparently due to the great insolubility of Zn phosphate; some of the primary precipitate is in this form and is not converted to cobalt phosphate, thus appearing in the section as colourless zinc sulphide. Moog (1943) has reported an inhibition of the histochemical reaction in the chick embryo by zinc ions.

(*e*) *Effect of Inhibitors.*

The effect on the histochemical density of a number of substances known to inhibit the enzyme in solution is shown in Table 9.

TABLE 9.

Effect of Inhibitors on the Activity of Phosphatase in Sections.

Inhibitor.	KCN.		Taurocholate.		Hydrosulphite.		Na_2S.		Nil.
Concentration (M.) ..	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	—
Density units	6	14	18·5	19·7	22	21·2	0	17·5	20

No Mg. M/100 glycerophosphate M/50 borate buffer. pH of solutions adjusted to 9·4 after addition of inhibitor. Incubation 30 mins. Kidney sections.

Bodansky (1936) has shown that kidney phosphatase is inhibited about 50% by taurocholate. There is little or no effect on the enzyme in sections. Moog (1943) has found an inhibition of the enzyme in chick embryo sections by taurocholate.

Sizer (1941) demonstrated that ox kidney phosphatase is inhibited strongly by hydrosulphite, but in guinea-pig kidney sections a slight activating effect is seen. Sizer was unable to demonstrate much inhibition by cyanide or sulphide, but Schmidt and Thannhauser (1943) found a 90% inhibition of purified intestinal phosphatase by M/60 cyanide. Fell and Danielli (1943) found ammonium sulphide a powerful inhibitor of skin phosphatase in both test tube and sections.

Emmel (1946) has described a reversible inhibition of rat kidney phosphatase in sections and noted an apparent activation of the enzyme after thorough removal of the cyanide. This could not be demonstrated in guinea-pig kidney, only about 75% of the original enzyme density being recovered after cyanide treatment.

(f) Effect of Calcium Concentration.

The effect of calcium concentration on the density resulting after the histochemical reaction is shown in Table 10; in both organs the density is less at M/10 than at M/100, and at concentrations less than M/100 there is an increased density with kidney sections but a decreased density with testis sections.

TABLE 10.

Effect of Ca Concentration on Apparent Enzyme Activity in Sections.

Ca Concentration.	M/10.	M/100.	M/1000.	M/10,000.
Kidney	12	34	42	36
Testis	32	55	35	1

Values quoted are density units. M/50 borate glycine buffer pH 9·4. M/100 Mg. M/40 substrate. Incubation 30 mins. kidney and 45 mins. testis. Testis sections 10 μ.

The interpretation of these phenomena is as follows. At calcium concentrations less than M/100 some phosphate diffuses from the enzyme site before it is trapped as calcium phosphate. Some of this calcium phosphate is secondarily adsorbed by the tissue section, and some escapes to the incubating solution (shown experimentally). In kidney sections the main site of enzyme activity is at the brush borders and the enzyme at this site is normally rapidly inhibited by the accumulated calcium phosphate (cf. Table 11). Owing to this fact loss of phosphate has little effect on the final density at this site and the slight loss of density is more than counterbalanced by the phosphate absorbed by other parts of the section. The overall density thus shows an increase at low calcium concentrations. In testis incubated for short periods the enzyme is very little inhibited by calcium phosphate at the enzyme sites, which are fairly evenly distributed in the sections (cf. Table 11). Practically all the phosphorus that the enzyme is potentially capable of splitting thus appears in the section: when any is lost

to the incubating solution, as happens with low calcium concentrations, there is an overall loss of density.

The lower density at Ca concentrations greater than M/100 is due to depression of enzyme activity; the cytological picture is identical with that found when the enzyme activity is lowered by other means (cf. Part II of this paper).

Significance of the Photometric Estimate.

(*a*) *Time Density Relation.*

It is evident from previous figures that when the logarithm of density is plotted against the logarithm of incubation time a rectilinear relation holds for kidney.

That the same relation holds in tissues with a different intracellular partition of the enzyme is shown in Text-fig. 7. The log./log. relation is evidently characteristic of the kinetics of the histochemical reaction and almost certainly describes the reaction rate in the various cell components which contribute to the overall density of the microscope field. Text-fig. 8 indicates that the relation is not due to any peculiarity of the histochemical visualization procedure or the photometric method.

A strong inhibition of the enzyme activity, increasing with incubation time, is implied by these figures. An indication of the degree and time course of the inhibition in testis and kidney sections may be obtained from Table 11.

Table 11.

Comparing the Enzyme Activity of Tissue Sections when the Phosphate is Trapped as Calcium Phosphate as in the Histochemical Reaction (+Ca) and when it is Free to Diffuse into the Incubating Fluid (−Ca).

Organ.		Ca Increase of Phosphorus (μg.) in Sections (+Ca) and Fluid (−Ca).		
		1 Hour.	2 Hours.	4 Hours.
Testis	+	75	117	185
	−	88	191	364
Kidney	+	111	145	178
	−	485	950	1780

M/50 borate buffer pH 9·4. M/100 Mg. Ca, when present, M/100. Substrate M/20. Approximate total section area, 11 cm.² for each organ.

There are two possible explanations of this inhibition: lowering of substrate concentration at the enzyme surface by a diffusion barrier due to the calcium phosphate or actual inhibition of the enzyme by the high concentrations of inorganic phosphate which is present at the enzyme site as calcium phosphate (cf. Schmidt and Thannhauser, 1943).

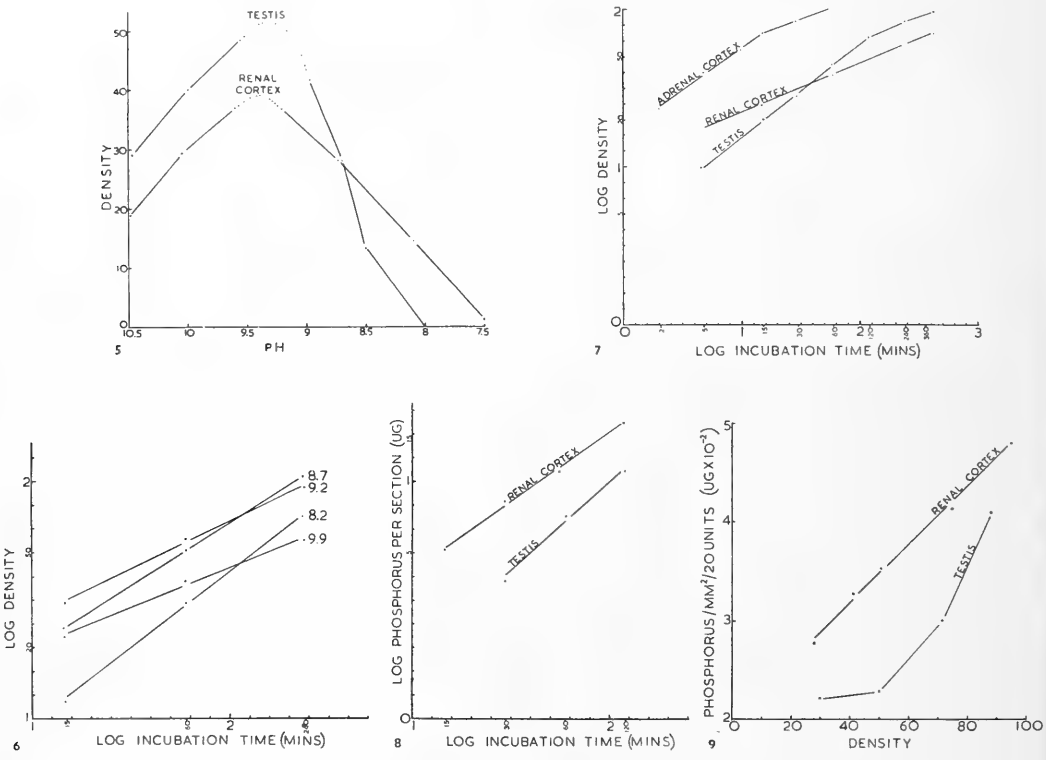
The only satisfactory way of deciding between these two alternatives has been by the use of substrates showing different affinities for the enzyme; this type of evidence favours the latter alternative (p. 43).

In Text-fig. 7 it will be noted that the kidney line has a lower slope than the adrenal or testis lines. This is due to the fact that the brush borders are very active, giving a high density at low incubation times, but, because of the inhibition by split products, the density contribution by this source increases only slowly with time of incubation. The slope of the kidney line is thus mainly determined by the reaction in the other parts of the cells of the proximal tubules, which occupy a relatively small area in the section. In the adrenal cortex high densities are reached at an early stage of incubation because of the high enzyme content of the cortical cytoplasm of the cells, which occupies a larger relative area than do the brush borders in the case of kidney. The slope of the adrenal line is higher than that of kidney, because the chief factor determining it, the reaction in the rest of the adrenal cortical cell. is

spread over a much larger relative area than was the case in kidney. In testis the density is low at small incubation times because the enzyme is not present at any site at concentrations comparable with that in kidney brush border or the cortical cytoplasm of the adrenal cortex cells, and the slope of the line is high because the enzyme is distributed over a wide area in the microscope field.

(b) Relation between Photometric Density and Inorganic Phosphate Content of Sections.

In a study of the time course of the histochemical reaction in sections of different thickness it was found that the slope of the time density plots varied with section thickness. The causes to be considered were variation of the adequacy of diffusion



Text-figures 5-9.

Figure 5.—The effect of hydrogen ion concentration on the phosphatase activity of testis and kidney sections, as measured by the histochemical technique. Incubation conditions: M/50 borate veronal buffer. M/100 Ca and Mg. M/100 substrate for kidney, M/40 for testis; incubation time 30 mins. kidney and 120 mins. testis. An excess of inorganic phosphate was present at all pH values.

Figure 6.—The time course of the histochemical reaction at different hydrogen ion concentrations. Note the apparent change in pH optimum between pH 8·7 and 9·2, due mainly to wandering of calcium phosphate from the enzyme site, as will be demonstrated in another paper.

Figure 7.—The time course of the histochemical reaction in sections of three different organs. Note the difference in slope, due mainly to differences in the cytological distribution of the enzyme, as explained in the text. The conditions of incubation are the same for the three organs.

Figure 8.—The time course of the phosphatase reaction in kidney and testis sections as measured by the amount of phosphorus extractable from the section after varying incubation times.

Figure 9.—The relation between phosphorus extractable from tissue sections and the photometric density of the sections. While the density is a valid estimate of phosphate content of model systems (fig. 1), this figure indicates considerable deviation from Beer's law in tissue sections, owing to the uneven distribution of the very intensely light-absorbing cobalt sulphide.

of substrate or visualizing reagents with section thickness or inadequacy of the density as a measurement of inorganic phosphate in sections. The latter was the simplest to test; in the experiment shown in Table 12 it will be seen that the amount of inorganic phosphate extractable from the section is proportional to the section thickness but the inorganic phosphate/density ratio is different in sections of different thickness.

TABLE 12.

Relation between Section Thickness, Inorganic Phosphate Content and Density in Kidney Sections of Different Thickness.

Thickness.	Density.	Phosphorus. (μg.)	Cortex Area. (Mm.)	μg. P/20 units/mm.^{2}
5 μ	63·5	9·3	91	0·032
8 μ	78	14	95·5	0·0379
11 μ	91	19·2	97	0·0435

M/50 borate glycine buffer pH 9·4. M/100 Mg, Ca. Substrate M/20. Incubation time, 2 hours.

In order to systematize this finding, experiments were conducted in which the density and inorganic phosphate content of sections incubated for various times was determined.

When the phosphorus content, per unit density and area, was plotted against density the curves shown in Text-fig. 9 resulted. It will be seen that in kidney sections a change of relation occurs even at low densities and is fairly constant in rate. Testis sections show a constant relation up to medium densities and then change rather rapidly. It is this change of relation which causes the deviations in testis sections, of the plot of incubation time/density in Text-fig. 7. A similar effect is probably responsible for the deviation in adrenal sections at the longer incubation times.

When the figures derived from this experiment are used to calculate the amounts of inorganic phosphate present in the sections of different thickness mentioned above, it was found that the calculated phosphate content was proportional to section thickness at all incubation times.

The most likely interpretation of these findings is as follows. In kidney the brush borders react very strongly at small incubation times. Very active parts of the brush border reach at an early stage a density such that all of the incident light is absorbed. As the incubation time is increased, more brush border areas become completely absorbing. Although completely absorbing the light in the finished preparation, these areas still produce phosphorus by substrate splitting, this extra phosphate not contributing to the total photometric density. The observed density/phosphorus ration implies that the rate of reaching this 100% light-absorbing state is fairly constant for kidney. In testis the situation is different. The apical ends of the Sertoli cells are the most active constituent but react at a much slower rate than the brush border of the kidney. Furthermore, they cover a larger relative area than the brush borders. For this reason the density-phosphorus relation is constant up to medium densities. After this, Sertoli cell areas become 100% absorbing and reach this stage much more synchronously than the brush borders, resulting in a rapid change in the above relation.

DISCUSSION.

To establish the possibility of a histochemical estimation of the enzyme content, either relative or absolute, of tissue sections is a difficult task requiring a thorough investigation of all the points involved.

The first requirement is a method of estimating the reaction products of the enzyme as they occur at the enzyme site. It has been shown in this paper that the cobalt conversion method of Gomori (1941), somewhat modified, is capable of giving

a reasonably accurate estimate of inorganic phosphate incorporated in cigarette papers. It is desirable to check conclusions derived from such model experiments in two respects—to show that Beer's law is obeyed not only in model systems where the substance is homogeneously distributed, but also, if possible, in tissue sections where the distribution is rarely homogeneous, and to show that any losses of the substance which occur during the colorimetric procedures are similar in both model system and tissue section. Both these points were shown to be important in the present study: Beer's law was not obeyed in tissue sections and losses of phosphate occurred in cigarette papers when they were subjected to exactly the same treatment as tissue sections. The fact that no significant loss of phosphate occurs in tissue sections during incubation implies that the calcium phosphate arising by enzyme action is adsorbed by the section. This adsorption, while it makes possible a clear demonstration of the enzyme in the section, suggests caution in interpreting the picture so obtained as a valid intracellular localization of the enzyme: it is quite possible that different cell components have different powers of adsorbing calcium phosphate.

The second requirement is that no loss of enzyme should occur during the incubation, or if it does, that no difference in the percentage lost occurs in different histological or cytological elements of the section. It is evident from the experiments detailed in this paper that little loss of enzyme need be expected at least over relatively short incubation times, and especially where, owing to high enzyme content, the enzyme is rapidly protected by a calcium phosphate shell. The enzyme at the brush borders, at first sight, appears to be more resistant to inactivation and less inhibited by the inhibitors used than the enzyme in other cell sites. This, however, is no evidence of differential loss or inhibition of the enzyme: because of the autoinhibitory nature of the phosophatase reaction, the enzyme at the brush borders may be strongly reduced in amount or activity without showing a comparable change in the density visible at this site in histochemical preparations. This same factor complicates the interpretation of Emmel's (1946*b*) results with cyanide inhibition.

In estimations of enzymes it is desirable, although not absolutely necessary, to determine the conditions of optimal activity of the enzyme. The conditions prescribed by Gomori (1941) call for a veronal buffer of approximately M/50 concentration and a substrate concentration have been found to show considerable decreases in pH over a period of days when in constant use, and the present study has shown that borate buffers of higher molarity are preferable. When the pH falls below the optimum, as can happen quite readily with veronal buffers, considerable changes in the apparent cytological partition of the enzyme occur. An inspection of published figures for various organs suggests that this factor has been operating. It has also been shown in this study that the substrate concentration recommended by Gomori is insufficient to saturate the enzyme. The saturating concentration of glycerophosphate appears to be in excess of M/10, a concentration considerably higher than that found necessary to saturate the fresh enzyme in test-tube experiments. It seems possible that, in the process of fixation, some barrier is formed around the enzyme site which obstructs the free diffusion of substrate molecules to the active groups of the enzyme. A difference in this barrier might explain the differences recently noted by Wang and Grossman (1949) between the enzyme activity of alcohol fixed and frozen dried sections of various organs.

A linear relation between incubation time and amount of substrate split makes for a convenient and accurate estimate of an enzyme. Non-linearity may be caused by enzyme inactivation, a fall in substrate concentration, or an inhibition of the enzyme by substrate split products. Schmidt and Thannhauser (1943) have found that the organic part of the phosphate ester causes little inhibition of alkaline phosphatase while inorganic phosphate has a considerable inhibiting effect. They have interpreted this to mean that it is the phosphate moiety of the substrate which combines with the enzyme, and have suggested that this is the reason for the relative non-specificity of the enzyme. In the alkaline phosphatase reaction in tissue sections the enzyme is strongly inhibited by the inorganic phosphate liberated from the substrate, which,

instead of diffusing away and thus becoming diluted, is retained at the enzyme site as calcium phosphate. This fact greatly complicates attempts to estimate the enzyme content of tissues or cells by the histochemical method, especially since the study of the time/density relation has indicated that the inhibition is somewhat anomalous. This inhibition can give rise to quite false visual estmates of the relative activity of different cell components: for example, it would not be suspected from inspection of a routine histochemical preparation that the brush borders of kidney tubules have an activity which must be some hundredfold that of any other component of the tubule cell. Estimates of the enzyme activity based on the alcohol moiety (Menten et al., 1944; Manheimer and Seligman, 1948; Loveless and Danielli, 1949) would theoretically be preferable to estimates based on the phosphate, since the autoinhibition would be less. These methods would be difficult to standardize, however, and would need much more critical examination than they have yet received.

The section on the time/density relation seems to indicate that the most satisfactory method of comparing the enzyme activity of two organs by histochemical means would be based on the density produced in incubation times sufficiently short to obviate the split product inhibition. Thus the density found in testis and kidney sections in Text-fig. 7 is, by extrapolation to 1 minute incubation, 2·5 and 10 units respectively, suggesting that testis has a relative activity about 23% that of kidney. It will be shown in another paper that this estimate is in accord with test-tube assays of the enzyme in fresh tissue homogenates.

An approximate calculation of the absolute enzyme activity of a fresh tissue equivalent of the kidney sections in Text-fig. 7 indicates that the calculated activity is of the same order as found in fresh tissue homogenates, and fails to indicate that much destruction of the enzyme can be ascribed to the preparation of the histological sections.

Reasons have already been given for thinking that the methods for estimating the enzyme content of tissue sections are also applicable to estimates of the intracellular partition of the enzyme.

Thus reasonably accurate estimates of the cytological distribution of alkaline phosphatase seem technically feasible. Whether such estimates would indicate a physiological reality depends on two points which have not been investigated in the present paper. These are that no movement of the substances used in localization of the intracellular enzyme occurs, and that there is no loss or no differential loss of enzyme between the time the cell is removed from the living animal and the time when the histochemical incubation begins. These two factors will be considered in Part II of this paper.

Summary.

1. A photometric apparatus suitable for the determination of the light absorption of microscope fields is described and its method of use indicated.

2. Loss of enzyme with time in paraffin blocks or in tissue sections on slides is very small.

3. The sources of variability in the photometric estimate are considered and the procedures adopted shown to give precise results.

4. In order to standardize the histochemical demonstration of calcium phosphate a number of modifications have been made to the technique, and the technique adopted is shown to give a valid estimate of the phosphate content of model systems.

5. The effect of pH on the solubility of calcium phosphate is stressed and the importance of this factor in the histochemical technique shown.

6. A number of fixatives have been compared for their ability to preserve enzyme, and acetone or 80% alcohol found to be the most suitable.

7. Factors influencing the stability of the enzyme during incubation of tissue sections are studied and the addition of 0·006M glycine found to be desirable. The presence of calcium phosphate in sections is found to have a stabilizing effect.

8. The enzyme activity increases with glycerophosphate concentration, the enzyme still being unsaturated at M/10 substrate.

9. The pH optimum of the enzyme in sections is the same as of the enzyme in solution, but owing to peculiarities of the histochemical reaction an apparent change of pH optimum occurs in kidney sections.

10. Borate buffers of high concentration are in general shown to be preferable to the classical veronal buffer.

11. Magnesium ion at a concentration of M/100 is the most effective enzyme activator.

12. The time/density relation in tissue sections is best expressed by a double logarithmic plot, different organs showing different slopes in such plots. This is shown to be due to differences in the cytological distribution of the enzyme.

13. There is a strong inhibition of the enzyme by split products retained at the enzyme site. The degree of inhibition is different in different organs, because of differences of cytological distribution of the enzyme.

14. The light absorption of tissue sections is often not a valid estimate of the phosphate extractable from the sections. The invalidity is different for different organs.

15. Difficulties in comparison of the enzyme activity of organs differing much in the cytological distribution of the enzyme are indicated. A valid comparison of the enzyme activity of different cell sites would be possible under certain conditions.

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A STUDY OF THE ALKALINE PHOSPHATASE REACTION IN TISSUE SECTIONS.

PART II. THE HISTOLOGICAL AND CYTOLOGICAL VALIDITY.

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(Plates I and II.)

[Read 26th April, 1950.]

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Introduction.

In a previous paper (Cleland, 1950) an investigation of the possibility of quantitative use of the histochemical reaction in tissue sections was made.

It was shown that a reasonable estimate of the enzyme content of tissues and cells was possible, provided that: (*a*) no movement of the localizing substances or enzyme occurred, and (*b*) no differential loss of enzyme occurred in different cell components or organs.

The present communication records a study of these two points.

Methods.

Unless otherwise stated materials and methods are the same as described in the previous paper.

Phosphatase activity was determined in distilled water homogenates prepared by means of a Waring blendor fitted with a microcup. With the following conditions: veronal buffer, M/20, pH 9·4; M/20 glycerophosphate; M/100 Mg.; temperature 40°C.; the activity was expressed as the amount of phosphorus split from the substrate (in μg.) by 100 mg. fresh weight in 30 minutes.

Homogenates for the centrifugal studies were prepared in normal saline. The activity remaining in the supernatant after the following centrifugal treatment: 150 x g.

for 3 minutes in an International SBI centrifuge; 10,000 x g. for 10 minutes in a Servall SSIa centrifuge and 20,000 x g. for 30 minutes in the same instrument: was expressed as a percentage of the activity in the unfractionated homogenate.

The absolute activity of the enzyme in tissue sections was determined with the same conditions used for determination of the homogenate activity except that Ca was rigorously excluded from the solution.

In the enzyme determinations phosphorus analyses were made by the method of Fiske and Subbarow.

Validity of the Cytological Localizations.

In a multistep technique such as this, false localizations can arise at any step and, to prove its validity, it is necessary to investigate each step individually, starting from the last and progressing to the first.

(a) Visualization of Cobalt Phosphate.

As the dilution of ammonium sulphide was decreased below 1/200 or the pH of sodium sulphide below 7·5, important changes in the physical state of the cobalt sulphide resulted. Instead of the usual homogeneous reaction in the ground cytoplasm in kidney sections the reaction was in the form of granules having the distribution and shape of mitochondria, the total density being slightly reduced. This could be interpreted in two ways: either that at higher ammonium sulphide concentrations a cobaltammine complex is formed which diffuses from the granules in which phosphatase is known to be present (see later); or that the slow conversion to sulphide favours solution of the cobalt phosphate which is then precipitated from solution in the tissue in the form of granules of cobalt sulphide. A decision between these two alternatives may be made with ordinary ammonium sulphide concentrations by increasing the ammonium concentration by addition of ammonium chloride, and by increasing the solubility of cobalt phosphate by dissolving the ammonium sulphide in glycine buffer of the same pH as the ammonium sulphide solution itself. The first procedure has no effect, but the second gives an accentuation of the picture found with low ammonium sulphide concentrations. The correct picture is, then, the homogeneous distribution normally found.

It is of interest to note that the nuclei do not adsorb the cobalt phosphate when it is made more soluble by the above method. As will be shown later, much of the cellular alkaline phosphatase is bound to the mitochondria, and these survive fixation. The fact that the phosphatase cannot be shown on these granules in tissue sections suggests that the method may be invalid for cellular localizations.

(b) Movement of Cobalt Phosphate.

This may move either during its formation from calcium phosphate or during the washing of the section after the conversion. In view of the fact that the cytological distribution is the same with the present technique, which greatly reduces the solubility of the cobalt phosphate in both steps, as with the classical technique, means that these steps are unlikely to be associated with any significant migration of the compounds. This conclusion is strengthened by the fact that an identical cytological distribution is found when the calcium phosphate is visualized directly with alizarin added to the incubating solution.

(c) Movement of Calcium Phosphate.

Danielli (1946) has reported experiments indicating that nuclei can adsorb calcium phosphate from supersaturated solution or can serve as condensation points under these conditions. It was not possible to reproduce the experimental conditions used by Danielli, but the observation has been confirmed by a number of different methods. Danielli reported that this calcium phosphate adsorbing property of the nuclei does not invalidate the cytological localization of the enzyme. The experiments reported here do not support the latter contention, which appears to be almost correct for kidney under optimal conditions of enzyme activity and short incubation times, but is not

correct for organs with lower activity than kidney, or for kidney at longer incubation times.

The experiments on which these views are based are detailed below.

(i) *Effect of Calcium Concentration.*

When sections of kidney and testis are incubated in substrate buffer solutions containing M/100 Ca and M/1000 Ca, for the time required to give equal density in the two series, it is found that the apparent cytological distribution of the enzyme is different. At M/100 Ca concentration the nuclear reaction in the proximal tubules is quite weak, the cytoplasmic reaction moderate and the brush border reaction intense. At the lower Ca concentration the nuclear reaction is much stronger in the proximal tubules and is increased in other cortical tubules, the cytoplasmic reaction is increased in intensity and the brush border reaction slightly diminished. In testis, the main difference between sections incubated at the two calcium concentrations is that the nuclei have a higher density at the lower calcium concentration, the effect being less noticeable than in kidney. These alterations in relative density of various cell sites become more marked as the calcium concentration is lowered even further (Figs. 1 and 2, Pl. i).

With low calcium concentrations the diffusion of calcium ions will be insufficient to trap the phosphate liberated by the enzymatic hydrolysis immediately it is formed. The effect of low calcium concentration will then be to favour trapping more uniformly in the section and the above observations thus provide independent proof of the ability of nuclei to adsorb calcium phosphate liberated in their vicinity. Nuclei do not adsorb calcium phosphate (in a form demonstrable by the histochemical reactions) from a substrate free incubating solution saturated with this compound.

This does not, in itself, prove that the calcium concentration usual in the technique (approx. M/100) is insufficient to cause immediate trapping of the phosphate at the enzyme site.

As has already been stated (Cleland, 1950), high concentrations of calcium cause a reduction in the final density of sections as compared with those incubated in solutions of normal calcium content. When sections incubated to the same final density in the presence of M/10 and M/100 Ca are compared microscopically, it is found that the differences are indistinguishable from those which occur in cases where the enzyme activity is depressed to a comparable degree by lowering the substrate concentration, or by the addition of KCN (Figs. 3 and 4, Plate i). This suggests that Ca concentrations of the order of M/100 are adequate to cause immediate trapping of the enzymatically liberated phosphate.

(ii) *Effect of Reducing Enzyme Activity.*

As the enzyme activity of kidney sections is reduced by various means, and the resulting cytological picture compared with that in sections of normal activity, both series being incubated for the times required to give the same final density, it is found that, in the sections in which the activity is reduced, the nuclei have a much greater density. Furthermore, the nuclear density increases with increasing enzyme inhibition.

Thus when slides incubated in M/100 substrate are compared with those incubated to the same histological density in M/1000 substrate, it is found that, in the latter, the nuclei are much more heavily impregnated and the brush border reaction is noticeably weaker (Figs. 5 and 6, Plate ii). With substrate concentrations of the order of 10^{-5} to 10^{-6} M the brush border reaction is very weak and the nuclei heavily impregnated. No increase in density occurs in sections incubated without substrate in buffer calcium solutions saturated with inorganic phosphate.

When the activity of the kidney sections is reduced by other means—e.g., by incubation in solutions containing KCN—exactly the same phenomena occur as were observed when the activity was reduced by lowering the substrate concentration. It seems very unlikely that the nuclear enzyme would be so different from that in the rest of the cell that it would simultaneously have so different a Michaelis constant and be activated by cyanide and high Ca concentration instead of inhibited.

These observations on kidney tissue sections are much less marked, but still demonstrable, in testis sections.

(iii) *Effect of pH.*

In kidney sections, reduction of the rate of enzyme action by lowering the pH results in a much more marked nuclear impregnation than that shown when other methods of reducing enzyme action were used (Fig. 7, Plate ii). Four hypotheses could account for this: (*a*) that the nuclear enzyme is more readily inactivated at the higher pH; (*b*) that the nuclear enzyme is activated at the lower pH; (*c*) that the nuclear enzyme has a lower pH optimum than the rest of the cell; (*d*) that the greater solubility of calcium phosphate at the lower pH (Cleland, 1950) favours more movement of this substance in the section during incubation, with subsequent adsorption of it by the nuclei. An experiment designed to test the first two alternatives is shown in Table 1. These two hypotheses are excluded. In some experiments, differences between slides 2, 4 and 6 suggested that there was some loss of final density in the nucleus caused by pretreatment of the slides at the higher pH; thus under certain conditions hypothesis (*a*) accounts for a small part of the difference.

TABLE 1.

Analysis of Cause of Higher Nuclear Reaction at Lower pH.

Slide.	Pretreatment without Substrate.	Incubation with Substrate.	Result.
1	1 hr. at pH 9·5	1 hr. at pH 9·5	Same as 3.
2	do.	1 hr. at pH 8·3	Same as 6.
3	nil	1 hr. at pH 9·5	Poor nuclear reaction.
4	1 hr. at pH 8·3	1 hr. at pH 8·3	Same as 6.
5	do.	1 hr. at pH 9·5	Same as 3.
6	nil	1 hr. at pH 8·3	Good nuclear reaction.
7	2 hr. at pH 9·5	nil	Usual diffuse blank reaction.
8	2 hr. at pH 8·3	nil	do.

All solutions saturated with inorganic phosphate. M/50 borate veronal buffer, M/100 subtrate and Mg. Kidney sections.

The third hypothesis is made unlikely by the observation that the relative nuclear reaction increases down to the lowest pH tested (7·7) and a pH optimum difference of this order seems very unlikely. A decision between the third and fourth hypotheses can, however, be made by a study of nuclei separated from fixed or fresh tissue. This would exclude the possibility of absorption by the nuclei of calcium phosphate formed at other cell sites, and make possible a study of the pH optimum of the nuclear enzyme itself.

Nuclei were separated from fresh guinea pig kidney by methods described later, smeared on slides, dried and fixed in acetone. The enzyme present in these separated nuclei has the same pH optimum as that found for whole tissue sections. This, then, means that the higher nuclear activity at the lower pH is most probably due to the fact that calcium phosphate has a higher solubility at the lower pH.

Testis sections show this pH effect to a lesser degree than kidney while adrenal cortex shows it in greater degree.

(iv) *Discussion.*

It can be shown by the following experiment that calcium phosphate once precipitated in the section does not redistribute itself. Kidney sections were incubated for one hour in the presence of substrate, thoroughly washed in three changes of buffer calcium, and then placed in buffer calcium at pH 9·5 and 8·0 (both saturated with phosphate) for periods up to 24 hours. An unincubated slide was placed with each of these in order to determine whether any substrate had been carried over with the slides. No redistribu-

tion of density could be demonstrated at either pH, indicating that any calcium phosphate movement must occur during its formation.

The following hypothesis is capable of integrating all the observations. The calcium phosphate is formed in the molecular state and is freely diffusible in this state. When the local concentration is such that supersaturation occurs, the calcium phosphate precipitates in the colloidal form, a state in which no diffusion is possible. Certain cell constituents because of their electrostatic properties, are capable of attracting the molecular form to give a supersaturation in their vicinity with subsequent precipitation of the colloidal form.

This hypothesis explains the effects observed on reducing the rate of enzyme action in kidney, since with lower activity, less of the molecular form will reach a concentration sufficient to give precipitation in the colloidal form, with consequent diffusion of the molecular form and its adsorption by the nuclei. When not only the rate of formation is reduced but also the precipitation threshold of the calcium phosphate is raised, as it is by reducing the pH, the effect is much more marked. In testis where the activity is normally considerably lower than kidney, diffusion of calcium phosphate plays a considerable part in the picture obtained under normal conditions of incubation, and the above effects observed in kidney are thus less evident.

That the molecular form of calcium phosphate is strongly adsorbed to the tissue section, and does not diffuse into the medium is shown by the following experiment. Sections of kidney and testis were incubated for four hours at two substrate concentrations. Analyses of the increase of phosphorus in the incubating fluid and the sections were made with the results shown in Table 2. The results obtained are best explained by diffusion of inorganic phosphate (untrapped by the calcium ions) from the section.

TABLE 2.

Loss of Phosphorus from Sections of Different Enzyme Activity.

Organ.	Substrate Concentration.	P in Sections. (μg.)	P Increase in Fluid. (μg.)
Testis	M/100	155	8
	M/1000	102	5
Kidney	M/100	180	13
	M/1000	145	4

Active tissue area approximately 11 cm^2 M/50 borate buffer M/100 Mg and Ca. Incubation 4 hours.

Figures quoted for increase of phosphorus in the incubating fluid are probably slight overestimates; bacterial substrate splitting is probably greater in the fluid containing sections than in the control fluid without sections.

This strong adsorption of the molecular form of calcium phosphate implies that this substance is probably capable of wide migration in tissue sections.

Since this work was done the paper of Jacoby and Martin (1949)* has been seen. The present work confirms their findings and defines the conditions under which the migration of the calcium phosphate is likely to give serious errors of cytological localization of the enzyme.

(d) Movement of Enzyme.

(i) *General Considerations.*

That it is desirable to entertain the possibility of adsorption of enzyme, at least as far as the nucleus is concerned, is shown by the following considerations.

(*a*) As will be shown later, a significant fraction of the alkaline phosphatase is present in a soluble form. The purified enzyme is not precipitated by acetone concentrations less than 50% (Schmidt and Thannhauser, 1943). Since the normal perme-

* See also *J. Anat.*, 1949, 83: 351.

ability properties of the nucleus (Callan, 1948) are likely to be changed during the process of fixation, there is a possibility of diffusion of the enzyme into the nucleus during fixation. In floating out the sections after cutting, this possibility would be even greater.

(*b*) The isoelectric point of the nuclear substance, at least when fixed or partly fixed, is quite low. In at least some groups of enzymes, a rough positive relation is found between the pH optimum and the isoelectric point. The possibility of complex formation between chromatin and alkaline phosphatase is therefore indicated.

(*c*) The adsorption of protein under certain conditions by tobacco mosaic virus has been shown to occur by Kleczkowski (1946) and by Lauffer (1948). The general aspects of the problem have been discussed by Pirie (1948). The adsorption of enzymes (lipase, catalase and phosphatase) from dilute solution by vaccinia virus, which contains similar quantities of the same type of nucleic acid as the cell nucleus, has been shown to occur by Hoagland et al. (1942).

(*d*) Dounce and Beyer (1948) have shown that nuclei can take up arginase from solution. Since they worked with whole nuclei, separated by relatively gentle means, this suggests that the permeability properties of mammalian nuclei may be different from those of the amphibian cocyte, Callan (1948), or else the properties are changed by relatively gentle means.

(*e*) Lison (1948) has considered the possibility of enzyme adsorption in the histochemical technique and thinks that it plays a considerable role. However, he presents no experimental evidence to support the view.

(ii) *Positive Evidence in Tissue Sections.*

The evidence in favour of an adsorption of enzyme by cell nuclei in a histochemically demonstrable form may be summarized thus:

(*a*) In almost all organs showing a positive cytoplasmic reaction for alkaline phosphatase the nuclei also show a positive reaction. In a few cases the nuclear reaction is greater than the cytoplasmic reaction.

In all these cases it is not possible to be sure that the observed picture is not due to adsorption of calcium phosphate by the nuclei.

(*b*) In guinea-pig kidney the nuclear reaction is usually confined to the cells of the proximal tubules, the other cortical and medullary tubules showing little or no reaction, except with very long incubation times. In the latter case this reaction is probably not due to enzyme action.

If the reaction in the nuclei of the proximal tubules is due to adsorption of enzyme from the cytoplasm of these tubules, the reaction in nuclei separated from whole kidney would be expected to differ from that found in tissue sections since, during preparation, all nuclei would be exposed to similar concentrations of soluble enzyme.

Whole guinea-pig kidney was homogenized in a Waring blendor microcup in 10 vols. of saline. The homogenation time was 45 secs. and the blendor was run at half voltage since full voltage resulted in complete fragmentation of the nuclei. The nuclei were separated from the homogenate by centrifugation with fields between 70 and 100 g., the separation being microscopically controlled for each run. The principal final contaminant was red cells, no attempt being made to remove these. The concentrated nuclear suspension was smeared on slides in the same manner as used for blood films, fixed for varying times in acetone, and subjected to the histochemical reaction. Good nuclear suspensions could also be prepared from fixed tissues, the blendor then being run at full voltage.

All nuclei so prepared show a slight positive reaction, which increased very little with increasing incubation time. While individual nuclei differ somewhat in their density, there was no evidence of the presence of the two populations of nuclei expected from the results in tissue sections.

These results are consistent with the hypothesis that enzyme may be taken up from solution by nuclei, and that this enzyme, under favourable conditions, may give rise to a slight reaction in the histochemical procedure.

The histochemical reaction is usually found to be denser in smears of nuclei separated from distilled water homogenates.

(iii) *Negative Evidence.*

The evidence against movement of enzyme being an important invalidating factor in the histochemical technique, may be summarized as follows:

(*a*) In cells with a very intense cytoplasmic reaction, in which the adsorption of calcium phosphate by the nuclei should be minimal, as indicated in the previous section, the nucleus should give no reaction if it contains no intrinsic enzyme and does not adsorb any from the cytoplasm during fixation. Such a situation occurs in the polymorphs of guinea-pig blood films fixed in acetone, where a jet black cytoplasmic reaction surrounds the colourless lobes of the nucleus. This only applies to short incubation time; longer incubation times result in a nuclear impregnation which is probably caused by calcium phosphate adsorption.

(*b*) If adsorption of enzyme occurred only during fixation, some difference in the nuclear reaction would be expected between blocks of different size. In very small blocks of tissue (1 mm. thick) fixation by acetone would be almost instantaneous, while in large blocks (8 mm. thick) the fixation would be slow and some time would elapse before the enzyme precipitating concentration of acetone was reached. In experiments of this type no differences in the nuclear reaction could be found.

The possibility of enzyme redistribution during the floating out process cannot, however, be excluded, since it will be demonstrated later that enzyme soluble before fixation does not become insoluble after fixation.

(*c*) If nuclei are capable of adsorbing enzyme in a form demonstrable by histochemical means, it should be possible to demonstrate a positive reaction in nuclei separated from a source which is completely phosphatase negative but which have been subsequently exposed to a solution of phosphatase. Avian red cells provide a convenient source of nuclei showing no trace of a reaction under normal conditions.

A solution of phosphatase was prepared from ox kidney in the following way. Minced ox kidney was suspended in 1½ vol. of 95% alcohol, stirred for 30 mins. and filtered. This process was repeated three times and the resulting tissue preparation stored in the refrigerator in closed containers. The preparation was found to be quite stable. The soluble enzyme was extracted from this by blending in four vols. of water and the resulting suspension filtered. Ammonium sulphate was added to 50% concentration and the precipitate collected. After dialysis against 1% NaCl solution overnight the solution was centrifuged at 20,000 g. for 15 mins. and diluted to an activity of 1200μg.P split/ml. enzyme/30 mins. under the conditions described in the next section. This enzyme activity is approximately double that of the soluble enzyme in the proximal tubules of guinea-pig kidney.

Pigeon red cells were washed in four lots of 10 vols. of 1% NaCl. They were suspended in four vols. of 1% NaCl and added to an equal volume of the above enzyme solution. The mixture was blended until about 90% of the red cells had been disrupted. The cell membrane was removed by this method, in contrast to other methods of cytolysis, where the cell membrane is found to surround the nucleus.

The nuclei were centrifuged down, the supernatant removed and replaced by 50% acetone. After 30 mins. this was replaced by absolute acetone which was allowed to act overnight in the refrigerator. After pouring off the acetone the preparation was taken up with sufficient distilled water to give a thick suspension which was then smeared on slides. These were subjected, either immediately, or after further acetone fixation, to the histochemical reaction. In neither case could any reaction be demonstrated.

(iv) *Discussion.*

Previous workers have approached this problem in other ways. Danielli (1946) has reported that the reaction is the same in rat kidney after a wide variety of fixatives, and Emmel (1946) has compared the reaction found in frozen dried material with that found after normal methods of fixation. While these results are suggestive, they do not eliminate the possibility that movement of soluble enzyme occurred during the floating-out process.

The evidence on the possibility of enzyme movement giving rise to false nuclear localizations in the histochemical reaction is thus mainly negative. The slight positive reaction found in all isolated kidney nuclei as against the reaction in tissue sections could be interpreted as due to inactivation of the enzyme in all the nuclei of the latter due to the paraffin embedding, the positive nuclear reaction found in the proximal tubule nuclei in tissue sections being due to adsorption of calcium phosphate.

The Histochemical Validity of the Reaction.

(i) *Phosphatase Activity of Different Organs.*

It has been demonstrated in the previous section that the phosphatase reaction has a number of grave defects making its use in cytological localization of the enzyme unsure. It remained to see what value the method has as a histochemical technique. Little objection can be raised to a qualitative localization of the enzyme to a given histological element, but the quantitative aspects, and, more particularly, the validity of negative results, has not yet been investigated satisfactorily.

Attempts at test tube assay of phosphatases in different animals and in different organs from the same animal appear to have been made rather infrequently. Some of the relevant data are collected in Table 3.

Table 3.

Animal.	Kidney.	Intestinal Mucosa.	Liver.	Lung.	Spleen.	Pancreas.	Testis.	Adrenal.	Author.
Rat ..	100		3	25					1
	100		0·27	1·4					2
	100		8	45				24	3
Mouse ..	100	260	0·3	3·3	1·6				2
	100	202	4·5	9	4·5	4·5		3	3
	100		4·5						9
Rabbit ..	100	43·5	9·5						4
	100	292	34·5	30·5	53·5				5
	100	267	47·5	43	44	19		19	3
Cat ..	100	212	8	54	6·5	5			5
	100	650	4	24	6	4		12	3
Dog ..	100	360	5·6		10	26·5			6
	100	630	15	12	22	27		7·5	3
Man ..	100	240	16·5					27	5
Chicken ..	100	360	29						7
Guinea-pig	100	650	6·5	31				103	3
	100		11·7		15·6		26		8
Number of animals	12		10		6		11		
Range ..			9–13		14–18		19–34		

Literature key: 1. Truhlar *et al.* (1939). 2. Greenstein (1941). 3. Macfarlane *et al.* (1934). 4. Hoffmeyer *et al.* (1946). 5. Kay (1928). 6. Gad (1946). 7. Moog (1946). 8. Present investigation. 9. Kochachian and Fox (1944).

The methods used for the data quoted in Table 3 are very diverse, a factor which probably explains some of the discrepancies in the estimation of the activity of the same organ in the same animal by different authors. The only data for guinea-pig which could be found were those of Macfarlane et al. (1934), the data being obtained by a method using very suboptimal pH and very long incubation time, and not including analyses for testis and spleen, organs very suitable for comparison by histochemical methods.

The results obtained in the present investigation are shown in Table 3 as percentages of the value of homologous kidney cortex. They may be converted to absolute values by reference to the mean value for kidney which was 1127μg.P/30 mins./100 mg. fresh weight, with a range of 950–1400.

A rough histochemical estimate of the enzyme in tissue sections was made for these four organs in three complete experiments, in which the histological procedures were the same for the organs in each experiment. The sections were incubated for 45 mins. under conditions identical with the test tube enzyme determinations. The results were as follows (expressed as density units defined in the previous paper): Kidney 51 (range 45–55), Testis 42 (range 38–45), Spleen 20·5 (range 18–23). In liver no consistent increase over the blank density could be detected at this incubation time, or after four hours' incubation.

The difficulties of making histochemical comparisons of the enzyme activity in sections of organs in which the distribution of the enzyme is different, has already been stated (Cleland, 1950), but it will be seen that the values given by kidney, testis and spleen are plausible comparisons of their relative enzyme content, while that given by liver is very much lower than would be expected from the test tube experiments.

(ii) *Soluble and Insoluble Enzyme in Different Organs.*

It is known that phosphatases occur in bound and free form, and it is of considerable importance for interpretation of the histochemical reaction to know whether the enzyme in liver is less tightly bound to the cell particulates, and, if so, to what extent it became insoluble after the fixation used in the histochemical reaction.

The first point was investigated by centrifugal fractionation of homogenates.

The results of two experiments are shown in Table 4, results from two other experiments, performed with different centrifugal fields, being completely in accord with those quoted. The value quoted is a percentage of the value in the whole homogenate.

TABLE 4.

Phosphatase Partitions in Various Centrifugal Fractions in Different Organs.

Organ.	Supernatant 1.	Supernatant 2.	Supernatant 3.
	%	%	%
Testis	75	27	22·5
	82	30	25·5
Kidney	40	19	14
	42·5	20	14
Liver	97	85	87
	97	100	91
Spleen	76	29	21·5
	81	36·5	25·5

The composition of the fractions removed was as follows:—Testis: (1) sperm heads, tails, middle pieces; some unbroken nuclei, small granules, developing acrosomes; (2) few sperm tails, many small granules; (3) very small granules. Kidney: (1) fibre, few nuclei, granules; (2) small granules; (3) microscopically unidentifiable. Liver: (1) occasional nucleus, granules; (2) small granules; (3) very small granules. Spleen: (1) fibre, some whole leucocytes, leucocyte nuclei, polymorph lobes; (2) few fibres, granules; (3) microscopically unidentifiable. Fraction I in all tissue contained a few unbroken erythrocytes.

It will be seen that, with the exception of liver, the major part the alkaline phosphatase is bound to the particulate matter of the cytoplasm, mainly to the large granule fraction. Fragmented brush borders of kidney are almost certainly included in this fraction as granules, since it was impossible to trace them as morphological entities. Chromosomes from nuclei occur in fractions I and II.

It seems unlikely that the low percentage of bound enzyme obtained for liver is due to autolytic freeing of the phosphatase from the granules, since available assays for cathepsin in different organs (rat) indicate that liver has a lower activity than spleen or kidney (Maver, Dunn and Greco, 1948).

(iii) *Effect of Fixation on Amount and Distribution of the Enzyme.*

Having indicated that guinea-pig liver phosphatase is present mainly in soluble form, it remained to be seen whether any binding of the soluble enzyme occurred during fixation. Pieces of the above four organs were removed from guinea-pigs, weighed, and placed in 20 vols. of cold acetone in a refrigerator. After 24 hours the acetone was replaced by cold absolute alcohol, this being allowed to act for periods of three days and 14 days in two experiments each containing organs from two individual guinea-pigs. After fixation, the organs were placed in 10 vols. of saline and homogenized in the blendor for two minutes. Two drops of octyl alcohol were added after blending to break the foam. The homogenate was removed, 1/10 vol. of M/5 veronal buffer pH 9·4 added, and allowed to remain at room temperature for one hour. After mixing, a sample of the whole homogenate was taken for assay. The remainder was centrifuged at 20,000 x g. for 15 minutes, and a sample of the supernatant, which was clear, taken for phosphatase assay. The largest component present in the homogenate in appreciable quantity was the cell nucleus, the majority of which appeared to survive disintegration. Granules retained their integrity.

The phosphatase activities of the whole homogenates were within the normal activity range for the organ concerned, indicating that little or no loss of activity is caused by fixation, a fact noted by other authors (Danielli, 1946, Stafford and Atkinson, 1948, Doyle, 1948), and no difference could be found between the tissues fixed for three and 14 days. No change in the solubility of the enzyme in kidney or spleen could be demonstrated, an increase of 5% in the solubility of the testis enzyme was noted, while the percentage of soluble enzyme in liver had declined to 60%.

It will be seen that fixation, while decreasing the solubility of the liver enzyme somewhat, cannot be considered to preserve soluble enzyme in a form likely to be demonstrated in the histochemical reaction, and, therefore, that the reaction demonstrates only the bound enzyme of the cell. This possibility has been considered by Lison (1948), but no evidence was adduced.

The enzyme solubility factor is still insufficient to account for the low values found for the activity of guinea-pig liver sections in the histochemical technique. In order to study this matter further, to see what degree of inactivation was produced by imbedding in paraffin and the preparation of tissue sections in a form suitable for histochemical examination, and whether this was different for different organs, the absolute activities of the phosphatase in tissue sections of the above four organs was determined. The results are shown in Table 5.

TABLE 5.
Phosphatase Activity of Tissue Sections for Various Organs.

Organ.	μg. P Liberated per 100 sq. mm. of Surface Area.			Percentage of Kidney.
	1 Hour.	2 Hours.	4 Hours.	
Testis ..	10·1	22·3	43	21
Kidney ..	50·2	106	204	100
Liver ..	1·91	3·25	4·6	2·3
Spleen ..	7·0	15·5	29·4	14·5

Three points emerged from this experiment: (1) Enzyme in the liver is inactivated during the incubation and the activity is sufficiently low under the conditions of the

histochemical technique to account for the low values found by that technique. (2) Other organs show little inactivation over the times used and their activity in comparison with kidney is within the normal limits found for homogenates. (3) Assuming a shrinkage in volume of 50%, a not unlikely value in view of the experiences of Stowell (1941) and Tarkham (1931), a calculation of the enzyme activity of fresh tissue equivalent to these sections fails to indicate that any loss of enzyme activity can be ascribed to the histological procedures involved in the preparation of spleen, kidney or testis sections. This is at variance with the findings of Stafford and Atkinson (1948) and Doyle (1948).

Discussion.

The demonstration that cobalt sulphide can precipitate in the section in granular form under certain conditions, implies that the interpretation of such structures as the perinuclear granules described by Deane and Dempsey (1945) as Golgi apparatus in kidney sections, is rather difficult; they do not appear to be sufficiently constant for the possibility of artefact to be excluded. No structure which could be unequivocally interpreted as Golgi apparatus has been found in the guinea-pig kidney sections studied here. The blackening in the Golgi apparatus in the epithelial cells of small intestine is constant enough to be regarded as valid, but whether it is due to enzyme is another matter. A blackening in what appears to be the Golgi apparatus of adrenal cortex cells (Bennett, 1940) has been noted in the present study.

The demonstration of blackening in the chromosomes by Willmer (1942), Krugelis (1942, 1946), Danielli and Catcheside (1946) and others, and the reports of nuclear reactions by a large body of histochemists, cannot with any certainty be related to the presence of phosphatase, because of the demonstration of adsorption of calcium phosphate by chromatin. This is especially so because in those cells in which the chromosome reaction is described, the cytoplasmic reaction is usually slight, a state of affairs which is shown by the present study to give the most troublesome calcium phosphate wandering. This fact also removes much of the grounds for the interesting speculation of Brachet and Jeener (1948).

In view of the property possessed by nuclei of adsorbing calcium phosphate from supersaturated solution in the incubating fluid (Danielli, 1946), the use of unusual substrates, such as nucleic acids, often at a relatively low pH, does not seem likely to give valid results. At low pH and in the presence of a source of nitrogen bacterial growth is quite rapid; splitting of the complex substrate by the bacterial enzymes would yield calcium phosphate in the vicinity of the section, with probable adsorption of it by the nuclei. To complicate matters further, these low pH solutions are not usually presaturated with calcium phosphate, which, in a previous paper (Cleland, 1950), has been shown to be desirable. The resulting picture in these cases is a balance between a number of factors: loss of calcium phosphate from enzyme sites to the solution before the latter becomes saturated due to bacterial splitting of substrate, wandering of calcium phosphate from the enzyme site after saturation of the solution, adsorption of calcium phosphate from the solution which has become supersaturated due to bacterial action, and destruction of the enzyme owing to the long incubation times used. The findings of Gomori (1949) are interesting in this connection.

These remarks are not meant to imply that there is no alkaline phosphatase in cell nuclei: merely that it is not possible to obtain unequivocal evidence of its presence by means of the histochemical reaction.

The evidence for the presence of phosphatase in the nucleus is, however, very slight. Apart from a statement by Mirsky and Ris (1946) not accompanied by any experimental details, and a footnote report in a paper by Potter (1947) to the effect that large quantities of apyrase occur in liver cell nuclei, the only report of which the author is aware is that of Dounce (1943), who has shown that isolated liver nuclei contain about twice as much phosphatase on a weight basis as whole liver cells. No other nuclei appear to have been examined for their phosphatase content, and if the nucleur arginase distribution is generally applicable (Dounce and Beyer, 1948), considerable variation between nuclei from different sources can be expected.

In view of the reports reviewed in the section on adsorption of enzyme, this obesrvation of phosphatase in liver nuclei must be regarded with some reserve until suitable adsorption controls on isolated nuclei have been performed. The evidence on non-adsorption of enzyme adduced in this paper will not necessarily be applicable to studies of isolated nuclei assayed in the test tube; in the histochemical reaction the greater proportion of the enzyme not firmly affixed to the protoplasmic structure will be lost to the incubating fluid, while in the test tube assay all the enzyme both fixed and adsorbed will be measured.

Three methods have been used to study the enzyme constitution of the nucleus; reactions in tissue sections with microscopic localization of a coloured product produced at the enzyme site (literature in Glick, 1949); test tube assays of enzyme in nuclei separated from fresh tissues (Dounce, 1943; Lan, 1943; Dounce and Beyer, 1948); test tube assays of enzyme in cell fragments produced by centrifugal stratification and breaking of whole cells, in which fragments without nucleus are usually compared with fragments containing the nucleus and a little cytoplasm. Shapiro (1935), Holter (1936), Holter and Kopac (1937), Ballentine (1940) and others.

The first of these methods seems unlikely, in view of the present experience with the alkaline phosphatase reaction, which has a primary localizing product of very low solubility, to yield a valid localization of an enzyme and proof of the validity or invalidity of such methods may be difficult or even impossible. The fact that some enzymes have to be studied in unfixed sections introduces serious possibilities of extraction of enzyme and tissue substance during the procedures. Quantitation of such methods presents considerable difficulty and the interpretation of negative results is unsure.

The second method has two drawbacks: the possibility of adsorption of enzymes, and, in view of the work of Pollister and Leuchtenberger (1948), the strong likelihood of extraction of enzymes.

Inasmuch as the cell is very little injured by the third method (sea urchin eggs so broken are capable of development, Harvey, 1932, et seq.), and the nuclear membrane, being still surrounded by some cytoplasm, can be expected to preserve its normal permeability, this method appears to be the most satisfactory of the three.

Phosphatase belongs to a group of hydrolytic enzymes whose physiological role is, in general, obscure. Members of this group, on present knowledge, appear to be a liability to the cell rather than an asset, inasmuch as they cause degradation of key substances in the energy forming mechanisms of the cell. The fact that histochemists have related phosphatase to, *inter alia*, glycogen synthesis, nucleic acid synthesis and breakdown and protein synthesis, is, perhaps, a measure of our ignorance of the physiological role of the enzyme rather than of any property of the enzyme itself. A very suggestive approach to the physiological role of one of this group of apparently inimical enzymes (one causing cozymase destruction) has been made by McIlwain (1949). It seems possible that phosphatases may play a similar role in the regulation of energy formation in the cell.

The finding that alkaline phosphatase is, except in liver, associated with the large granules of the cytoplasm of the guinea-pig organs studied, is of interest in view of the current intensive enzymological study of this cell fraction. It appears that, with the exception of the glycolytic system, most of the energy-yielding reactions of the cell are present on this granule fraction (Cross, Taggart et al., 1949).* In an unpublished study of the cytoplasmic distribution of a number of the Krebs cycle enzymes in oyster eggs, it has been found that the enzymes are associated with the cytoplasmic granules, probably on that fraction identified cytologically as yolk (Cleland, 1947). Mitochondria evidently retain their enzyme integrity even on undergoing a cytological differentiation; Du Buy et al. (1949) have shown that the enzyme composition of melanoma granules is very similar to that of amelanotic granules, both resembling the enzyme spectrum reported by other authors of large granules from normal tissues. It would appear likely that this

* Since cytologically validated by Kennedy and Lehninger, *J. Biol. Chem.*, 1949, 179 : 957.

segregation of the energy-yielding reactions of the cell on the large granule fraction is one of the prime laws of the cytoplasmic structure of the animal cell.

The extraordinary resistance of the kidney enzyme to extraction from tissue sections is reminiscent of the firm binding of such enzymes as cytochrome oxidase and succinic dehydrogenase to the tissue particulates; the latter enzymes have not been obtained in a truly soluble form. As further evidence of the strong binding of the alkaline phosphatase the methods used for the preparation of the purified enzyme may be cited. These involve digestion of the tissue with trypsin (Schmidt and Thannhauser, 1943; Ehrensvard, 1933). It would appear that enzymes such as these are built into the structure of the granule; others, such as malic and lactic dehydrogenases, which may be extracted in a soluble form from acetone powders, are evidently retained by forces of a more secondary nature.

Moog and Steinbach (1946) have reported that significant quantities of the alkaline phosphatase of the chick embryo are bound to granules. When their fraction 2, which must contain most of the large granules, is included in the estimate of the particulate phosphatase, the percentage of soluble enzyme, up to 10 days' incubation, is between 25% and 30%, a figure similar to that found for guinea-pig kidney, spleen and testis in the present study. Between 10 and 12 days' incubation the percentage of soluble enzyme doubled in the chick embryo. Moog and Steinbach (1945) have reported that much of the apyrase activity of the chick embryo is also associated with the particulate matter of the cytoplasm.

The demonstration that the percentage of bound phosphatase may change during development (Moog and Steinbach, 1946)* and the demonstration in the present study that lyoenzyme is not made insoluble by histological fixation, when taken together, indicate that caution is required in interpreting reports of changes in alkaline phosphatase activity during development (Brachet, 1947, *a*, *b*; Krugelis, 1947, *a*, *b*; Moog, 1943; Zorzoli, 1947). Thus while Moog (1943) records decreases in histochemical alkaline phosphatase activity of most organs of the chick embryo after eight days' incubation, estimates of total alkaline phosphatase in the test tube show a continuous increase over this period (Moog, 1946). The discrepancy is evidently due to change in the proportion of bound enzyme.

In a previous study of oogenesis in the oyster (Cleland, 1947) no alkaline phosphatase reaction could be demonstrated. Later test tube studies have indicated that the mature oocytes have about 15% of the activity of guinea-pig spleen. This is sufficient to be demonstrable histochemically if the enzyme were bound. Since it is all present in soluble form, no histochemical reaction is demonstrable.

Similar difficulties of interpretation arise where a change in the intensity of the histochemical reaction occurs in a pathological condition or is associated with some physiological change.

Summary.

(*a*) Low ammonium sulphide concentrations can give rise to spurious granular precipitates of cobalt sulphide.

(*b*) Wandering of calcium phosphate and its adsorption by nuclei is a factor which makes interpretation of the cytochemical reaction virtually impossible. This factor becomes more serious when the enzyme activity is low, and is very serious when the incubation of the sections is conducted at a low pH.

(*c*) The weight of evidence is against any false localization of the enzyme being due to movement of the enzyme during or after fixation.

(*d*) The alkaline phosphatase activities of guinea-pig kidney, testis, liver and spleen, in test tube experiments, is compatible with the activity displayed in the histochemical reaction for all organs except liver.

(*e*) The enzyme is associated with the large granule fraction in testis, kidney and spleen, but the greater part of the liver enzyme is not bound to granules.

* Mazia (1949) has recently shown that desoxyribonuclease is progressively bound to tissue particulates during Arbacia development.

(*f*) Histological fixation causes virtually no change in the solubility of unbound enzyme, and results in little or no enzyme inactivation.

(*g*) The enzyme activity in tissue sections is almost entirely due to enzyme which was bound before fixation. No loss of enzyme could be ascribed to the embedding process.

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ADDENDUM.

Since this paper was written the paper of Ruyter and Neumann (1949)* has been seen. While their presentation is a little difficult to follow, their main point appears to be that the reaction, as usually practised, is likely to give invalid results because of extraction and wandering of enzyme in the lower concentrations of alcohol. With the tissue sections used in the present study we have been unable to find any evidence of extraction of enzyme by 30% alcohol even after 30 hours in this reagent.

EXPLANATION OF PLATES I AND II.

All photomicrographs are of guinea-pig kidney sections at a final magnification of 130 diameters. Orthochromatic plates were used throughout and the exposures were chosen to give maximum contrast for the nuclei, this causing considerable loss of contrast in the brush borders and cytoplasm of the proximal tubules. Exposure and development times were identical for all figures.

* Ruyter, J. H. C., and Neumann, H., 1949.—A critical examination of the histochemical demonstration of the alkaline phosphomonoesterase. *Biochim. Biophys. Acta*, 3 : 125.

PLATE I.

Figs. 1 and 2. Comparing the distribution of density in sections incubated to the same visual density in the presence of different concentrations of Ca. Borate buffer (M/50) pH 9·4, M/50 substrate, M/100 Mg. Ca concentration M/100 for Fig. 1 with incubation time 30 minutes; M/100,000 for Fig. 2 with incubation time 4 hours.

Note the increased cytoplasmic and especially nuclear density at the lower calcium concentration. This indicates that nuclei are able to adsorb calcium phosphate generated in their vicinity by enzyme action.

Figs. 3 and 4. Comparing the distribution of density in slides incubated to the same final density, with different rates of enzyme action. Borate buffer (M/50) pH 9·4, M/50 substrate, M/100 Mg. Ca concentration M/100 for Fig. 3, with incubation time 90 minutes, and M/10 for Fig. 4, with incubation time 4 hours.

When the rate of formation of calcium phosphate is slowed, this results in a greater migration of calcium phosphate to the nuclei.

PLATE II.

Figs. 5 and 6. Comparing the distribution of density in slides incubated to approximately the same final density, with different rates of enzyme action. Borate buffer (M/50) pH 9·4, M/100 Ca and Mg. Substrate concentration M/10 for Fig. 5, with incubation time 30 minutes, and M/1000 for Fig. 6, with incubation time 4 hours.

Even though the final density of the slide incubated at the lower substrate concentration is less than that of the control, the nuclear density is both absolutely and relatively greater. Similar results to those shown in Figs. 3 to 6 were also obtained when the rate of enzyme activity was depressed with potassium cyanide.

Fig. 7. Showing the distribution of density in slides incubated at pH 8·5 (veronal borate buffer). Other conditions same as in previous figures. When not only the rate of enzyme action is depressed but the precipitation threshold of calcium phosphate is raised by lowering the pH, adsorption of calcium phosphate by the nuclei is greatly increased. With comparable overall density, the nuclear impregnation decreases with increasing pH, as also does the solubility of calcium phosphate.

Fig. 8. Showing a typical control section, after four hours' incubation under the same conditions as Figs. 1, 3 and 5, but in the absence of substrate. The nuclear impregnation shown here is more than in the control sections corresponding to the other figures because this was prepared on the same day as it was photographed, while the others were over a month old and some fading had occurred in these. The dark bodies in the glomeruli and between the tubules are red cells.

DILATION OF THE FOOT IN UBER (POLINICES) STRANGEI (MOLLUSCA, CLASS GASTROPODA).

By Muriel C. Morris, Linnean Macleay Fellow in Zoology.

(Three Text-figures.)

[Read 26th April, 1950.]

Introduction.

For the past forty to fifty years it has been universally accepted that the phenomenon of turgescence and extension of certain parts of the body, in particular the foot, in most Molluscs is due to the forcing of large quantities of blood into the organs concerned. Thus Pelseneer writes in regard to the blood in Molluscs (Pelseneer, 1906): "The volume of blood in some groups, particularly in the Lamellibranchs and Gastropods, is so great that it plays a very important part in the turgescence of various parts of the integument, by filling the tegumentary sinuses during the relaxation of their muscles."

However, various workers of the early and middle nineteenth century held that water from the surrounding medium, taken up either directly into the blood vessels or into a set of "aquiferous" tubes completely separate from the blood system, was responsible for this turgescence. This theory was applied almost exclusively to the phenomenon of turgescence of the Molluscan foot. Many different theories accounting for the uptake and output of this water and its transmission through the foot were put forward—some of them backed with experimental evidence and some of them purely theoretical. The story of the birth, life and death of this "Wasseraufnahme" theory, as it was called, is a particularly interesting one, and Justus Carrière has left a very good and comparatively detailed account of its development from its birth in the work of Delle Chiaje (Descrizione di un nuovo Apparato di canali acquosi scoperto negli animali invertibrati marini delle due Sicilie, in Memorie sulla storia e anatomia degli animali senza vertebre del regno di Napoli 1823-29. Tom. 11, p. 259 ff., 1826) to its death at the hands of some six or seven workers during the 1880's and 1890's (Carrière, 1882). These workers of the latter part of the nineteenth century proved conclusively for *certain* species of Lamellibranch and Gastropod Molluscs that the expansion of the foot and other parts could be adequately explained by the forcing of blood into the parts concerned. Their assumption that a similar state of affairs existed in all other Lamellibranch and Gastropod Molluscs is understandable when one considers the large range of specimens investigated and the development of the study of comparative anatomy.

However, in 1884, just as the old "Wasseraufnahme" theory was beginning to lose popularity, Schiemenz published a paper in which he claimed to have demonstrated the existence of an "aquiferous" system in a family of Prosobranch Molluscs—the Naticidae (Schiemenz, 1884). Up to this time he had strongly opposed the "Wasseraufnahme" theory and had decided to carry out a series of experiments to prove his point, using *Natica josephina*, because of its large and extensible foot. To his amazement, his results seemed to show that water from the external medium was being used by the animal for expansion of the foot. He later (Schiemenz, 1887) carried out an investigation of the mode of uptake, transmission and expulsion of this water and claimed to have demonstrated the existence of pores on the edge of the foot opening into a series of water cavities completely separate from the blood vessels.

When Schiemenz's papers first appeared, they were quite readily accepted by a number of workers, for the old "Wasseraufnahme" theory still had a small following, but when this theory finally lost its hold it must have been obvious that here was a startling and isolated exception to what was by then an accepted theory, namely that the expansion of the foot in Lamellibranchs and Gastropods was due to the forced flow of blood. However, a survey of the literature of the past sixty years reveals that Schiemenz's theories regarding water-uptake in the Naticidae are still accepted by some writers, even though no workers have touched seriously on this problem of expansion of the Naticid foot since that time.

The common occurrence of a Naticid, *Uber* (*Polinices*) in the neighbourhood of Sydney drew the attention of the late Professor W. J. Dakin, of the Zoology Department, Sydney University, to this anomalous situation, and particularly to the strange hesitancy in the literature to give a full explanation of the phenomenon of foot expansion in the Naticidae. For instance, in several standard text-books the authors lead the reader to understand that the uptake of sea-water may be accepted as a factor in the extension of the Naticid foot, without giving any details or correlating the phenomenon with the entirely different means of extension in all other Molluscs in which expansion of the foot has been investigated. It seemed clear that here was a strange gap in regard to our knowledge of the physiology of the Molluscs, the existence of an isolated phenomenon which was not merely a problem of the Naticidae, but one which should be linked up with the fundamental physiology of the phylum. When it was realized that no check had been made on Schiemenz's work and that practically nothing original had appeared on the subject of water systems in the Molluscs during the past forty to fifty years, the advisability of an inquiry became obvious.

On Professor W. J. Dakin's suggestion it was decided that an investigation of the conditions in the local species of Naticids should be undertaken. It was felt that if the existence of an "aquiferous" system in the Naticidae was refuted, the one real stumbling block to the theory that turgescence in *all* Molluscs is due to the forcing of blood into the parts concerned would be removed. If, on the other hand, such a system was proved to be present, a very desirable confirmation of at least part of Schiemenz's work would be achieved, and possibly an interesting field for further research would be reopened.

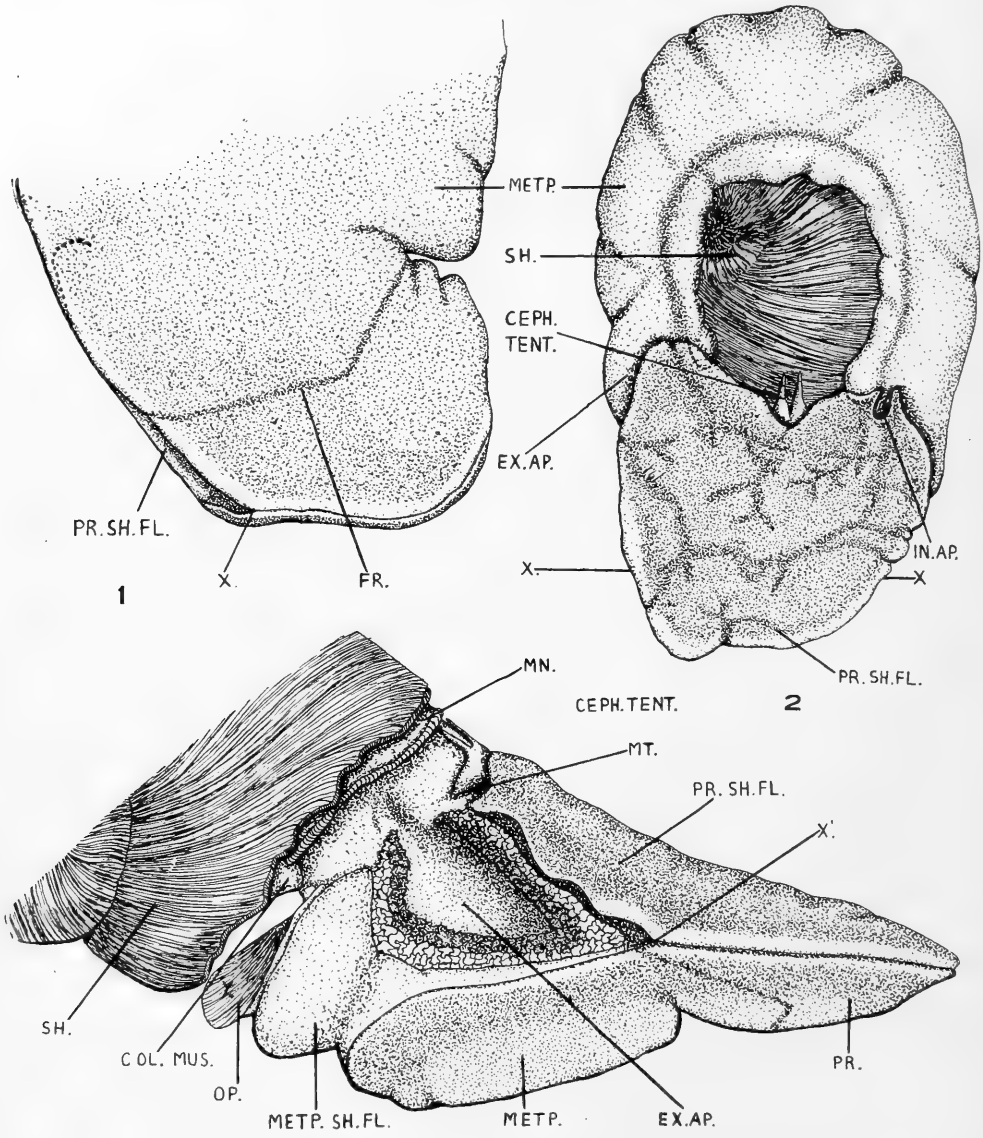
General Notes on the Naticidae.

The Naticidae are Prosobranch Gastropod Molluscs belonging to the order Mesogastropoda, which, in the modern classification of the Gastropod Molluscs, includes all the members of the old tribe Taenioglossa of the Monotocardia.

As in all Prosobranchs, the foot is divided by an oblique furrow into an anterior propodium (PR., Text-fig. 1) and a posterior metapodium (METP., Text-fig. 1). It has become highly modified for burrowing in the mud and sand, the propodium developing a "shell-fold" (PR.SH.FL., Text-fig. 2) shaped like a ploughshare, which can completely cover the head and anterior half of the shell; whilst the metapodium has also developed a "shell-fold" (METP.SH.FL., Text-fig. 2), which, when expanded, is present as a collar round the back edge of the shell. Whilst the "shell-fold" of the metapodium goes straight over into the metapodial tissue proper, the edge of the propodial "shell-fold" is separate from the edge of the propodium proper except for a region X–X′ (Text-fig. 2) at the anterior edge of the propodium. The actual point of junction, X, of this "shell-fold" with the propodium proper is shown more clearly in Text-fig. 3.

In *Uber* the propodium and the propodial "shell-fold" are darkly pigmented except at the edges. The edge at the anterior tip and back along the sides to just behind the junction of the propodium and the propodial "shell-fold" is a creamy yellow to orange, whilst the edges of the rest of the "shell-fold" and of the propodium proper are light grey (Text-fig. 2). The metapodium and metapodial "shell-fold" are usually not as darkly pigmented as the propodial tissue, the edges occasionally being more lightly pigmented than the bulk of metapodial tissue (Text-fig. 2).

It is probably correct to say that in no other Gastropod is there such a remarkable extension of the body from the shell as that observed in the family Naticidae, and the volume change is certainly such as to arouse interest and inquiry. When fully expanded the foot appears as a frill of oedematous flesh completely surrounding the shell, whilst



Text-figures 1-3.

Fig. 1, under-surface of anterior half of fully expanded foot of *Uber strangei* (× 2). Fig. 2, fully expanded *Uber strangei* (× 1·5). Fig. 3, postero-lateral view of *Uber strangei* from right side (× 2). CEPH.TENT., cephalic tentacles; COL.MUS., columella muscle; EX.AP., exhalant aperture; IN.AP., inhalant aperture; MN., mantle; MT., mouth; METP., metapodium; METP.SH.FL., metapodial shell-fold; OP., operculum; PR., propodium; PR.SH.FL., propodial shell-fold; SH., shell; X., point of separation of propodial shell-fold from propodium, right side; X.′, point of separation of propodial shell-fold from propodium, left side; Y., space enclosed by operculum, metapodial shell-fold and shell where water is sometimes trapped.

the shell is almost covered with the "shell-folds" (Text-fig. 2). In some of the biggest specimens observed, in which the shell measured five cm. from anterior to posterior edges when placed with the operculum down, the foot has been found to measure as much as sixteen cm. from anterior tip to posterior tip. In specimens of average size, with a shell size of three cm., the foot in the normal expanded condition usually measures about ten cm.

When the animal contracts, this large mass of foot and "shell-folds" is withdrawn into the space of the comparatively small shell, which is closed by the operculum attached to the upper side of the metapodium (Text-fig. 3).

Three species of Naticids occur commonly in the Sydney area—*Uber* (*Polinices*) *strangei* (Reeve), *Uber* (*Polinices*) *conicus* (Lamarck) and *Uber* (*Polinices*) *aulacoglossa* (Pilsb. and Van.), whilst very occasionally *Mamilla* (*Polinices*) *melanostoma* (Swainson), which occurs commonly in Queensland but does not extend much beyond the Queensland–New South Wales border, makes its appearance. Generally *Uber strangei* is confined to the estuarine flats, whilst *Uber conicus* and *Uber aulacoglossa* occur most commonly on the sandy beaches. They occur at the low-water level of spring tides, and consequently are seldom found on anything but very wet sand, usually preferring to be at least half covered with water. While collecting specimens it has been observed that they make their appearance about an hour before low tide, and then as the tide begins to rise again they disappear very quickly. Those species found on the estuarine flats seem to prefer the pools hollowed out in the sandy areas to the more muddy *Zostera* covered areas, and it is in these pools that the largest specimens are to be found.

The large ploughshare-shaped propodium and the propodial "shell-fold" are obviously adaptations of the foot for progression through the sand and mud. The propodium forces its way through the sand half an inch to an inch below the surface, so that all of the propodium and the propodial "shell-fold" and part of the shell are covered with mud. The only part of the animal which is visible is the metapodium and posterior part of the shell.

The tracks left in the sand by the animal are very characteristic. They consist of a smooth depression, one-half to two inches wide, depending on the size of the animal, with raised edges formed by the large bulk of propodial tissue burrowing under the sand, which, when it comes in contact with the shell, falls away on either side, building up a raised edge to the depression made by the propodial tissue. The metapodium following along behind simply smooths over the depression. At one end of the track there is usually a mound which, on closer investigation, proves to be the animal itself burrowing along beneath the sand. If disturbed in any way they will immediately burrow well down below the surface. It is not certain whether they continue to creep along once they are well down below the surface of the sand, or whether they remain stationary and then burrow up to the surface later on to continue their progression. The fact that a large number of the specimens found burrowing well beneath the surface at the end of a track are in a contracted or semi-contracted state seems to indicate the latter course.

The estuarine flats are criss-crossed with literally hundreds of these tracks at low tide, each of them pursuing a devious course and forming an interlacing network with other tracks. *Pyrazus ebeninus* leaves a very similar track, but in this case it is formed mainly by the shell dragging along behind the animal and not so much by the foot itself.

Material.

Uber strangei was used almost exclusively for the experiments presented in this paper. The specimens were collected from Gunnamatta Bay on Port Hacking and from Sailor's Bay on Middle Harbour, Port Jackson, and the actual work was carried out mainly at the Zoology Department, Sydney University. On two occasions, 17/1/49 to 25/1/49 and 14/3/49 to 17/3/49, when there was a need for good aquarium conditions

and a constant supply of specimens, the work was carried out at the Fisheries Division of the Commonwealth Scientific and Industrial Research Organization at Cronulla.

Experimental Results.

When an expanded specimen of *Uber* is disturbed so that the foot contracts and the soft parts are withdrawn into the shell, the effect is rather striking. The extended parts of the animal disappear from view quickly and at the same time a large volume of fluid escapes from the "mouth" of the shell. It will be seen later that Schiemenz (1884) claimed that the greater part of this fluid had come from the "aquiferous" spaces in the foot, where it had played a part in the expansion of that organ.

The propodium is withdrawn first with no loss of fluid, and it is only when the metapodium, bearing the operculum, is withdrawn that fluid is seen to escape from the "mouth" of the shell. This is very noticeable when observing an animal in the field, where it is continuously encountering obstacles in its path. On closer observation, however, a slight increase in the size of the metapodium as the propodium contracts, or a corresponding decrease in the size of the metapodium as the propodium is stretched out, is noticeable—pointing to the fact that a fluid is being alternately compressed into or withdrawn from the metapodium as the propodium is withdrawn or stretched out.

The amount of fluid given off naturally varies with the size of the specimen, but it is somewhere in the order of 4–7 ml. for an average-sized specimen when fully expanded (Table I). In this connection it should be pointed out that Schiemenz's figures for the total fluid given off when *Natica josephina* contracted (Schiemenz, 1884, table, p. 535) are amazingly large when compared with those given in Table 1 for

Table 1.

Fluid Expelled when Fully Expanded Specimens of Uber strangei *of Various Sizes Contracted.*

Ml. Fluid Expelled.	Size of Specimen.
9·0	Large specimen.
8·0	,, ,,
7·5	,, ,,
7·5	,, ,,
7·0	,, ,,
7·0	,, ,,
6·5	,, ,,
6·0	,, ,,
6·0	,, ,,
5·8	Medium sized specimen.
5·525	,, ,, ,,
5·5	,, ,, ,,
5·0	,, ,, ,,
5·0	,, ,, ,,
4·5	,, ,, ,,
4·0	,, ,, ,,
3·0	Small specimen.
2·5	,, ,,
2·0	,, ,,

Uber strangei. The figures for shell volume which he gives (Schiemenz, 1884, table, p. 535) show that most of the specimens he was using were larger than the specimens of *Uber strangei* used here; but even so the figure which he gives for the total fluid volume given off by a *Natica* of a certain size is much greater than that observed for an *Uber* of corresponding size. It seems very likely that he did not remove as much of the surface moisture and mucus as possible before measuring the amount of fluid given off when the specimens contracted.

When measuring the amount of fluid given off, care must be taken to make sure that the animal is fully expanded before inducing it to contract, because the amount of fluid varies with the degree of expansion. This fact would at first seem to indicate clearly that at least some of the fluid given off has played a part in the expansion of the foot; but there is another factor, the "free-space" of the shell, which has to be considered.

It was soon realized that when the animal expands, water from the outside will be sucked into the space, termed here the "free-space", between the animal and its shell—a space which, when the animal is in a contracted state, is filled with foot tissue but, when the foot is expanded, forms a partial vacuum and so sucks water in from outside. This water, of course, is pushed out when the animal later contracts and the space is again filled with foot tissue. The size of the "free-space" and consequently the amount of water taken up into it and given off from it will vary, for the one specimen, with the degree of expansion. Thus the fact that more fluid, i.e. total fluid, is given off by a fully expanded specimen than by a semi-expanded specimen could be attributed to the variation in the size of the "free-space" under these conditions, and so need not necessarily point to the fact that the fluid given off has played a part in the expansion of the foot.

The existence of this "free-space" can be quite easily demonstrated—for instance, if a hole is drilled in the shell and the animal induced to expand and then contract again, fluid which normally would have escaped *via* the "mouth" of the shell will be seen to escape through this hole.

Although it did not seem likely that this "free-space" could account for all the fluid given off when the animal contracts, it was deemed wise to investigate the matter by repeating an experiment carried out by Schiemenz, who had also realized the significance of this "free-space" (Schiemenz, 1884). The experiment had actually been devised by Agassiz (Agassiz and Siebold, 1855). The volume of fluid given off by a fully expanded specimen was measured and compared with the volume of the shell, the latter being determined by immersion of the empty shell in a graduated cylinder of water. It was realized that if the volume of the fluid was greater than the volume of the shell it would mean that some of the fluid must have been given off by the animal itself, because the "free-space" obviously could not accommodate it all. The figures for the volume of the shell give a liberal estimation of the size of the "free-space" because a lot of the space inside is normally occupied by the visceral hump, so the "free-space" will always be less than the volume of the shell. Schiemenz's figures for this experiment (Schiemenz, 1884, table, p. 535) show that the fluid given off was far in excess of the volume of the shell—as much as two to three times as great in many cases. Agassiz (Agassiz and Siebold, 1855) himself states that the volume of fluid given off when *Natica heros* contracted was two to three times as great as the shell volume, but he gives no figures to support this. The figures obtained when this experiment was repeated are given in Table 2.

In most cases the volume of fluid expelled is slightly greater than the volume of the shell, but it is especially to be noted that in no case is the difference as great as that observed by Schiemenz, and in some cases it is less than the volume of the shell.

At first it was assumed that the fluid given off was sea-water, either from the "free-space" of the shell or possibly from "aquiferous" spaces in the foot—if such spaces existed. However, when the fluid given off by four specimens was examined it was found to contain blood corpuscles. This pointed to the fact that some of the fluid given off was blood, which, although it might not be a normal component of the fluid expelled, but only present when the animal contracts under stress (which will always be the case when handling an *Uber*, no matter how much care is taken), must be taken into account in all volume measurements. Schiemenz had observed the presence of blood corpuscles in the fluid (Schiemenz, 1884) and had assumed that they had gained entry into the water cavities (and so to the outside when the animal contracted) by bursting of blood vessels during rapid contraction. However, he did

TABLE 2.

Comparison of Volume of Fluid Expelled by Fully Expanded Specimens of Uber strangei *with the Volume of their Shells.*

Ml. Fluid Expelled.	Volume of Shell. Ml.	Presence (*p*) or Absence (*a*) of Blood Corpuscles.
9·0	8·0	?
7·5	6·0	?
7·5	5·0	?
7·0	6·75	?
6·5	4·0	?
6·0	4·0	p
5·8	3·25	?
5·525	5·0	?
5·5	6·0	?
5·0	6·0	p
3·0	3·0	p
2·5	2·0	p
2·0	2·5	?

not mention the possibility that this blood component of the fluid given off, together with the water from the "free-space" of the shell, and any moisture and mucus adhering to the animal, could make up the whole of the fluid given off and that possibly no water was being expelled from the foot itself. This possibility must not be overlooked, especially as in no case did the present set of observations show that the amount of fluid expelled was much in excess of the volume of the shell. As regards the surface moisture and mucus, Schiemenz makes no mention of having wiped the outside of the animal to remove as much as possible before measuring the fluid given off. (The surface fluid, especially that lying in the space behind the shell (Text-fig. 3), the floor of which is formed by the operculum, the roof by the shell itself and the walls by the metapodial "shell-fold", is a very important factor and care was always taken to remove as much as possible.)

As it seemed at this stage that the fluid given off consisted of three, and possibly four, parts—(*a*) "free-space" water, (*b*) surface water and mucus, (*c*) blood, (*d*) water from "aquiferous" system, if present—it was decided to try removing the shell so that the "free-space" component (*a*) of the fluid could be eliminated and the surface water and mucus (*b*) component greatly decreased, as removal of the shell facilitates removal of surface moisture and mucus. It was realized that if a large quantity of fluid was still given off, parts (*a*) and (*b*) of the total fluid given off when the shell was still on could be regarded as unimportant, in which case the relative importance of parts (*c*) and (*d*) would have to be determined. If, on the other hand, no fluid was given off after removal of the shell, it could be assumed that parts (*a*) and (*b*) formed all of the fluid given off when the intact animal contracted. If a little was still given off it would mean that, whilst (*a*) and (*b*) made up most of the fluid, (*c*) and (*d*) also played a part, and their relative importance would have to be ascertained.

The shell was removed by carefully cutting it away with bone forceps until only the region where the columella muscle is attached remained. If care is taken and the animals kept for two to three days under good conditions, they recover from this operation quite successfully. Once the animals had recovered and expanded again, they were removed in an expanded condition from the aquarium, as much as possible of the surface moisture and mucus removed, and the fluid given off collected. In one case the fluid was examined for blood corpuscles. The results are given in Table 3.

These results show that even when component (*a*) and the major part of component (*b*) are removed, quite a large quantity of fluid is still given off. In the case of the one specimen in which the fluid was examined for blood corpuscles, the latter

TABLE 3.

Fluid Expelled by Fully Expanded Specimens of Uber strangei *after Removal of their Shells.*

Ml. Fluid Expelled.	Presence (*p*) or Absence (*a*) of Blood Corpuscles.
5·0	p
4·0	?
3·0	?
1·0	?

were found to be present in considerable numbers, which meant that the five ml. of fluid given off consisted of two, and possibly three, components: (*b*) surface moisture and mucus, almost negligible, (*c*) blood, (*d*) water from "aquiferous" system, if present.

The question was whether components (*b*) and (*c*) could together account for all of the fluid given off when the shell was removed, or whether part of this fluid could come from a set of "aquiferous" cavities in the foot.

The results of another experiment, carried out on a specimen with shell removed, are interesting in this connection. The animal was weighed in a contracted state and then in an expanded state. It was realized that if any increase in weight occurred during expansion it would be due to, firstly, extra moisture adhering to the enlarged surface (as the surface area of the foot would naturally increase on expansion) and, secondly, to water taken up into the foot for expansion (if such a process existed). The results are given in Table 4.

TABLE 4.

Differences in Weight between Contracted and Fully Expanded Specimens of Uber strangei *after Removal of their Shells.*

Weight when Contracted. Gm.	Weight when Expanded. Gm.	Ml. Fluid Later Given Off.	Ml. Sea-water of Density 1·02 Necessary to Account for Weight Increase.
6·530	8·930	1·0–2·0	2·35
6·230	15·280	5·0	8·87
6·230	10·270	?	3·99
5·475	9·445	?	3·89
3·646	5·455	1·0	1·77

These results show that there is a definite increase in weight in each case. The last column in Table 4 gives the amount of sea-water which would have to adhere to the surface of the animal if the extra surface water adhering to the expanded foot alone were to account for the weight increase. It is quite obvious, in the case of the first four examples, that such a volume of sea-water could not have been adhering to the surface at the time of weighing, especially as great care was taken to remove as much as possible before weighing. Part, and probably most, of the weight increase must have been due to something that was taken up by the animal during expansion. It is significant that the increase in weight in each case is roughly proportional to the amount of fluid given off when the animal later contracted.

Although this last experiment indicated that water was taken up by the foot during expansion, it was realized that obviously the animal must be able to expand to quite a considerable degree without taking up water in order that the foot can be brought into contact with the water.

It was thus decided to see if the animal would expand, or at least protrude the foot through the "mouth" of the shell, on a dry or slightly moist surface. After many unsuccessful attempts, one animal placed on perfectly dry linoleum did expand its foot to assume the normal creeping position and to move about. However, the foot tissue, especially the metapodium, had a flat, thin appearance instead of the rounded oedematous appearance of a normally expanded foot. The propodium was heavily creased, while the edges of the metapodium were curled up. When the animal later contracted, it did so without loss of fluid. Several other specimens behaved similarly when placed on damp blotting paper, damp sand or slightly moist glass. It might be argued that the animals did not expand fully because of the unusual environments of dry linoleum, damp blotting paper, etc., and not because there was no water present to be taken up into the "aquiferous" spaces. However, an animal which has expanded in sea-water and has been placed on either dry linoleum or damp blotting paper will crawl about and react quite normally.

It would thus appear that if water is used in the expansion of the foot it is needed only in the final stages of this process.

Discussion.

The experiments described on the measurement of the fluid given off when the specimens contracted, both with shell intact and with shell removed, together with the observations on shell volume and those on weight change during expansion, indicate that water, in this case sea-water, is taken up during the expansion of the foot, whilst the observations on the expansion of specimens in dry or slightly moist locations show that this water is used only during the final stages of expansion.

However, in the author's opinion it is not possible to be as certain about the matter as Schiemenz was because of the comparatively small volume and weight differences observed in the first experiments described in this paper compared with the large volume differences that he observed. The experiments merely indicate that Schiemenz's statement that water is taken up into the foot during expansion is probably correct and that this uptake occurs to a lesser degree than Schiemenz believed.

It is quite obvious that before a completely accurate picture of the mechanism of expansion can be obtained a full study of the blood system and the mode of circulation will have to be undertaken, in order that the relationship between blood system and "aquiferous" spaces can be fully understood. Schiemenz (1887) did inject the blood system of several specimens of *Natica josephina*, and as a result claimed the existence of a rather special type of blood system. This consisted of a closed system of arteries, capillaries and veins in the foot, completely separated from the water cavities and with none of the sinuses that are usually present in the Molluscan foot. All the histological elements, such as muscles, glands and nerves, were completely surrounded by sheaths protecting them from the sea-water taken in during expansion of the foot. (The water cavities were merely the spaces between the muscles and other tissue elements of the foot. The sheaths surrounding the muscles and glands enclosed a blood sinus (Gewebe sinus) which bathed these elements and received its blood from the capillaries via a sub-epithelial sinus. Because of the comparatively crude histological techniques in use at the time, his injection pictures, and consequently his whole theory of the blood system, must be regarded with doubt.

It will also be necessary to demonstrate definitely the existence of pores opening from the outside into the foot. Although a large series of sections of the foot were made, the author has not been able to demonstrate the existence of any pores opening from the outside into the foot. Obviously pores must be present if water is going to be used in the expansion of the foot. The uptake of water osmotically would be much too slow a process to account for the rapid expansion of the foot of *Uber strangei*. Schiemenz (1884, 1887) claimed to have demonstrated the presence of pores at the junction of the propodium and the propodial "shell-fold" in *Natica josephina*, but his description of the pores was not very convincing—possibly because he found it necessary

to go to great lengths to prove that they *were* pores and not merely rents in the epithelium.

Schiemenz admitted (Schiemenz, 1887) that his theory of the actual mechanism of water-uptake through these pores was weak. It would appear that he propounded his theory because he was unable to demonstrate the existence of an effective pump system or to show that the beating of cilia on the ciliated surface of the epithelium of the foot was sufficient to cause a current of sea-water to pass through the pores into the "aquiferous" cavities. Obviously there must be some better means of ensuring the uptake of water into these "aquiferous" cavities than that described by Schiemenz, but as yet it has not been demonstrated.

As regards the jets of fluid which Schiemenz (1884) described as spraying out from the foot at the point of junction of the propodium and the propodial "shell-fold", similar jets have been observed only once or twice during the course of this work, and Schiemenz himself admitted only having seen them on rare occasions. He was quite sure that these jets were due to water coming out through the pores rather forcibly, but each time the author observed them the animal was contracting rather vigorously, and it seems very probable that they were caused by water or the body fluids escaping through rents formed in the epithelium during violent contraction. It is understandable, of course, that if pores are present along the edge of the propodium, rents are more likely to occur near or around these pores than elsewhere during violent contraction.

There is one aspect of the question which should be stressed. This is in reference to the misleading statements on the subject of turgescence in the Molluscs which appeared in the standard text-books at the beginning of the century, and which are still appearing in almost unaltered form in the modern text-books.

The standard text-books (Lang, 1896; Pelseneer, 1906) side-stepped the issue when dealing with the phenomenon of turgescence in the Molluscs. The fact that these early authors were willing to accept the work of others, even on a controversial subject, is understandable. It was inevitable that, when dealing with such a wide field as the phylum Mollusca, they would strike new and still controversial subjects which, because of lack of time, they themselves could not investigate. It is unfortunate, however, that they did not state the case as it really stood at that time. It perhaps would have been wiser if they had pointed out that, while the forcing of blood into the parts concerned could almost certainly account for the phenomenon of turgescence in the Lamellibranchs, this theory could not be applied unreservedly in the case of the Gastropods because the situation was complicated by the fact that turgescence in the Naticidae had recently been shown to be due to the uptake of water into a series of "aquiferous" cavities.

A survey of recent editions of standard text-books (Borradaile, Potts, Eastham, Saunders, 1935; Kukenthal, 1925; Parker and Haswell, 1940; Thomson, 1944) reveals practically the same attitude to the phenomenon of turgescence in the Molluscs as that held by the authors of text-books at the beginning of the century. Most of them definitely state that turgescence in Lamellibranchs is due to the forcing of blood into the parts concerned, but none of them give an explanation of the phenomenon of turgescence in the Gastropods. A few refer to the "aquiferous" system in the Naticidae—giving no details and without correlating the phenomenon with the state of affairs in other Molluscs—but even this startling exception is mostly ignored.

As a result of this failure to state the case as it really stands, there has grown up among all except specialists in the field the idea that turgescence in *all* Molluscs has been proved to be due to the forcing of blood into the parts concerned. The state of affairs in the Naticidae has consequently either been overlooked by research workers or simply accepted as an exception which does not warrant investigation.

Summary.

(1) The explanation of turgescence and expansion of parts in the Molluscs is briefly considered. It is pointed out that in one small group of Prosobranch Molluscs, the

Naticidae, expansion of the foot is still considered to be due to the uptake of water into a set of "aquiferous" cavities in the foot, whilst in *all* other Molluscs it is universally accepted nowadays that the forcing of blood into the parts concerned is the chief factor in expansion.

(2) Some general notes on the Naticidae are given.

(3) Observations on the amount of fluid expelled during contraction by specimens, both with shell intact and with shell removed, together with observations on shell volume and on weight change during expansion, point to the fact that water is taken up during expansion.

(4) Observations on the partial expansion of specimens on dry linoleum, moist sand, etc., show that this uptake only occurs during the latter stages of expansion and to a lesser degree than was formerly thought.

(5) Two factors leave a doubt in the author's mind. Firstly, the volume of fluid expelled by an *Uber* of a certain size was much less than that observed by Schiemenz for a *Natica* of similar size; secondly, the differences between shell volume and volume of fluid expelled were smaller than those observed by Schiemenz for *Natica*. Possible explanations for these variations are given.

(6) Lines for further research are indicated.

(7) The discussion of the phenomenon of turgescence in text-books dealing solely with the Mollusca, and also in text-books of invertebrate zoology, is considered.

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REDUVIOIDEA FROM NEW SOUTH WALES.

NOTES AND DESCRIPTIONS (HEMIPTERA).

By Petr Wygodzinsky, Instituto de Medicina Regional, University of Tucumán, Argentine.

(*Communicated by J. W. T. Armstrong.*)

(Thirty-four Text-figures.)

[Read 29th March, 1950.]

Introduction.

It is to be supposed that Australia is one of the regions of the Earth least known as to its fauna of the *Reduvioidea*. There is no doubt that the continent possesses many highly interesting elements, the study of which might shed new light on many questions of phylogeny and zoogeography.

It was therefore with great pleasure that we accepted for determination the specimens sent by Mr. J. W. T. Armstrong, of Callubri, Nyngan, New South Wales. All of these have been collected in New South Wales and several species and even genera have proved to be new to science. Our sincerest thanks are due to Mr. Armstrong, whose excellent field work is of great value to Australian entomology.

The present paper, being the second of a series (the first being Wygodzinsky, 1949), contains locality records and notes for some known species, as well as the description of two very interesting new genera, one belonging to the Enicocephalidae, the other to the reduviid subfamily Emesinae. We are obliged to Professor Dr. R. L. Usinger, of Berkeley, California, for information regarding the enicocephalid.

Following Mr. Armstrong's wishes, the types of the new species and certain other specimens are being deposited in the Australian Museum, Sydney. We thank Mr. Armstrong for the kind permission to retain some specimens for our own collection.

Dr. I. Mackenzie Lamb very kindly assisted us in linguistic matters, for which our thanks are due to him.

Family Enicocephalidae.

USINGERIELLA, gen. nov.

Enicocephalinae. Medium-sized, slender species; body surface shining, with sparse delicate bristles, and small setiferous tubercles at certain regions of the body.

Head elongate, constriction behind eyes not very pronounced, the posterior lobe subrectangular, somewhat broader posteriorly than in front. Eyes of moderate size. Ocelli situated laterally at anterior border of posterior lobe of head. Rostrum short and stout, first joint short, second about twice as long, third short again, delicate in lateral view. Antennae not quite as long as head and pronotum together, the first joint shortest, the other three subequal, the last one very faintly spindle-shaped.

Pronotum unarmed, strongly flattened on disc above. Anterior lobe transversely rectangular, with a fovea on disc on either side, anterior and posterior lobes very short, the former subtrapezoidal, the latter transversely band-shaped, shallowly but distinctly emarginate on hind border. Anterior and median lobe with a median longitudinal impression. Pronotum laterally with 1 + 1 carinae beset with numerous small setiferous tubercles. Scutellum broad at base, subtriangular, somewhat pointed posteriorly, its disc flattened, smooth.

Forewings entirely membranous, all veins with slender bristles, which are more numerous at margins of wing. Venation characterized by its closed discal cell, basal cell wanting; stigma not perceptible.

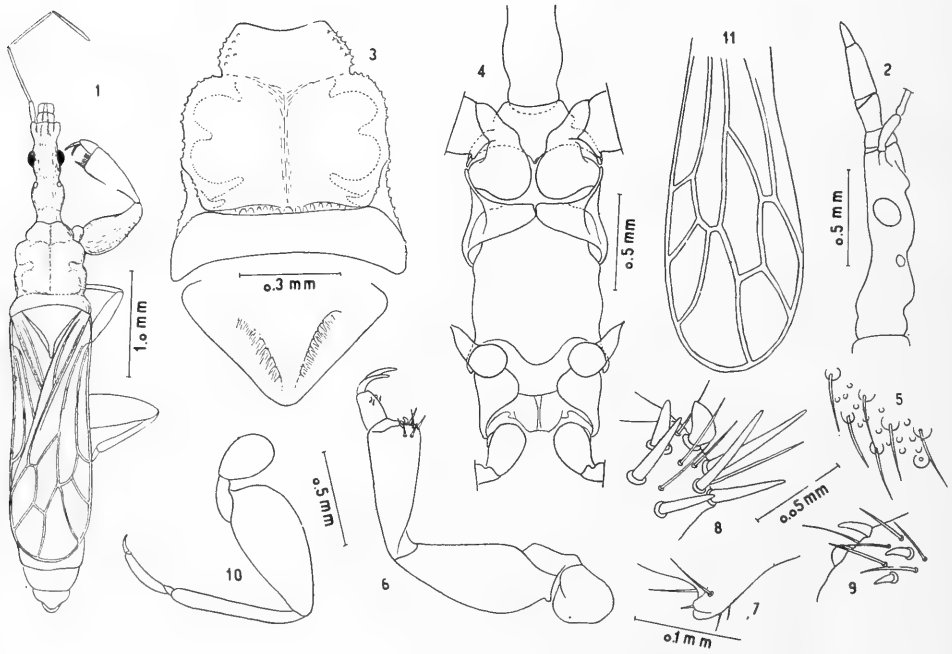
Anterior coxal cavities closed behind. Front femora strongly incrassate, with a small process ventrally near its base. Fore-tibiae strongly dilated. Anterior tarsi one-segmented, with two strong subequal claws. Mid-legs slender and short; hind-femora strongly thickened. Mid- and hind-tarsi two-jointed.

Abdomen without special features. Male genital characters unknown.

Genotype: *Usingeriella boganensis*, sp. n.

We have great pleasure in dedicating this new genus to Professor R. L. Usinger.

It is somewhat difficult to place *Usingeriella* in one of the tribes of the Enicocephalinae adopted by Jeannel (1941), owing chiefly to the impossibility of examining the genitalia of the male; most probably it belongs to the *Systelloderini*. By its wing venation, *Usingeriella* approaches *Nesenicocephalus* Usinger, 1938, from the Pacific region, a genus which was not known to Jeannel (1941). Ours differs from *Nesenicocephalus* by numerous characters, such as the much longer head, the very differently shaped pronotum, the strongly thickened front legs, the posteriorly closed anterior coxal cavities and the presence of small setiferous tubercles on various body regions. We are informed by Dr. Usinger (in litt.) that our new genus also seems to have affinities with the species described by Bergroth (1907) as *Gamostolus tonnoiri*, from New Zealand.



Wygodzinsky del.

Text-figs. 1-11. *Usingeriella boganensis*, gen. nov., sp. n., female, holotype.

Fig. 1, general aspect. Fig. 2, head, lateral view. Fig. 3, pronotum and scutellum, as seen in microscopical preparation. Fig. 4, thorax, seen from below. Fig. 5, setiferous tubercles of pronotum (high magnification). Fig. 6, foreleg. Fig. 7, process at base of fore-femur. Fig. 8, group of spines at apex of fore-tibia. Fig. 9, group of spines at ventral surface of fore-tarsus. Fig. 10, hind leg. Fig. 11, veins of forewing.

USINGERIELLA BOGANENSIS, sp. n.

Female.

Length to apex of abdomen 4·5; maximum width of pronotum 0·75 mm.

Colour of head to base of antenniferous tubercles, as well as pronotum, fusco-testaceous; anterior portion of head, rostrum, antennae, legs, scutellum, abdomen and

membrane testaceous; fore-tarsi and claws ferrugineous; setiferous tubercles dark brown. Eyes and ocelli reddish.

Body surface slightly shining, beset with sparse bristles of moderate length. Setiferous tubercles in small number, present on lateral and ventral surface of posterior lobe of head, ventral surface of fore-trochanter and femur, prosternum (rather numerous), lateral regions of pronotum, anterior acetabula, meso- and metapleura, sides of carina of scutellum and thoracic sterna.

General shape as in Text-fig. 1. Forewings somewhat shorter than extended abdomen.

Head as in Text-figs. 1–2. Eyes relatively small; their distance dorsally about three times their width, seen from above. In lateral view the eye does not attain the dorsal and ventral surface of head. Postocular region of head slightly rounded, about as wide as long. Rostrum as in Text-fig. 2, curved backwards in life. Length of first joint of antennae 0·2 mm.; relative length of joints I–IV = 1:2·7:2·5:2·3. All joints with slender bristles in small number, rather inconspicuous. Last joint slightly spindle-shaped.

Pronotum as in generic description; shape and relative size of the three lobes as in Text-figs. 1 and 3. Suture between mid- and hind-lobe with small shallow excavations. 4 + 4 apodemes visible in microscopical preparation (Text-fig. 3).

Legs as in generic description and Text-figs. 6-10. Process at base of fore-femur as in Text-fig. 7. Spines at inner apical angle of fore-tibia as in Text-fig. 8, five rather slender and two very short ones, one of the latter widened apically; spines of inner surface of anterior tarsus as in Text-fig. 9.

Venation of forewings as in generic description and Text-figs. 1 and 11.

Type: One female, holotype, in the Australian Museum, Sydney.
Locality: Bogan River, N.S.W.* Armstrong coll.

It is to be hoped that the comparison with additional species of the genus will allow us later to establish the truly specific characters of the insect in hand.

Family Reduviidae.

Subfamily Emesinae.

empicoris rubromaculatus (Blackburn, 1889).

Localities: Bogan River, N.S.W., J. W. T. Armstrong coll. (one male, author's collection); Acacia Plateau, N.S.W., J. W. T. Armstrong coll. (one male, in coll. Armstrong, one female, Australian Museum).

This is a cosmopolitan species.

armstrongula, gen. nov.

Rather small, delicate species. Body surface smooth, opaque, not hairy. Adults winged or apterous.

Head very short, rounded behind eyes; ante- and postocular region of about equal size. Ocelli wanting; transversal furrow between eyes present. Ventral surface of head with 1 + 1 rows of numerous long and delicate spines. Rostrum slender, not elbowed, its joints of subequal length; upper (morphologically ventral) surface of first joint with several long spine-like bristles.

Antennae inserted dorsally and laterally at anterior third of anteocular portion of head; antenniferous tubercles rather prominent, stout. Antennae long and very slender, second and third joints shorter than first one, the fourth very short.

Pronotum (in winged and apterous form) constricted near hind margin; not pedunculate. Anterior lobe subovate, convex dorsally, posterior lobe not overlapping mesonotum except at anterior extremity. Superior lobe of anterior acetabula with a forwardly and downwardly directed process. Mesonotum subrectangular. Scutellum simple, subtriangular. Metanotum distinct in winged form, knobbed apically. Hind border of prosternum slightly emarginate.

* Specimens of this insect were taken singly during the spring on the underside of logs, mostly on wet, swampy ground; though recently (November) one was taken in a much drier situation under a board on "red" ground. J. W. T. Armstrong.

Fore-coxa slender, dorsal surface basally and ventral surface apically with some spine-like erect bristles. Femur rather slender, slightly curved at base. Trochanter and femur with regularly arranged spines of various size; those on femur arranged in four longitudinal series, all of which commence near base of joint: one antero-ventral and one postero-ventral series, the former interrupted near base; the postero-ventral series composed of large spiniferous processes, the antero-ventral series composed of small spiniferous tubercles and slender spines. Between these two series there are on the ventral surface two more complete series, composed of smaller spines only. Fore-tibia over half as long as fore-femur and about as long as coxa, straight, rather stout, somewhat widened apically, ventrally with two series of rather long spine-like bristles. Tarsus much shorter than tibia, together with the latter as long as trochanter and femur together; three-jointed, rather heavily chitinized, ventrally with short simple setae, some of them spine-like, practically bare above. Two short simple claws present. Mid- and hind-legs simple, very delicate, the hind-femora surpassing considerably the apex of the abdomen. Mid- and hind-tarsi three-jointed, the joints of about equal size, not strongly chitinized, hairy on all surfaces; claws simple.

Venation of forewing of the *Ploiaria* type, viz., with one large elongate discal cell, at its base laterally a small elongate submarginal cell. Stigma carried almost to apex of forewing.

Abdomen slender, elongate, subfusiform, somewhat widened near middle. Sternites convex, without median carina.

Genotype: *Armstrongula tillyardi*, sp. n.

This very pretty new genus, which we have pleasure in naming for its collector, is nearly related to the African *Tinna*, specimens of which are before us. *Armstrongula* differs from *Tinna* chiefly by the considerable number of spines on the underside of the head, the slender spines on the upper surface of the first rostral joint (absent in *Tinna*) and the four distinct rows of spines on the ventral surface of the fore-femora.

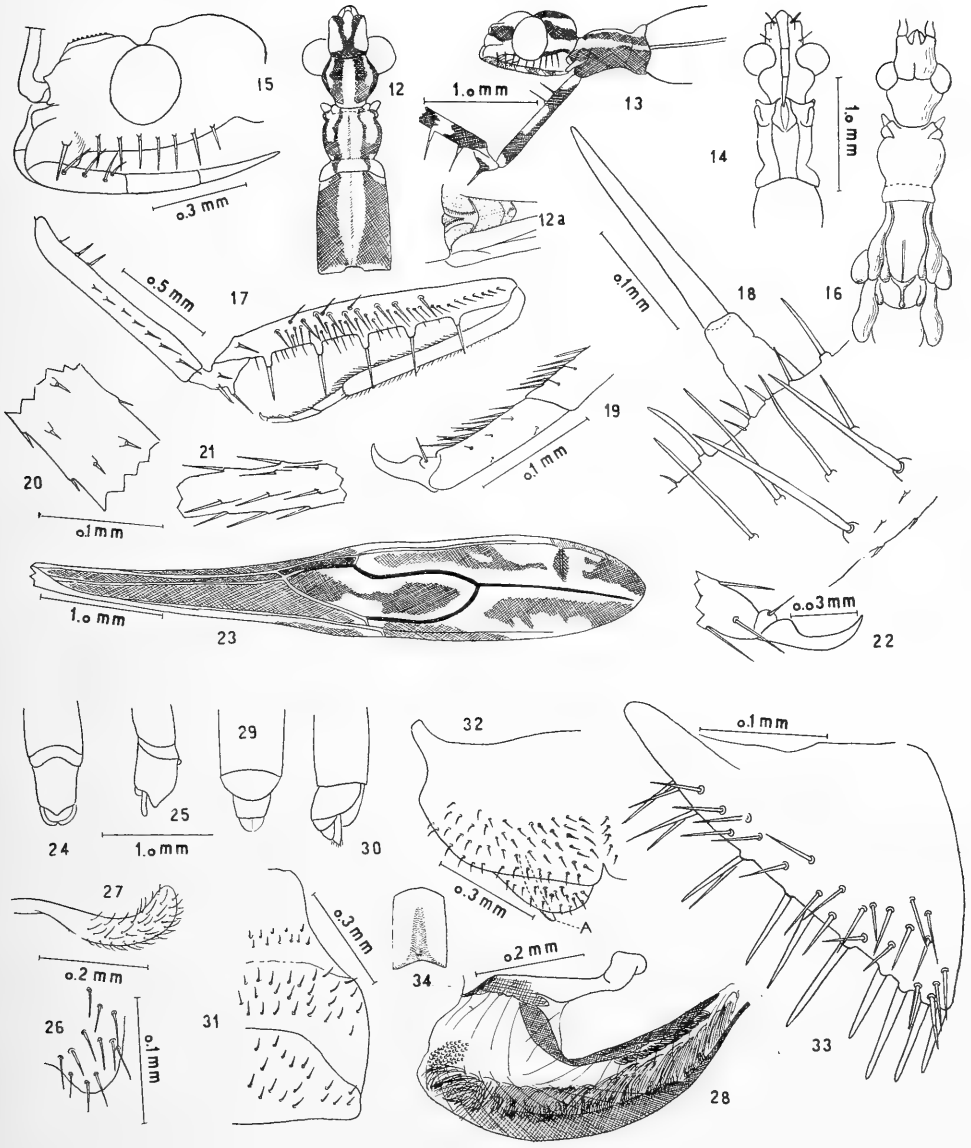
ARMSTRONGULA TILLYARDI, sp. n.

Winged Male.

Length to apex of forewings 7·0–7·5, maximum width of thorax 0·7–0·8 mm. General colour stramineous. The following regions piceous: head dorsally and laterally (Text-figs. 12–13), rostrum with all of the first, apical two-thirds of the second and basal two-thirds of the third segment laterally; all joints of antennae, with exception of one small whitish annulus at base and apex of first and apex of second joint; pro- and meso-thorax dorsally and laterally as in Text-figs. 12–13; metanotum (post-scutellum) with exception of the whitish apex; two large annuli of fore-coxa which join ventrally; ventral half of fore-trochanter; three large annuli of fore-femur, one sub-basal, one submedian and one subapical, these much wider than the intervening whitish spaces; two large annuli of fore-tibia, one sub-basal and one apical; second and third joint of fore-tarsus; meso- and meta-pleura; lower half of median and posterior acetabula; a streak on lower half of median and posterior coxa; median and hind-femur and tibia; ventral surface of abdomen. Ground colour of forewings greyish stramineous, mottled with brownish (Text-fig. 23); veins of anterior half of wing creamy white, on posterior half, piceous. The dark pigment sometimes fading to reddish, the extension of the reddish pigment obviously variable individually.

Surface of head and pronotum minutely wrinkled, of mesonotum very minutely granulous; at low magnification both appear dull. Pubescence absent. Surface of abdomen ventrally as on mesonotum, beset sparsely with very short bristles that become somewhat more numerous towards apex.

Head as in Text-figs. 12–14; ante- and post-ocular region of about equal length. Transverse constriction between eyes only faintly curved. Eyes large, dorsally about as wide as interocular space, ventrally somewhat more approximated; in lateral view the eyes attain ventral and dorsal surface of head. Antenniferous tubercles stout. Ventral surface of head laterally with 1 + 1 rows of 7–8 ventrally directed slender spines inserted at regular intervals (Text-fig. 13, and as in Text-fig. 15). Rostrum shining,



Wygodzinsky del.

Text-figs. 12-34. *Armstrongula tillyardi*, gen. nov., sp. n.

Fig. 12, head and thorax of male, seen from above. Fig. 12*a*, scutellum and metanotum of same. Fig. 13, same, lateral aspect. Fig. 14, same, seen from below. Fig. 15, head of female, lateral view. Fig. 16, head and thorax of female, seen from above. Fig. 17, foreleg of male, antero-lateral view. Fig. 18, detail of fore-femur. Fig. 19, detail of fore-tarsus, one claw shown only. Fig. 20, detail of hind-femur. Fig. 21, detail of apical portion of hind-tibia. Fig. 22, hind-pretarsus with one claw shown. Fig. 23, forewing of male. Fig. 24, genital region of male, seen from above. Fig. 25, same, lateral view. Fig. 26, posterior process of male hypopygium, with its bristles, high magnification. Fig. 27, clasper. Fig. 28, aedeagus, lateral view. Fig. 29, genital region of female, seen from above. Fig. 30, same, lateral aspect. Fig. 31, posterior tergites. Fig. 32, seventh sternite, with lobe of eighth sternite and anterior gonapophysis. Fig. 33, posterior gonapophysis (high magnification). Fig. 34, tergite V of female.

slender, only very faintly curved at base, its joints of about equal length (Text-fig. 13); first joint on upper surface with two rows of four upwardly directed spines each. Antennae very slender, without distinct pilosity; length of first joint 6·0 mm.; relative length of joints = 1:0·58:0·47:0·17.

Pronotum distinctly shorter than head, about as wide as long, its sides rounded, its hind margin emarginate; antero-lateral processes short, truncate apically; slightly convex above, its posterior two-thirds with a delicate median longitudinal furrow. Lateral aspect as in Text-fig. 13. Ventral aspect of prothorax as in Text-fig. 16; apparent posterior border emarginate. Mesonotum dorsally as in Text-fig. 12, longer than pronotum, its sides subparallel; slightly convex above, its median longitudinal furrow extending for its whole length; hind margin almost straight, sharply excavate at centre. Mesonotum laterally with two subparallel carinae. Scutellum and post-scutellum as in Text-fig. 12*a*; the former semicircular, its centre elevated longitudinally, the elevation bifid at anterior margin. Disc of metanotum flattened, its apex tuberculiform, somewhat elevated.

Forelegs as in generic description and Text-figs. 17–19. Coxa dorsally at base with 2–3 spines, its posterior half on antero-ventral surface with a series of about eight spiniform bristles. Trochanter with 2–3 spines, the largest one strongly inclined, inserted upon a stout ventral process. Femur more or less parallel-sided; postero-ventral series beginning at base of joint, composed of five stout setiferous processes on which there are inserted strong spines, the latter being distinctly longer than the diameter of the joint. Antero-ventral series composed of one basal spine, inserted near base of joint, and 8–9 additional ones, which are much shorter and more delicate than those of the other series. The two intermediate series composed each of about 25–30 strong bristles of variable size, several of them being distinctly lateral of the principal row. Tibia and tarsus as in generic description and Text-figs. 17 and 19. Median and posterior legs simple, slender, posterior femur surpassing considerably apex of abdomen; bristles of tibia and femur stout and short (Text-fig. 20), those of apical portion of tibia slender and much longer (Text-fig. 21). Length of median femur 6, posterior femur 8·8, median tibia 9·1 and posterior tibia 10·2 mm. Median and hind-tarsi and claws as in generic description; pretarsus and claws as in Text-fig. 22.

Forewing attaining apex of abdomen, its venation and pattern as in Text-fig. 23; large discal cell a little longer than apical vein.

Abdomen elongate, slender, widest at middle. General aspect of genitalia as in Text-figs. 24–25. Hypopygium beset with rather numerous short bristles, apically with a very short rounded process (Text-fig. 26). Clasper slightly curved, not strongly chitinized apically, beset with numerous bristles of moderate length (Text-fig. 27). Aedeagus elongate, with a rather complex internal structure (Text-fig. 28).

Apterous Female.

Rather similar to male, which makes a detailed description unnecessary.

Length 7·0 mm. Pattern much as in male, dark pigment less extensive; abdominal sternites stramineous to whitish, darker near sides and hind border. Tergites stramineous, with an elongate median longitudinal stripe of subtriangular shape that does not attain the anterior margin of the segment (Text-fig. 34).

Eyes much smaller than in male (Text-fig. 15), their distance dorsally corresponding to a little less than twice their width. In lateral aspect the eyes do not attain dorsal or ventral surface of head. Meso- and meta-notum as in Text-fig. 76; the former with a median longitudinal furrow behind, the latter with a median longitudinal carina which is somewhat widened behind. Wing pads of fore- and hind-wings distinct, but very small (Text-fig. 16).

Abdominal tergites somewhat longer than wide, slightly emarginate behind, at hind margin in centre with a small but distinct tubercle which is not included in the dark stripe mentioned above (Text-fig. 31). General aspect of genital region (difficult to make out) as in Text-figs. 29–30. Seventh sternite (Text-fig. 32) with 1+1 subrectangular antero-lateral processes; posterior border indented in centre. Lobes of

eighth sternite very short, rounded. Anterior gonapophyses reduced to small chitinized rods (Text-fig. 32, A). Median gonapophyses (not figured) very delicate, of the usual fan-shaped type. Posterior gonapophyses (Text-fig. 33) well developed, subtriangular, their disc anteriorly with several short and stout bristles, their hind margin with some large and heavily chitinized spines.

Types: One male, holotype, one female, allotype, Australian Museum; one female, paratype, in coll. Armstrong; one male, one female, paratypes, author's collection.
Locality: Bogan River, N.S.W., J W. T. Armstrong coll.

The species is dedicated to the memory of that great Australian entomologist R. J. Tillyard.

Subfamily Holoptilinae.

ptilocnemus lemur Westwood, 1840.

Localities: Bogan River, N.S.W., J. W. T. Armstrong Coll. (one male, author's collection); Inverell, N.S.W., Armstrong coll. (one female, author's collection; one male, one female in coll. Armstrong; one female, Australian Museum).

This pretty species is rather common in Australia. Its biology is not well known and it would be worth further study.

Subfamily Stenopodinae.

sastrapada australica Stal, 1874.

Locality: Acacia Plateau, N.S.W., Armstrong coll. (one male, author's collection).

This specimen corresponds well to Stal's description, differing only in its slightly larger size (12·5 mm.).

Subfamily Reduviinae.

archilestidium ornatulum Breddin, 1900.

Locality: Acacia Plateau, N.S.W., J. W. T. Armstrong coll. (one male, one female, author's collection; two males in coll. Armstrong; one male, Australian Museum).

This is the genotype; only one additional species has been described (*cinnabaricum* China, 1925, from Victoria).

Subfamily Harpactorinae.

endochus (pnirsus) cinctipes Stal.

Locality: Inverell, N.S.W., Armstrong coll. (one female, author's collection).

The species was described from "Australia borealis". Though we have not seen the type, we are fairly sure of our determination, owing to the complete agreement of the specimen before us with the original description.

nyllius Stal, 1859.

Nyllius Stal, 1859, Oefv. K. Vet.-Akad. Foerh., 16 (8): 365.
Nyllius Stal, 1868, Kongl. Sv. Vet.-Akad. Handl., 7 (11): 98.
Nyllius Stal, 1874, Kongl. Sv. Vet.-Akad. Handl., 12 (1): 42.
Orgetorixa China, 1925, Ann. Mag. Nat. Hist., 9 (11): 486.

There is nothing in China's description that would differentiate his *Orgetorixa* from Stal's *Nyllius*. It is possible that China's only species, *australicus*, is different from *Nyllius asperatus* Stal, considering its much larger size. We therefore maintain China's species for the time being.

nyllius australicus (China, 1925).

Locality: Acacia Plateau, N.S.W., J. W. T. Armstrong coll. (one female, author's collection, one female in coll. Armstrong).

Summary.

The author gives distributional and synonymical notes on some *Reduvioidea* of New South Wales. In the Enicocephalidae there is described *Usingeriella boganensis*, gen. nov., sp. n., apparently related to *Nesenicocephalus* Usinger, 1939, from the Pacific

(Hawaii and the Philippines). In the Reduviidae there is described *Armstrongula tillyardi*, gen. nov., sp. n., an emesine related to the African *Orthunga* Stal. The harpactorine *Orgetorixa* China, 1925, is considered a synonym of *Nyllius* Stal, 1859. Distributional notes on some other reduviids are given.

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THE HAIR TRACTS IN MARSUPIALS.

PART IV. DIRECTION CHARACTERISTICS OF WHORLS AND MERISTIC REPETITION OF RADIAL FIELDS.

By W. Boardman.
(Department of Zoology, University of Melbourne.)

(One Text-figure.)

[Read 26th April, 1950.]

Introduction.

An analysis of tract pattern as exhibited by the collection of marsupial pouch young on which this investigation is based throws into relief the importance of the radial field (convergent and divergent) and its whorled variant as a factor in pattern formation and pattern evolution. The results of this analysis will be discussed subsequently. In the present contribution a group of generalizations is submitted relative to some attributes of these fields.

Direction Characteristics of Whorls.

Both divergent and convergent radial fields often appear as the familiar divergent (centrifugal) and convergent (centripetal) whorls. The direction of whorling may be either clockwise or counter-clockwise, using the terms as Schwalbe (1910, 1911) defined them. Whorling of a radial field may occur invariably at a particular site as with the genal convergence found in the genus *Wallabia*, or only infrequently as in the case of the commonly present inter-ramal median convergence. Median divergent radial fields in the marsupial groin, but not elsewhere, appear always to be free of whorling; bilateral pairs of radial fields in the same situation are constantly present in whorled form. Sometimes whorled and unwhorled conditions may alternate in the species, as with the occipital divergent whorl of *Trichosurus vulpecula*.

The direction of whorling is found to conform to definite rules having applicability to the species in the case of median unpaired structures but to the order Marsupialia as a whole for bilateral pairs.

Median Divergent Whorls.

Median divergent whorls occur both on the dorsal and ventral surfaces. Dorsal whorls, mostly situated in the cranial half of the body, are characteristic of the Macropodinae and Phascolarctidae, and are also found in some of the Phalangerinae. A ventral whorl at the root of the throat is present in *Vombatus hirsutus*. The strong, nearly median whorl in the groin of *Onychogalea fraenata* (Boardman, 1946, Fig. 39) is difficult to interpret considering the certainly abnormal arrangement of the surrounding fields: it is probably morphologically equivalent to the whorl found in the Macropodinae (except *Dendrolagus*) on each side behind the knee.

The Dorsal Surface. A survey of the material indicates that the directional character, clockwise or counter-clockwise, of dorsal median whorls is constant within the limits of the species. The series of *Trichosurus vulpecula vulpecula* and of *Phascolarctos cinereus*, in which the median dorsal whorled system is clockwise and counter-clockwise respectively, may be quoted as examples. The material available does not present any exceptional cases, though their occurrence might be anticipated in view of the statistical studies carried out on the crown whorl of man (Schwarzburg, 1927),

where a clockwise condition has been shown to be genetically dominant to the less frequently occurring counter-clockwise form. The fact that nearly related species of the same genus (*Trichosurus vulpecula* and *fuliginosus*, and *Wallabia agilis* and *bicolor*, for instance) may show opposite directions of rotation supports this possibility.

The Ventral Surface. There is insufficient evidence to contend that direction of rotation is similarly constant for the species in the case of median whorls on the ventral surface, since only a single specimen of Vombatidae, *Vombatus hirsutus hirsutus*, has been recorded as possessing the medially placed divergent whorled system. Considering, however, the clear rules that exist for paired whorled systems on both the dorsal and ventral surfaces (*v. infra*) it seems unlikely that species constancy for direction is not applicable equally to both the dorsal and ventral surfaces when the whorl is single and medial.

Median Longitudinal Pairs of Whorls. Occasionally a median whorl may appear doubled in a longitudinal direction. The phenomenon has not been observed in marsupials other than in *Trichosurus vulpecula* and *T. fuliginosus*.* In two of the *vulpecula* series an additional whorl occurs on the vertex in front of the normal whorl between the ears; its direction in both specimens is counter-clockwise, that is, opposite to its partner. In one of the two examples of *fuliginosus* a median longitudinal pair is substituted for the single whorl; the additional whorl, in this case apparently the hinder one, has the same direction of rotation as its partner (see Text-figure 1). No explanation can be offered for this anomaly.

MEDIAN CONVERGENT WHORLS.

Median convergent radial fields frequently show whorling in three situations—on the crown (as in the Macropodinae), in the inter-ramal position (as in *Trichosurus vulpecula*), and at or in the vicinity of the umbilicus (as in *Sarcophilus harrisii*). Where two or more specimens of a species show whorling of a given convergent field only similarity in direction characteristics has been recorded. In *Sarcophilus harrisii*, for example, the umbilical convergence is, in a litter of four females, clockwise. While the evidence is not as ample as in the case of median divergent whorls, a like rule—species constancy of direction of rotation—would seem to hold.

BILATERALLY PAIRED DIVERGENT WHORLS.

Rules for direction are applicable to bilateral pairs of whorls wherever they occur on the body. In the Marsupialia separate rules are determined for the dorsal and ventral surfaces.

The Dorsal Surface. There are only three locations on the dorsal surface where bilaterally paired divergent whorled systems develop. Considering these from before backwards, the first will be the small whorls immediately behind the caudal margin of the rhinarium that give rise to the well-known rhinal reversal present in some phalangers and in all except one (*Macropus major*) of the Macropodidae before me. The second site is the occiput and neck region, as in some Macropodinae, where, however, it is commoner to find the whorled system single and medial (the relationship between single and double whorled systems in the same location is discussed below). Finally, paired whorls may be present in the sacral region; *Phascolarctos* is the only marsupial recorded as having them. Divergent radial systems are frequent at the median angle of the eye but they appear not to be subject to whorling. In such bilateral pairs of whorls the two members have the relationship of mirror images the one to the other and the whorl on the left side is counter-clockwise. No exceptions to this rule were observed.

The Ventral Surface. As with dorsal paired systems, bilaterally paired whorls on the ventral surface have the relationship of mirror images. However, in contradistinction to the direction of rotation on the dorsal surface, the left member of a ventral pair is clockwise when viewed from the ventral aspect. Here also there are

* Compare, however, repetition (which may be simultaneously longitudinal and lateral) of the dorsal whorl in *Phascolarctos cinereus* (*v. infra*).

only three situations for the occurrence of the whorls—on the throat or cranial part of the thorax (Peramelidae, Tarsipedinae, Vombatidae, Potoroinae), in the axilla or on the upper arm adjacent (Phalangerinae, Phascolarctidae, Macropodinae), and in the groin (Thylacininae, Peramelidae, Macropodinae). A single exception has been noted in the axillary whorls of *Onychogalea fraenata*, which have the opposite direction of whorling.

BILATERALLY PAIRED CONVERGENT WHORLS.

Convergent whorls having the relationship of bilateral pairs (mirror images) are found only infrequently in marsupials. On the dorsal surface they do not occur elsewhere than in the vicinity of the lateral canthus (some Phalangerinae, some Macropodinae). On the ventral surface a pair is present one on each flank of *Thylacinus* and *Thylogale*. The present material would seem to point to the same directional rules holding for both convergent and divergent systems, that is, on the dorsal surface the left member of a pair is counter-clockwise, on the ventral surface it is the right member that is counter-clockwise. The only exception is in the whorl between the eye and ear in *Pseudocheirus*; on the left side it is clockwise and not counter-clockwise, as might be expected from a position so near to the mid-dorsal line.

The genal convergent radial field of some Macropodinae appears invariably to be whorled, the direction of rotation being in accord with the rule for the dorsal surface. A single specimen of the *Trichosurus vulpecula vulpecula* series (Boardman, 1946, Fig. 19) shows this whorl as an abnormality and conforming to the rule for the ventral, not the dorsal, surface.

THE CASE OF SUPERNUMERARY (ABNORMAL) WHORLS.

Isolated single whorls additional to the normal complement of the species may occur in the marsupial skin. Such whorls may be convergent or divergent; occasionally both types are found in a single individual (see, for example, *Perameles gunnii* (Boardman, 1946, Fig. 11). Certain individuals seem particularly subject to development of these supernumerary structures, the majority of examples being recorded from two specimens: the bandicoot *Perameles gunnii* already mentioned, and a wallaby, *Onychogalea fraenata* (Boardman, 1946, Fig. 39). The direction characteristics of supernumerary whorls situated to the right or left of the middle line support the validity of the rules enumerated above, that is, they most frequently conform to the rules for the position occupied as if they were members of bilateral pairs. Of eleven such whorls only one is exceptional in this respect.

LIMB WHORLS.

In *Phascolarctos cinereus* and in two macropods, *Thylogale* sp. and *Onychogalea fraenata*, whorls develop on the hind-limbs. *Phascolarctos* has only a single whorl (clockwise on the left, counter-clockwise on the right limb) situated medially just above the ankle. The two macropods, each represented by only a single specimen, display a pair of whorls distally on the shank, one placed on its lateral and one on its medial aspect. *Thylogale* sp. has the left lateral whorl clockwise, the right counter-clockwise, the left medial whorl counter-clockwise, the right clockwise. For each corresponding whorl site in *Onychogalea fraenata* the opposite direction of whorling holds. The directional constancy of the whorls in the *Phascolarctos* series indicates that direction of rotation of limb whorls is probably species constant. The data provided by the three species taken together suggest that the direction of whorling on the hind-limbs is independent of that of whorled structures of the trunk.

Whorls occur medially on the forelimbs in *Thylogale* sp. and *Macropus major*. *Thylogale* has the whorl on the forearm, but in *Macropus major* it lies proximal of the elbow. In both the direction of whorling conforms to the rule for the ventral surface of the body—clockwise on the left, counter-clockwise on the right. This is not coincidence and might in fact be anticipated by a consideration of the homologies of these particular whorls, concerning which there seems little doubt that in both

cases they are to be classified as laterally displaced axillary whorls. Axillary whorls occur in the Macropodinae except in the genus *Dendrolagus* and in the two species having forelimb whorls.

THE RULES ESTABLISHED FOR OTHER MAMMALIAN ORDERS.

Schwalbe (1911) has summarized his findings in the Cercopithecidae and Simiidae. In these two families of Anthropoidea it appears that on the vertex, belly and nape whorls are clockwise on the right side of the body, counter-clockwise on the left side; on the side of the head, the breast, side of the abdomen and on the elbow the opposite holds. The rules cover both centrifugal and centripetal types of whorls. Voigt (1857) has enunciated a similar rule for man.

In man attention has further been concentrated on the crown or so-called occipital whorl, and several studies of its distribution both in the single and bilaterally double condition have appeared. Gates (1946) has summarized the work in this field. The contribution of Schwarzburg (1927) on school children in Germany may be taken as typical of the results obtained. Predominantly the whorl is medial and clockwise; cases in which it was counter-clockwise were only a quarter as frequently encountered. Of all cases examined, 7% had the whorled system bilaterally double, the majority having the left member clockwise and the right counter-clockwise.

Meristic Repetition of Radial Fields.

Bateson (1894) has classified an immense number of cases of serial duplication of parts which he differentiated from other fluctuations in structure under the term *meristic variation.* The term is applicable to a group of cases in the marsupial material before me in which radial fields are, in a minority of specimens of some species, duplicated either laterally or longitudinally. The duplication, when it occurs, is independent of whether the radial field involved is or is not whorled in the single condition.

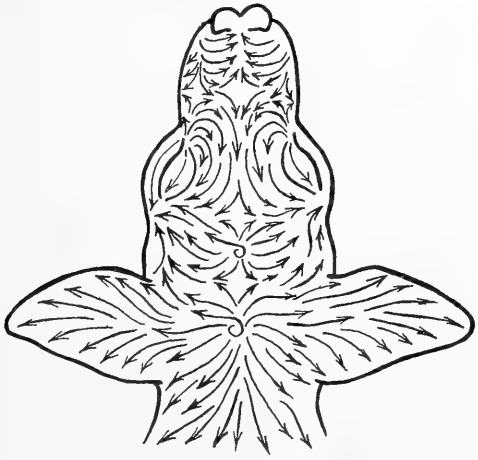
DUPLICATION OF DORSAL DIVERGENT FIELDS IN THE DIPROTODONTIA.

A median dorsal whorl is characteristic of the Phascolarctidae and Macropodinae, and is also found in some of the Phalangerinae. The whorled system is most usually situated on the nape or occiput, less frequently between the shoulders, and occasionally, as in the genus *Dendrolagus* (Rothschild and Dollman, 1936), at the middle of the back or near the root of the tail. When the whorl occurs between or cranial of the shoulders it is subject to repetition which may be transverse or longitudinal or, more rarely, in both directions in one and the same specimen.

The case of the koala, *Phascolarctos cinereus*, illustrates the phenomenon in its simplest and also in its most complex expression. The hair tracts of ten specimens have to date been recorded in the literature. Of these, seven agree with the original account of Wood Jones (1923) in that there is present a single counter-clockwise whorl in the mid-dorsal line between the attachments of the forelimbs; one specimen (Boardman, 1943*b*) has the dorsal whorled system duplicated so that a bilateral symmetrical pair is substituted; two specimens (Boardman, 1943*a*) have instead of the single median whorl a series of three bilateral pairs, of which the middle pair would appear to correspond to the single median structure.

A bilaterally doubled condition of the same whorl is apparently common in the Macropodinae (*Osphranter robustus* and *Wallabia dorsalis*, for example). As only a single specimen is available of the species showing the double condition it cannot be said whether or no the single and double forms of the whorled system are alternatives for the species, as in *Phascolarctos cinereus*. No case can be cited of a macropod with longitudinal doubling of the whorl. In *Macropus major*, where two dorsal divergent centres do in fact occur in the middle line of the back, there seems little doubt, in view of the distance separating the centres of the two fields, that the hinder of the two is a new development in the genus.

In the Phalangerinae the dorsal whorl appears not to be subject to lateral twinning but in two species longitudinal duplication occurs. Thus two of the series of *Trichosurus vulpecula* and one of the two examples of *T. fuliginosus* have the occipital whorl longitudinally doubled. In *T. vulpecula* the additional whorl has a rotation direction opposite to that of the partner, but in *T. fuliginosus* the two whorls are similarly orientated (Text-figure 1).



Text-figure 1. Longitudinal doubling of the median dorsal divergent whorl in *Trichosurus fuliginosus*. Both whorls have the same direction characteristics.

Except in the case of *Trichosurus vulpecula* duplication, whether lateral or longitudinal or both simultaneously, produces pairs of whorls which conform to the rules for direction of rotation on the dorsal aspect of the body (*v. supra*).

DUPLICATION OF THE GULAR DIVERGENT FIELD IN *Vombatus hirsutus*.

A mid-ventral whorled field occurs at the root of the neck of *Vombatus hirsutus hirsutus* and has the same capacity for longitudinal duplication described above for dorsal systems in the Phalangerinae. The case for longitudinal doubling rests on a single specimen of the subspecies (Boardman, 1943*a*). No comment can be made on the directional relationship of ventral longitudinal pairs, since in this single specimen displaying them they are in the form of unwhorled divergent fields.

Bilaterally paired whorls, presumably homologous with this gular whorl, occur in both subspecies of *Vombatus ursinus*, but as the ventral surface is known from only a single specimen in each case it is not possible to state whether or no the doubling is a variable feature of the species.

INGUINAL DIVERGENT FIELDS IN *Thylacinus cynocephalus*.

The male of *Thylacinus cynocephalus* shows a well-developed unwhorled divergent radial field having the scrotal stalk at its centre. In the female a bilateral pair of whorled fields occurs (Boardman, 1945); the left member is clockwise, the right counter-clockwise. This difference can be interpreted in one of two ways: it either represents an example of meristic repetition or of sex-dimorphism. Sex-dimorphism of hair tracts is a phenomenon of extremely restricted occurrence and is not known to be concerned with whorl duplication. It seems most likely, then, that the condition in the female *Thylacinus* is a further case of meristic variation.

EVOLUTIONARY SIGNIFICANCE OF DUPLICATION OF RADIAL FIELDS.

Most of the radial fields, both diverging and converging, and their whorled variants described for marsupials in this work occur either as a single median system or duplicated. The alternative conditions may, in the case of certain fields (*v. supra*), be

present among the members of one and the same species. Meristic repetition of this kind is probably commoner than the cases cited would indicate. In the Macropodinae, for example, both the single and duplex conditions of the nuchal whorl occur in the related species of the genus *Wallabia*, but the material includes only one example of each species. It is likely that in such related groups examination of series would provide further cases. It must not be assumed, however, that alternative forms of radial fields within the species limits are at all general. A survey of the material points to there being no grounds for doubting that these fields, whether in the single or doubled condition, are constant in character in the majority of species.

Two facts emerge when the gross distribution within a group (say family or subfamily to take an arbitrary unit) of single and double versions of the same radial system is considered. Firstly, for any particular field presenting these two alternatives there are more species with the single than with the double condition. The inguinal radial field system in the Peramelidae and the nuchal whorl in the Macropodinae may be cited as illustrating this point. Further, in the cases of meristic repetition so far recorded the duplex condition affects only a minority of the members of a species. Secondly, there is a marked tendency within a group where both conditions exist for the single condition to have a greater frequency of occurrence in the lower members. Thus *Perameles gunnii* alone among the peramelids examined has regularly a duplex condition of the inguinal whorled system. Bensley (1903) has stated that this species is "undoubtedly a derivative of *nasuta*"; it must, then, represent a relatively advanced stage of evolution within the Peramelidae. The large wallabies (*Wallabia* spp.) represent a later phase of evolution than the smaller Macropodinae (Bensley, 1903). It is in these larger species that the nuchal whorl may appear as a double system. It is significant, too, that meristic repetition is most commonly and probably only found at higher evolutionary levels. *Thylacinus*, *Trichosurus* and *Phascolarctos*, for example (*v. supra*), all represent terminations of evolutionary trends in modern marsupials.

Taking these facts together, it would seem highly probable that of the two expressions of a radial field, single and doubled, the single condition is the more primitive, and that generally, doubling, whether it be a constant or occasional feature of a species, coincides with a later stage of marsupial evolution.

Summary.

1. The direction characteristics (clockwise or counter-clockwise) of median divergent whorls on the dorsal surface are constant for the species. This is probably true also of median divergent whorls on the ventral surface and of median convergent whorls on both dorsal and ventral surfaces.

2. Bilaterally paired whorls have the relationship of mirror images the one to the other. On the dorsal surface the left whorl is counter-clockwise; on the ventral surface it is clockwise. This appears to be true for both convergent and divergent systems.

3. Supernumerary whorls conform to the direction rules for their situation as though they were members of bilateral pairs.

4. The direction of rotation of whorls on the limbs is probably constant for the species, but is independent of that of whorled structures on the trunk.

5. The phenomenon of meristic repetition of the radial field, frequent in marsupial material, is discussed. Where duplication occurs the pairs of whorls conform to the directional rules for the dorsal and ventral surfaces respectively.

6. It is considered on the available evidence that, in general, the single median radial field represents the primitive condition, the equivalent duplex system being a later development in pattern evolution.

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A HYBRID EUCALYPTUS.

By L. D. Pryor, M.Sc., Dip. For.

(Plates III and IV.)

[Read 26th April, 1950.]

A good deal of interest has been created from time to time in the possibility of the occurrence of natural hybrids of *Eucalyptus*. Ewart (1930) states: "the occurrence of hybrids between certain species is well established". Mueller (1882) says: "Natural cross-fertilization of flowers only exceptional and then partial, artificial hybridization easy." Further, Mueller (1884) says: "Hybridism does not seem to explain the origin of these aberrant forms in a genus where cross-fertilization is guarded by a calycine lid."

Baker and Smith (1902) support this view in the following statements:

". . . it appears difficult to understand how natural hybridization pertains in the origin of Eucalyptus species, the essential organs being protected by an operculum, and in almost every instance pollen grains are found adhering to stigmas before the operculum falls off." . . . "Cross-fertilisation in the case of eucalypts is, in our opinion, quite exceptional. . . ."

In the same connection Maiden (1924) says: "I have tested all the following points as regards *E. vitrea* . . . and its characters are possessed in about equal proportions by *E. coriacea* and *E. amygdalina*. I think I have produced sufficient evidence to show that my suggestion as to the hybrid character of *E. vitrea* is a very reasonable one. That hybridization occurs in the genus and that there is much evidence of it, I consider to be absolutely proved."

The general difficulties associated with the classification of the genus Eucalyptus have been mentioned in several places by various writers. This has been partly responsible for the suggestion that hybrids are of frequent occurrence on the one hand, and for the relatively small amount of reliable information which has been published concerning their existence or otherwise, on the other hand. In making an examination of any individual plant, it is always necessary to reject the tendency to describe an individual as of hybrid origin simply because it has characters which are intermediate between those of other known groups. It is true this is one of the characters which many hybrids may be expected to possess, but in itself it is only one of the factors to be considered in establishing the hybrid origin of a given individual. The determination of a specimen as a hybrid between two established species may be supported by evidence in the following way:

1. From morphological characters, as described above, its position may be indicated as intermediate in some degree between the putative parents.
2. Evidence relating to its field occurrence.
3. Evidence of its genetic constitution deduced by breeding or examining progeny from the individual concerned.
4. The synthesis of a similar type by controlled hybridizing.

There are few, if any, cases for which information on all of these aspects is available. From Maiden's comments on *E. vitrea*, and subsequent examination, it is known that individuals occur in several areas which present considerable variation and which have, with some reserve, been referred to *E. vitrea* R. T. Baker. There are several forms which are thus described occurring on the Southern Tablelands, and one of them has been examined in some detail. This tree has morphological characters which are intermediate between *E. dives* Schauer and *E. pauciflora* Sieber. Firstly the bark, while characteristically of the persistent Peppermint kind at the butt, does not extend far into the larger limbs which become smooth as *E. pauciflora*. The fruits are intermediate in

size between *E. dives* and *E. pauciflora* and the leaves have venation which is somewhat more longitudinal than *E. dives* but less so than *E. pauciflora*, while again the texture of the leaves is somewhat coarser than that of *E. dives* but not so coriaceous as that of *E. pauciflora*. The peduncle also is shorter than *E. pauciflora* but longer than *E. dives* and the wood, macroscopically, shows the same intermediate position, being lighter in colour than *E. dives* but darker than *E. pauciflora*, apart from other intermediate characters. In short, in the major botanical features, specimens are found which are intermediate in several important morphological characters between these well-established species.

Trees of this particular kind are found fairly widely spread in many parts of the Southern Tablelands south of Goulburn and Canberra. Trees are frequently youthful, possibly around 40 to 50 years old, and they are invariably found in a site which is the junction between *E. pauciflora* and *E. dives*. This distribution is fairly frequent in some parts of the Tablelands where stony ridges adjoin cold plains. The tree is never found as a distinct population, but always as an odd specimen among either *E. dives* or *E. pauciflora*. From a specimen such as this, seed was collected. This was open-pollinated and presumably, as it was from an isolated tree, was either selfed or back-crossed with one or other of the putative parents, viz., *E. dives* Schauer and *E. pauciflora* Sieber. Progeny secured from this seed has shown considerable segregation of characters and has resulted, after three years, in the production of young plants (only 16 were preserved) ranging from forms very close to *E. pauciflora* to forms close to *E. dives*. As the juvenile foliage of *E. dives* carries broadly elliptical or ovate leaves for an indefinite number of pairs, and that of *E. pauciflora* changes after a few pairs (3–5) to the alternate condition, a very good character is available for estimating degree of segregation. Characters of venation, stem colour, leaf texture, also correspond with the other characters apparent in the segregates.

The synthesis of such a hybrid has not yet been achieved experimentally, but the evidence available suggests strongly that this form described as *E. vitrea* is, in fact, a naturally occurring hybrid between *E. dives* and *E. pauciflora*. It may be added that there is no general difficulty in supposing that such a hybrid could be formed as firstly the two species are relatively closely related in their morphological characters, the cross is not a wide one within the genus, they occur naturally side by side in the field, and they also flower together in early spring.

The occurrence of isolated specimens only and the fact that many of the trees are approximately the same age, suggest perhaps the extensive regeneration which followed heavy land clearing and ringbarking some 50 or 60 years ago, may have released many seedlings and caused a mass regeneration which would permit the survival here and there of occasional hybrids, as has been suggested by Brett (1949). Assuming this hybrid is of the F_1 generation, the absence of extensive natural populations of segregates between the apparent parents is simply explained by the fact that up to the present date there has been practically no regeneration under sheep grazing conditions since the one which initially followed the first heavy clearing.

The figures (Plates iii and iv) show firstly the intermediate characteristics in leaf structure and fruits and buds in a naturally occurring tree, compared with the putative parents, and also the type of variation obtained in the leaves of progeny from one of these trees.

Summary.

Evidence from field occurrence, morphological affinity and open-pollinated progeny tests is produced which strongly indicates the natural occurrence of a hybrid between *Eucalyptus pauciflora* Sieber and *Eucalyptus dives* Schauer, in the Australian Capital Territory and Southern Tablelands of New South Wales. The hybrids have been previously referred to *Eucalyptus vitrea* R. T. Baker.

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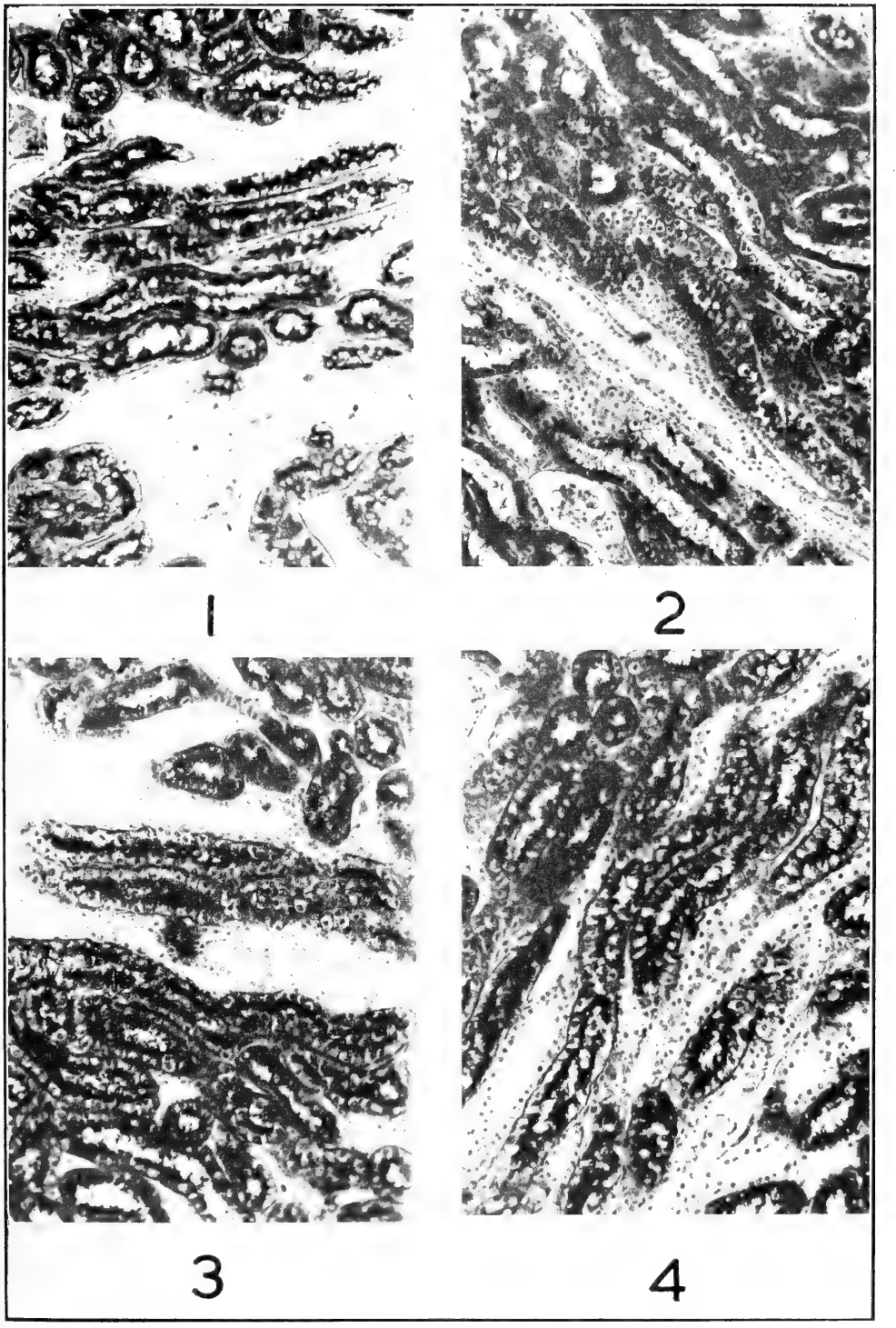
EXPLANATION OF PLATES.

Plate III.

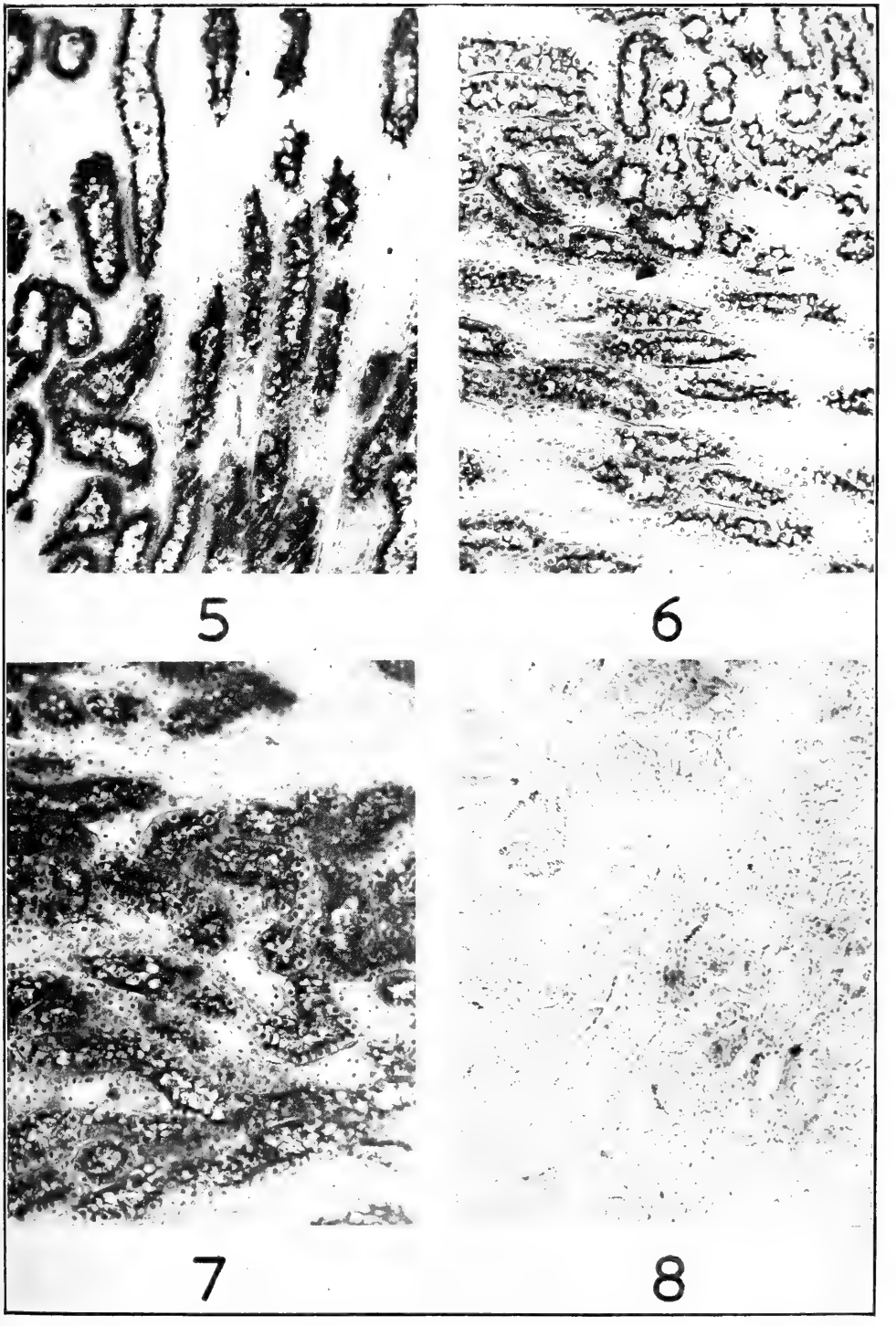
Upper specimens show for comparison, mature foliage, fruits, and buds of *Eucalyptus pauciflora* Sieber, *Eucalyptus vitrea* R. T. Baker, *Eucalyptus dives* Schauer, with characteristic juvenile foliage of *Eucalyptus dives* and *Eucalyptus pauciflora.*

Plate IV.

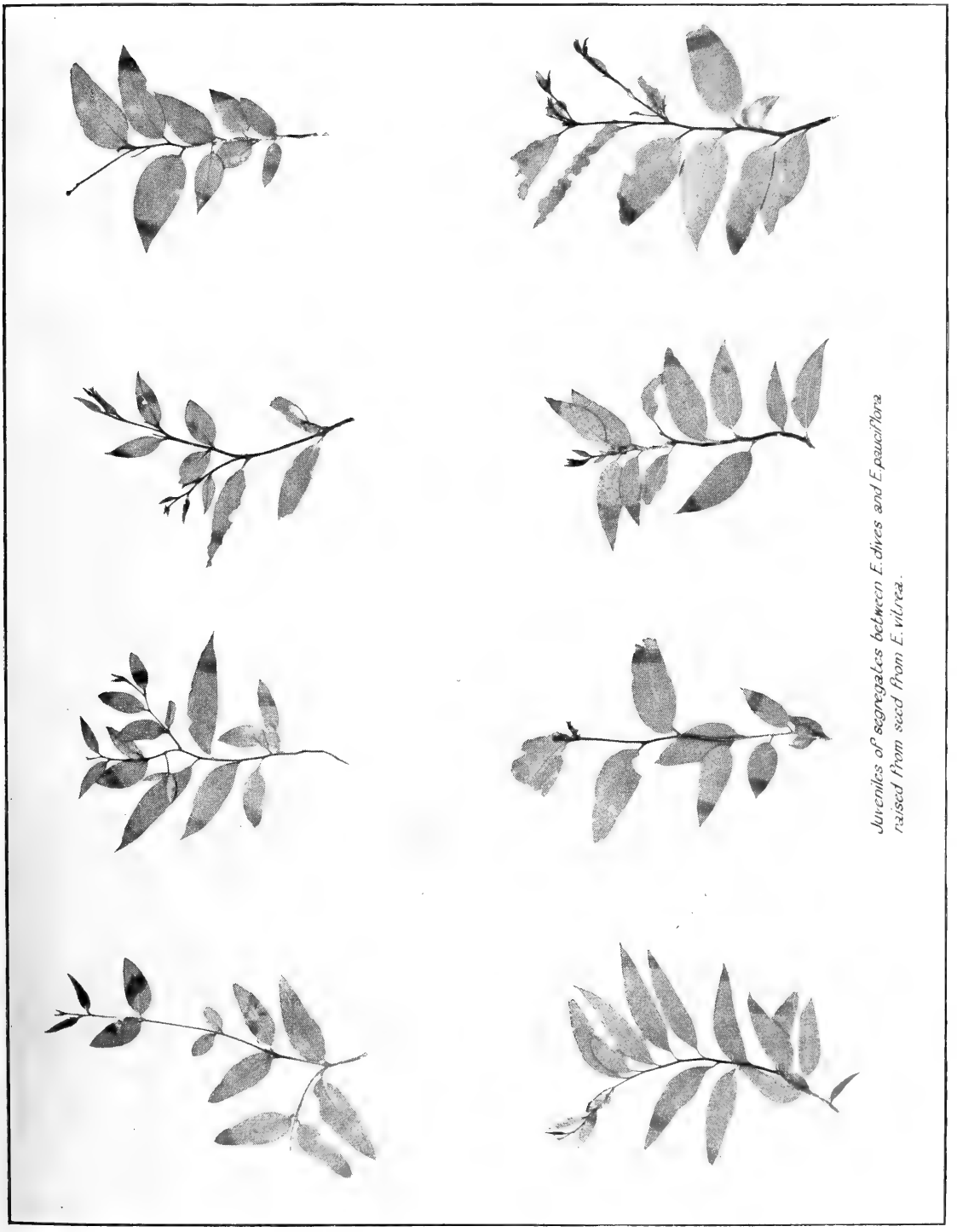
The range of juvenile foliage obtained from seedlings raised from a single tree of *Eucalyptus vitrea,* arranged from a type approaching *Eucalyptus dives,* upper left, to a type approaching *Eucalyptus pauciflora,* bottom right. Note the change from approximately opposite to markedly alternate leaves, the very short petiole or practically sessile leaves to markedly petiolate leaves, the thin stems of the *Eucalyptus dives* type, to the thick, almost fleshy stems of the type tending to *Eucalyptus pauciflora.*



Alkaline phosphatase reactions in tissue sections.

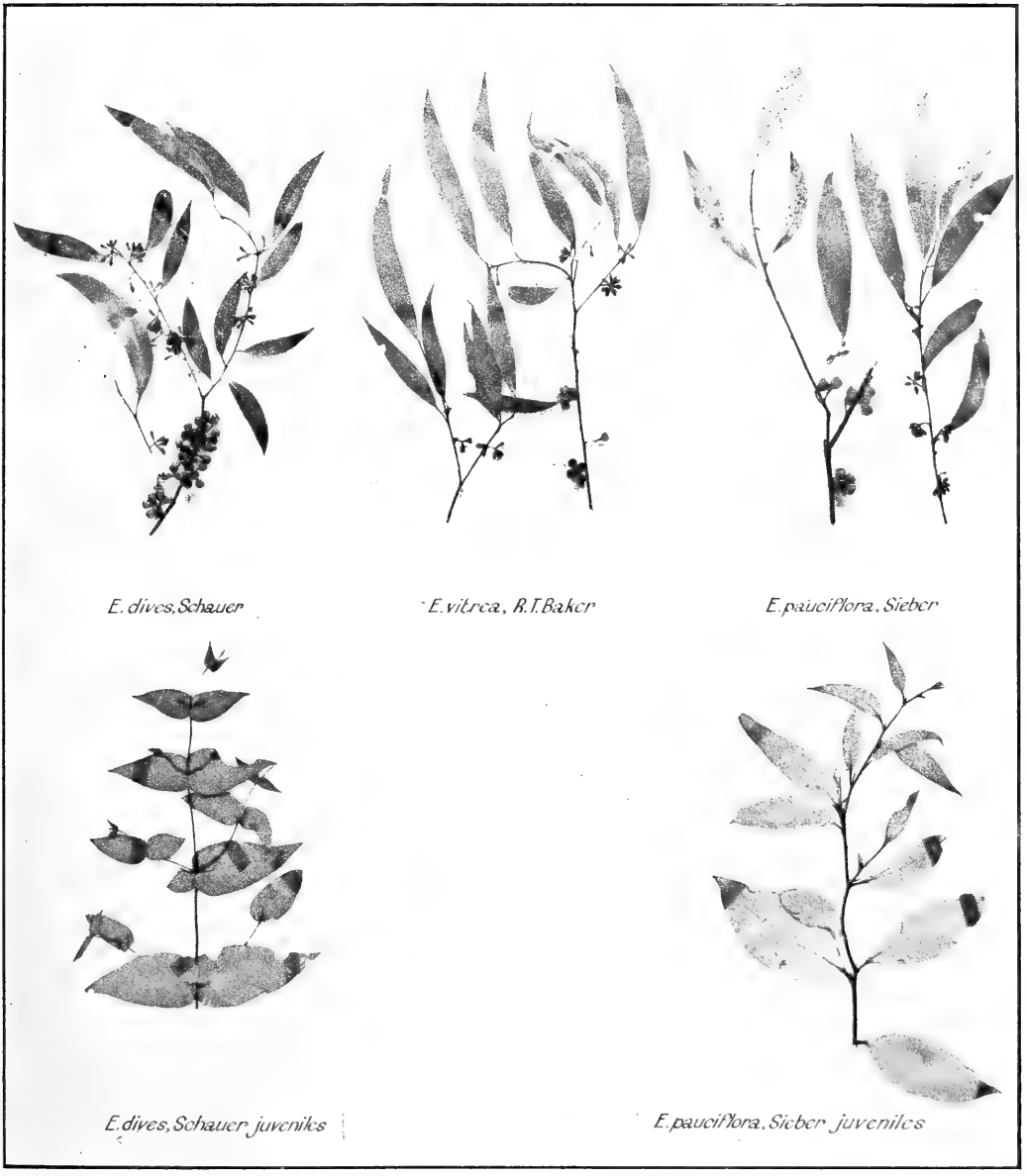


Alkaline phosphatase reactions in tissue sections.



Juveniles of segregates between E. dives and E. pauciflora raised from seed from E. viridea.

A hybrid Eucalyptus.



A hybrid Eucalyptus.

CYTOLOGICAL STUDIES IN THE MYRTACEAE.

III. CYTOLOGY AND PHYLOGENY IN THE CHAMAELAUCOIDEAE.

By S. Smith-White,
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(Plates v and vi; One hundred and one Text-figures.)

[Read 31st May, 1950.]

Introduction.

It has been shown that the basic chromosome number in the larger subdivisions of the Myrtaceae—the Leptospermoideae and the Myrtoideae—is eleven (Atchison 1947; Smith-White 1948), and this number is remarkably constant. The occurrence of secondary association, and other conditions, suggested that this basic number is itself a derived one, probably from an original set of six. The present study was undertaken with this hypothesis in mind, and in the hope that it might yield evidence with a bearing on the phylogenetic relationships between the three tribes of the family.

Cytology has been used in a number of cases to indicate the phyletic relationships within and between groups, and to show the nature of the processes that have controlled speciation (e.g., Babcock 1942, 1947; Anderson 1937). In a natural group evolutionary trends may remain discernible, and depending on the nature of the processes involved, cytological data may be of considerable value in the interpretation of phylogeny.

Taxonomy and Distribution of the Chamaelaucoideae.

The Chamaelaucoideae has usually been associated systematically with the Leptospermoideae, and the two tribes have much in common both in morphology and in geographical distribution. Both are essentially Australian, but the Chamaelaucoideae is much more restricted both in its range and in the number and diversity of its species. Whereas the Leptospermoideae extends to New Zealand, Oceania, and through Indonesia to South-East Asia, the Chamaelaucoideae is entirely restricted to Australia, and shows its maximum diversity only in the south-western corner of the continent.

The Chamaelaucoideae is, however, of considerable interest in connection with the origin of the Australian myrtaceous flora. Andrews (1913) considered the group to be derived, through the Leptospermoideae, from some "generalized" *Eugenia*-like or *Myrtus*-like tropical mesophytic ancestor, the development of xerophytic characters being a response to the gradually increasing aridity of the Australian climate. Bews (1926) supported this opinion, maintaining that the xerophytic habit in angiosperms is derived from a primitive mesophytic one. On other grounds, including floral morphology and geographical distribution, the tribe has sometimes been considered the more primitive. Woolls (1884) expressed the opinion that the typically Australian genera of the Myrtaceae originated in south-west Western Australia, and quoted Hooker in support of that opinion. Woolls to some extent foreshadowed the theory put forward by Vavilov (1926, 1939) that the primary regions of plant groups are characterized by great botanical diversity. Since the Myrtoideae is absent from Western Australia, and is centred in tropical America, the distribution of the three tribes would suggest at least a dual phylogenetic origin of the family from primitive types of the Myrtiflorae, with the Chamaelaucoideae either giving rise to or derived from the Leptospermoideae through connecting genera such as *Scholtzia*.

The number of genera and approximate number of species in the Chamaelaucoideae and their distribution as between Eastern and Western Australia are given in Table 1. This table has been compiled from the Flora Australiensis (Bentham 1866), from the Floras of the various States by Bailey (1900, 1909), Black (1926), Ewart (1930) and

Maiden (1916), and from various papers by Black, Cheel, Gardner, and White. No claim is made for any finality or completness in the figures.

Material and Methods.

This paper deals with the cytology of 17 species and taxonomic varieties of the Chamaelaucoideae, representing seven genera and all three subtribes of the tribe. A summary of the data presented is given in Table 2, in which the arrangment of the species is essentially that of Bentham (1866). Most of the species are native to the

Table 1.

Distribution of the Genera and Species of the Chamaelaucoideae.

Genus.	Number of Species.			Total.
	Eastern.*	Western.†	Cosmopolitan.	
Euchamaelauceae.				
Actinodium	—	1	—	1
Darwinia	10	30	—	40
Homoranthus	3	—	—	3
Rylstonea	1	—	—	1
Verticordia	1 (?)	46	—	47
Pileanthus	—	3	—	3
Chamaelaucium	—	12	—	12
Calythriceae.				
Calythrix	12	34	2	48
Lhotzkya	3	7	—	10
Thryptomeneae.				
Homalocalyx	2‡	—	—	2
Thryptomene	4	26	2‡	32
Micromyrtus	5	12	1	18
Total Chamaelaucoideae ..	41	171	5	217

* Includes the northern areas of the Northern Territory.
† The south-west part of the Continent.
‡ Species with a strictly northern distribution.

environs of Sydney, but *Chamaelaucium uncinatum* has been studied from cultivated material, and other material of Western Australian species has been forwarded by Miss Baird and Mr. G. Smith of the University of Western Australia, and by Miss Eardley of the University of Adelaide. The identification of this material is due to the collaborators mentioned.

Most of the work recorded was concerned with meiosis in pollén mother cells, and the observations were made chiefly from temporary acetocarmine crushes. Aqueous fixation, followed by Feulgen staining gave satisfactory preparations with the smaller anthers of *Darwinia* but was less satisfactory with *Chamaelaucium*. The introduction of a treatment with 4% ammonium oxalate, as suggested by Hillary (1940) was found to be an advantage. More detailed observations of meiosis in *Darwinia fascicularis* and *Chamaelaucium uncinatum* were also made on material fixed in Randolph's CRAF modification of Navashin's fluid, embedded, and cut at 14 and 20 microns. The Feulgen-Light Green method of Semmens and Badhuri (1939) proved useful in the study of chromosome-nucleolus relationships, although such preparations were found to fade rapidly.

Pollen fertility was determined in the dextrin-sorbitol stain mountant previously described (Smith-White 1948).

Observations were made with a 2 mm. apochromatic objective, an achromatic condenser of 1·3 n.a., and a 20× compensating eyepiece. Drawings were made with an Abbe camera lucida, giving a magnification at bench level of ×3900, and have been reduced to ×2600 in reproduction. Photographs are at the magnifications indicated.

Meiosis: General Outline.

For the present purpose the species *Darwinia fascicularis* and *Chamaelaucium uncinatum* are considered as representative of the 6- and 11-chromosome types within the tribe.

(i) *Darwinia fascicularis* Rudge. n = 6.

At the time of meiosis the anthers are minute and the pollen mother cell mass is usually only 3–4 cells across and little more in length. The cells are tightly packed

Table 2.

Summary of Cytological Data.

Tribe Chamaelaucoideae.

Species.	Distribution.	Localities.	No. of Plants Examined.	Haploid Chromosome No.	Seasonal Occurrence of Meiotic Stages.
Euchamaelauceae.					
Actinodium Cunninghamii ..	W. Aus.	King George's Sound.	1 (?)	6	Sept.
Darwinia citriodora	W. Aus.	Perth.	1 (?)	6	Sept.–Oct.
D. taxifolia	E. Aus.	Blackheath.	10	6	May–July.
D. biflora	E. Aus.	Pymble.	10		
		Epping.	10	6	May–July.
		Ryde.	10		
D. grandiflora	E. Aus.	Berowra.	10		
		St. Ives.	3	6	June–Aug.
D. intermedia	E. Aus.	St. Ives.	10		
		Loftus.	10	6	Aug.–Dec.
		National Park.	5		
D. taxifolia v. *grandiflora* ..	E. Aus.	Bulli.	10		
		Helensburgh.	10	6	April–June.
D. taxifolia f. *tetraflora* ..	E. Aus.	King's Tableland.	10	6	July.
D. fascicularis	E. Aus.	Pymble.	10		
		St. Ives.	10		
		National Park.	10	6	Aug.–Dec.
		Helensburgh.	10		
		Kuring-gai.	10		
Verticordia sp.	W. Aus.	Perth.	1 (?)	8	Aug.
V. plumosa	W. Aus.	Perth.	1 (?)	8	Aug.
V. farnesiana	W. Aus.	Adelaide (Cult.).	1	11	
Chamaelaucium uncinatum ..	W. Aus.	Sydney (Cult.).	8	11	May–July.
Calythriceae.					
Calythrix tetragona	E. Aus.	Pymble.	5		
		National Park.	10	11	Aug.
C. Fraseri	W. Aus.	Perth.	1 (?)	11	Oct.–Nov.
Thryptomeneae.					
Thryptomene Mitchelliana ..	E. Aus.	Melbourne (Cult.).	2	11	Aug.
Micromyrtus microphylla ..	E. Aus.	Wahroonga.	5	11	Aug.

and do not separate easily (Text-fig. 1). The nuclei measure only 8–10 microns in diameter (Text-fig. 2), and are of the vesicular type reported by McAulay and Cruickshank (1937) in *Eucalyptus*, and described by Manton (1935). There is present in each only a single nucleolus, 3–4 microns in diameter, and the nucleoplasm is clear except for a number of minute Feulgen-positive bodies, probably heterochromatic in nature.

The early stages of prophase are normal. There is the usual increase of nuclear size, to a maximum of 17–20 microns at pachytene. At zygotene, pairing of the heterochromatic granules, which may represent the resting stage prochromosomes, is often apparent. At pachytene, the paired threads may be distributed uniformly near the surface of the nucleus (Text-fig. 3), or they may form a more or less compact mass which may or may not include the nucleolus (Text-figs. 4, 5). It is generally assumed that synezesis is artefactual, but the impression is gained from the present material that it may represent some real substage of pachytene. Whatever its significance, it provides a useful condition for determining the attachment of chromosome threads to the nucleolus, as illustrated in Text-fig. 4. Never more than one attached chromosome (bivalent) thread was observed in *D. fascicularis* or any other species of the genus. At pachytene, the nucleolar-associated chromatin body is of relatively large size (cf. Plate v, fig. 1), and it is usually closely applied to the nucleolus.

Contraction of the bivalents during diplotene-diakinesis is often irregular (Text-fig. 6). Individual bivalents may possess one, two or occasionally three chiasmata, but any complete analysis of chiasmata in diplotene nuclei has not been accomplished. At diakinesis and at metaphase, interstitial chiasmata may occur (Text-fig. 7).

TABLE 3.
Chromosome Arrangement in Darwinia.

	Configurations.		Irregular.	Total.
	5 (1).	6 (0).		
D. fascicularis—				
1–M	3	5	—	8
	5	0	—	5
1–A	12	1	—	13
2–M	3	1	—	4
2–A	23	7	—	30
D. grandiflora—				
1–M	53	9	2	64
2–M	35	5	1	41
Total	88	14	3	105
Grand total	111	21	3	135

Following diakinesis there is a short condition of prometaphase (Text-fig. 8), when the bivalents lie in a close group in the centre of a clear region of the cytoplasm. It is considered that this stage provides an opportunity for the development of secondary association if the necessary chromosome relationships exist.

At first metaphase (Text-figs. 9, 10, Plate v, fig. 3), pairing is complete. Univalents and multivalent associations are not found. The bivalents are of approximately uniform size, although some other species have one bivalent distinctly larger than the others (vide infra). Both at first and second metaphases secondary association is notably absent, in contrast with the condition found in the 11-chromosome genera studied. In the absence of secondary association the bivalents at first metaphase and the chromosomes at second metaphase are evenly spaced in the configurations shown to be stable by Kuwada (1929) and Maeda and Kato (1929). The most frequent arrangement consists of five chromosomes in a ring, with one centrally placed (Text-fig. 9), and a much less frequent arrangement is of all six chromosomes in an evenly spaced ring (Text-fig. 10, Plate v, fig. 4). The frequency of these arrangements is indicated by the data in Table 3.

At early first anaphase the chromosomes may be associated by one or two terminal chiasmata or by interstitial chiasmata (Text-figs. 11–14). In the latter case the

chromosomes may show late disjunction, and this behaviour is apparently responsible for the lagging and false "bridges" illustrated in Text-figs. 15, 16 and 17. Rarely it offers the possibility of non-disjunction and unequal partition. The stretching of the region of a chromosome between the centromere and an interstitial chiasma is often considerable and the submedian position of the centromere becomes quite evident in such cases.

Late anaphase (Text-fig. 18) is normal, and telophase is notable only for the marked clumping of the chromosome groups before the development of the interphase nuclei (Text-fig. 19). In the interphase nucleus the dispersion of the chromosomes is not complete and heterochromatic granules remain distinctly larger than in fully resting nuclei. The nuclei are considerably flattened (Text-fig. 22) and the chromosomes tend to lie on the inner surfaces of the nuclei relative to the cell as a whole, giving the "saucer" stage described by McAulay and Cruickshank in *Eucalyptus*. With the smaller number of chromosomes in *Darwinia* this appearance is less conspicuous. Never more than one nucleolus per nucleus has been observed at interphase, and this is usually eccentrically placed and is associated with only one chromosome or prochromosome.

Interphase passes without conspicuous change into second prophase. At second metaphase (Text-figs. 23, 24, Plate v, fig. 5) and anaphase (Text-figs. 25, 26) the spindles may lie in the same plane or at right angles to one another, giving either a quartite or tetrahedral arrangement of the microspores. These stages again illustrate the absence of secondary association. Second telophase shows also a clumping of the chromosomes before the microspore nuclei open out (Text-figs. 27–29). In each microspore nucleus soon after formation only a single nucleolus is developed, in association with one of the chromosomes. The nuclei are now similar to the original pollen mother cell nuclei, but are smaller and have fewer heterochromatic granules (Text-fig. 30).

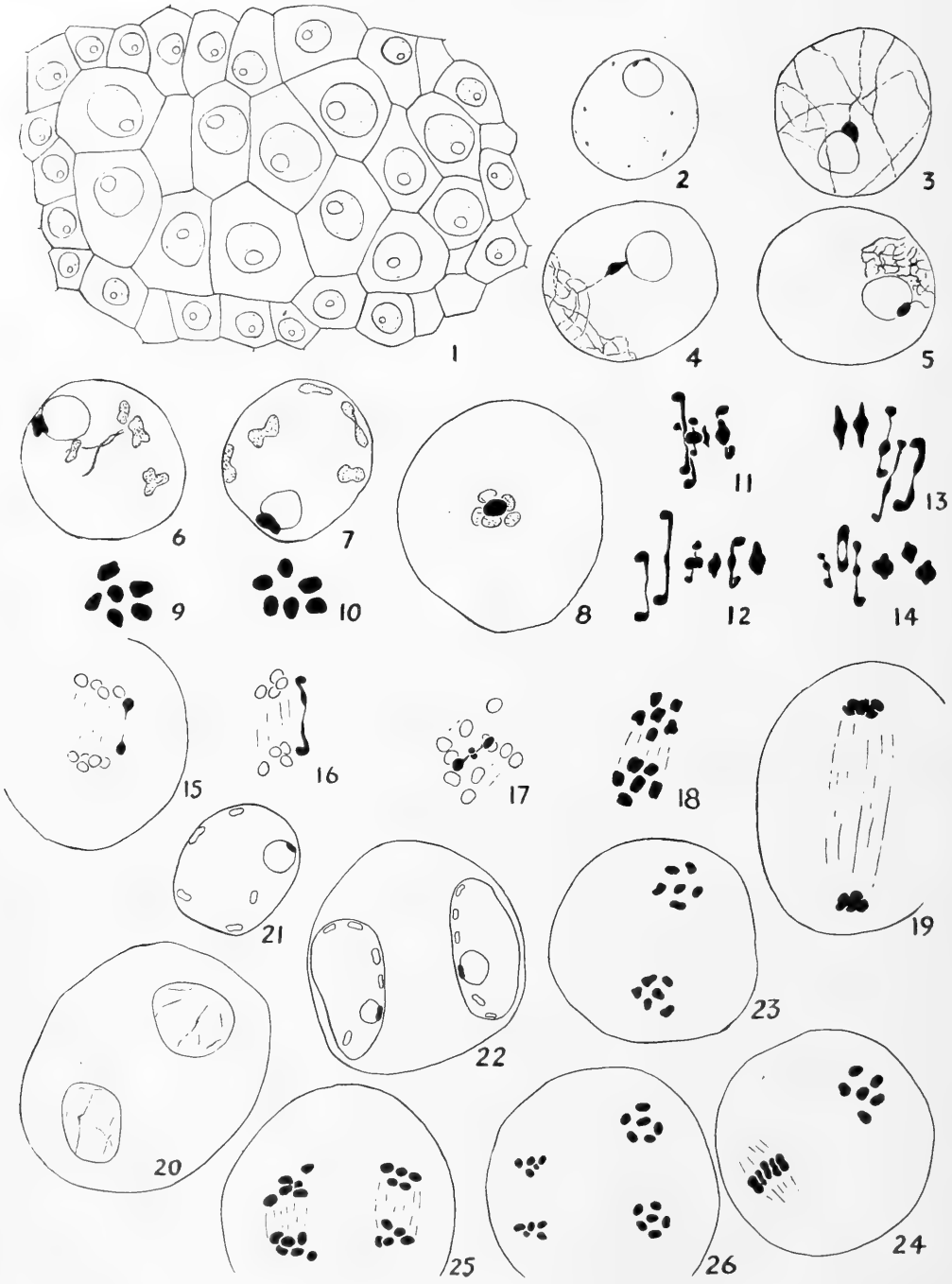
Cytokinesis does not commence until after the full development of the microspore nuclei. It is then accomplished by furrowing, a method apparently normal to many dicotyledons, first described by Farr (1916, 1918).

(ii) *Chamaelaucium uncinatum* Schau. n = 11.

Except for the difference in chromosome number, and for details dependent on this, meiosis in *Ch. uncinatum* follows a similar pattern as in *Darwinia*. Resting premeiotic nuclei, ca. 10 microns in diameter, have a larger number of heterochromatic granules, and four of these may be associated with a single large nucleolus, although this association has not been consistently evident.

Prophase development is again similar, but at pachytene two bivalent threads may be attached to the nucleolus (Text-figs. 32, 33; Plate v, fig. 11), one by a larger heterochromatic mass than the other. This attachment is again most readily determined at synezesis. At diakinesis (Text-fig. 34) two bivalents still show nucleolar association.

Chiasma conditions are similar to those found in *Darwinia*. At diakinesis and at first metaphase (Text-figs. 35, 36) there may be one or two terminal chiasmata, or occasionally interstitial chiasmata in each bivalent. At metaphase, 11 bivalents are regularly formed, and these are arranged in a compact equatorial plate on the spindle (Text-figs. 37–42, Plate v, fig. 12). The bivalents are of uniform size, but are distinctly smaller than in *Darwinia*. The arrangement of the chromosomes on the plate is considerably affected by the occurrence of secondary association. In the absence of secondary attraction, the stable arrangement of 11 chromosomes would be as a ring of eight, with three centrally placed, as demonstrated by Kuwada (1929), Muto (1929), Ogawa (1929), and Alam (1936). Alam (1936) and Raghaven (1938) have demonstrated how the occurrence of secondary association can upset such an arrangement. In *Ch. uncinatum* the arrangement 8 (3) is distinct only in those cases showing a minimum of secondary association (Text-fig. 37). This grouping reaches a maximum of five pairs (Text-fig. 41). Lesser degrees of association are more common, and sometimes associations of threes may occur (Text-fig. 42). Table 4 indicates the frequency of secondary association in *Ch. uncinatum*. Following Alam and Raghaven, secondary



Text-figures 1-26. *Darwinia fascicularis* Rudge. 1. L. sect. of an anther just before meiosis (×1300). 2. Pollen mother cell in premeiotic resting stage. Two heterochromatic bodies are shown associated with the nucleolus. 3. Pachytene. 4 and 5. Pachytene showing the synezetic knot, when the nucleolar associated chromatin is distinct. 6. Late diplotene, with irregular condensation of the bivalents. 7. Diakinesis. There is one nucleolus-associated bivalent. 8. Prometaphase. 9 and 10. 1-M plates showing different configuration. 11-14. Early anaphase stages. In 12 the chromosomes have been separated in drawing to show the chiasma

pairs have been recorded as representing one association each, and triple groups as two associations, for the purposes of the table.

First anaphase and telophase (Text-fig. 43) closely resemble the corresponding stages in *Darwinia*, but interphase shows an important difference. Frequently two nucleoli may be formed in one or both nuclei of a pollen mother cell, and these may be of similar or of widely different size (Text-fig. 44, Plate v, fig. 13). The occurrence of the two nucleoli becomes very conspicuous after the use of Semmens' and Badhuri's Feulgen-Light Green technique, which also demonstrates the association with each nucleolus of a single heterochromatic granule. Single nuceoli, presumably originating

TABLE 4.
Secondary Association in Chamaelaucium uncinatum.

No. of Secondary Associations.	0	1	2	3	4	5	Total.
1-M	1	6	9	14	11	7	49
2-M	2	1	2	4	2	0	11
Total	3	7	11	18	13	7	60

by fusion, possess two heterochromatic granules. Two nucleoli also occur in the microspore nuclei following the second devision (Text-fig. 51, Plate v, fig. 15). The high frequency of only single nucleoli would suggest that fusion occurs soon after the formation of the nucleoli.

Second prophase (Text-figs. 45, 46), metaphase (Text-figs. 47–49, Plate v, fig. 14), anaphase and telophase resemble the same stages in *Darwinia*, except for the occurrence of secondary association. At second anaphase (Text-fig. 50), occasional laggards may be seen. Cytokinesis also occurs by furrowing.

CYTOLOGY OF THE GENERA AND SPECIES.

I. Subtribe EUCHAMAELAUCEAE.

Of the seven genera of this subtribe, four, containing over 60 species, are confined to Western Australia, and two, with five species, are entirely eastern. *Darwinia* is the only genus extending throughout the continent, but it is also predominantly western.

1. *Actinodium.* n = 6.

A monotypic western genus. Material of *A. Cunninghamii* Schau. was forwarded by Miss Baird and was obtained from the King George's Sound district.

In premeiotic mitoses in sporogenous tissue a diploid number of 12 was determined (Text-fig. 52). The chromosomes are of uniform size and show no conspicuous features of morphology apart from submedian centromeres. Failure to observe satellites or secondary constrictions in the material cannot be accepted as critical.

In meiosis in the pollen mother cells the similarity with *Darwinia* is very apparent. The nucleolar-associated heterochromatin is particularly conspicuous at pachytene (Plate v, fig. 1). From first metaphase to second metaphase (Text-figs. 53, 54, Plate v, fig. 2) the chromosomes show the same type or arrangement and give no evidence of secondary association.

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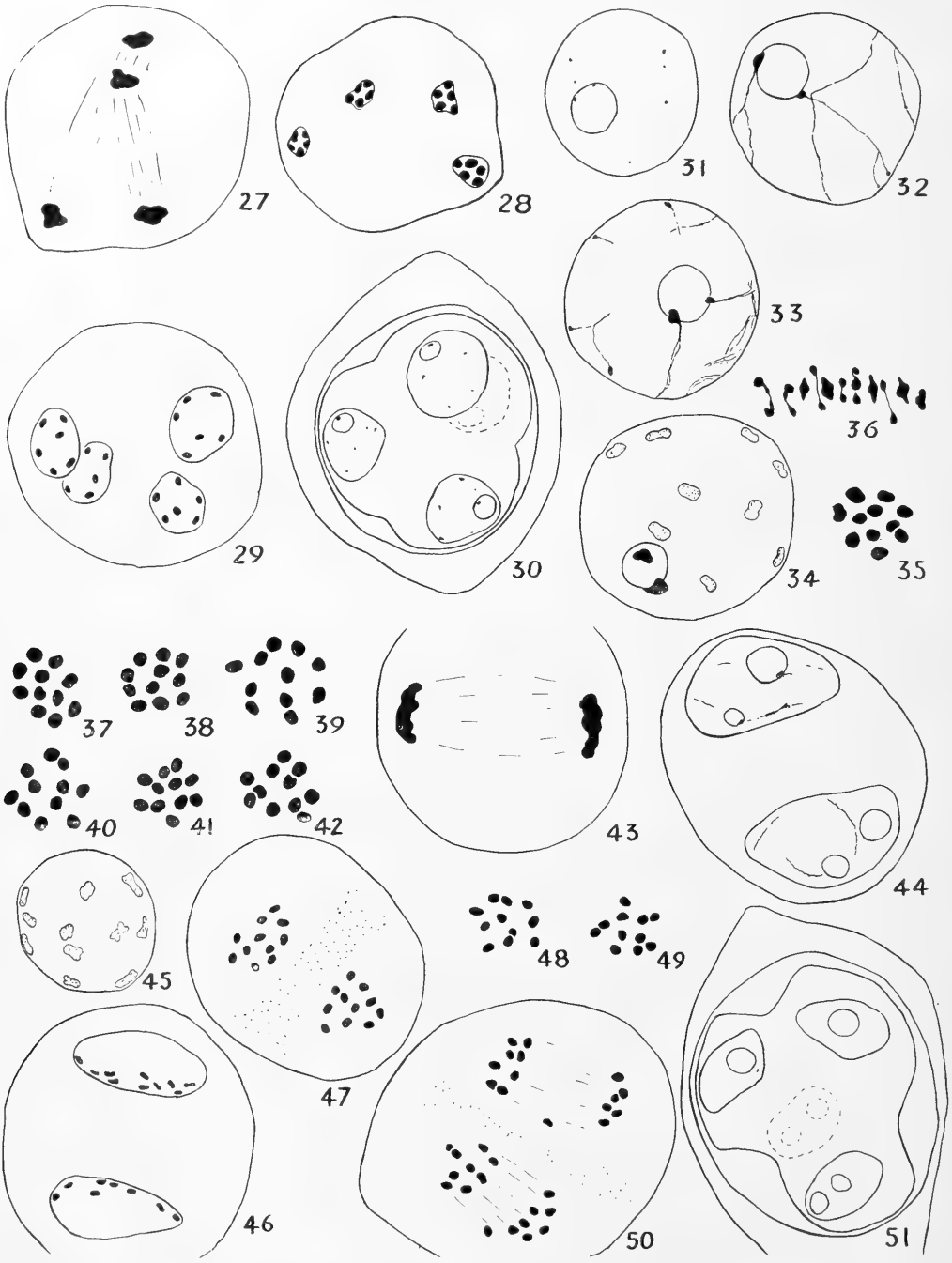
relationships. 15-17. Anaphase stages showing late disjunction of a bivalent, giving the appearance of false bridges. 18. 1-A in polar view. 19. 1-T, showing the clumping of the two chromosome groups. 20. Interphase. 21 and 22. Second prophase. 22 shows the development of the "saucer" stage. 23. 2-M, showing the typical chromosome configuration. 24-26. Second anaphase. All text-figures are at × 2600 unless otherwise stated.



Text-figures 27-51. 27-30. *D. fascicularis,* showing a series of developmental stages from second telophase to cytokinesis. 31-36. *Chamaelaucium uncinatum.* 31. Pollen mother cell nucleus prior to meiosis. 32. Pachytene. There are two pairs of nucleolar organizers. 33. Another pachytene nucleus. 34. Diakinesis. Two bivalents are associated with the nucleolus. 35. 1-M, in polar view. 36. 1-M or early 1-A in side view to show normal chiasma relationships. *Chamaelaucium uncinatum.* 37-42. Different 1-M plates showing different degrees of secondary association. 43. First telophase. 44. Interphase, showing two nucleoli per nucleus, prophase, showing the "saucer" stage. 47-49. 2-M plates showing different degrees

The close relationship assumed between *Actinodium* and *Darwinia* on morphological grounds (Cheel 1922) is supported by the limited cytological evidence. Undoubtedly one is derived from the other, but *Actinodium* may be a relic type, restricted by reason of genetic homogeneity (Stebbins 1942) or a new form of recent origin.

2. *Darwinia.* n = 6.

A genus of about thirty-seven species, divided into two sections. The section *Genetyllis* is probably the more primitive and is represented in both eastern and western floras. All the species dealt with in this paper belong to this section. The section *Schaumannia*, entirely western, connects the genus with *Verticordia* and with the eastern genus *Homoranthus.*

D. citriodora Benth. n = 6.

Vicinity of Perth; Miss A. M. Baird, Mr. G. Smith. Cytological conditions are similar to those described for *D. fascicularis* (Text-figs. 55–59). There is no evidence of secondary association, and meiosis is quite regular. At first metaphase the chromosomes are of rather larger size, and one bivalent is distinctly larger than the others of the complement. This large bivalent may be late in disjunction, but no other abnormalities were seen.

Darwinia taxifolia sensu lata.

D. taxifolia was first described by A. Cunningham, but Bentham (1866) included as synonymous with it another species, *D. intermedia*, also described by Cunningham. Cheel (1922) has shown the two species to be distinct in both morphological character and geographical range, and at least deserving of varietal rank. In the author's experience the species of *D. taxifolia* as understood by Bentham is a complex group and contains a number of forms of more or less doubtful specific rank, viz.:

D. taxifolia A. Cunn., sensu strictu.
D. taxifolia var. *biflora* Cheel.
D. grandiflora Baker and Smith.
D. taxifolia var. *grandiflora* Bentham.
D. intermedia A. Cunn. (= *D. taxifolia* var. *intermedia* Cheel).
D. taxifolia f. *tetraflora.*

All these forms appear to possess (1) district geographical ranges and perhaps also ecological requirements, and (2) constant morphological differences. No natural hybridization between them can occur and they would appear to constitute separate genetic systems and to possess the properties of species as accepted by Huxley (1939, 1942), Darlington (1940), Timofeeff-Ressovsky (1940), Muller (1940), Dobzhansky (1941) and Mayr (1942, 1948).

(*a*) *Darwinia taxifolia* A. Cunn., sensu strictu.

According to Cheel (1922) this form has a wide distribution from the Blue Mountains to Jervis Bay. The material studied, from an isolated population at Blackheath, agreed essentially with the description given by Cheel, except for a rather higher proportion of flowers in pairs than in fours. The character of the floral bracts distinguishes the form sharply from *D. biflora.*

Except that the chromosomes are slightly smaller, cytological conditions in the form are normal and typical of the genus. Meiosis did not appear irregular, but in view of the pollen condition found, a more careful examination is necessary. In ten plants selected at random from the population, so little pollen was produced that satisfactory counts could not be made, but the pollen grains seen appeared small and deficient. It is not possible to decide at the present stage whether this lack of fertility is cytologically, genetically or physiologically determined. The isolation of the population,

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of secondary association. 50. 2-A, showing laggard chromosomes in each spindle. It is not clear whether these are due to misdivision at first anaphase. 51. Newly formed microspore nuclei in a pollen mother cell. Some show two nucleoli, but in two these have already fused. All text-figures are at × 2600 unless otherwise stated.

allowing no opportunity for hybridization, did not give any expectation of the pollen sterility found.

(*b*) *D. taxifolia* var. *biflora* Cheel. n = 6.

Populations of this form from localities extending from Ryde to Pymble were examined. The species was found in association with *D. fascicularis*, but not with any other forms of the *D. taxifolia* group.

The meiotic divisions show six bivalents of small but even size (Text-fig. 60), and cytological behaviour appears typical of the genus. In some plants univalents may occur with a frequency of from 1% to 5% of the pollen mother cells, and microcytes may be formed with a similar frequency at the tetrad stage. In the population examined for pollen fertility a high figure was obtained (Table 7), but it is probable that other populations are more variable.

(*c*) *D. grandiflora* Baker and Smith. n = 6.

A tall shrubby species quite distinct from the var. *grandiflora* of Bentham, and with a distinct range, from St. Ives northwards to Hawkesbury River. Material from St. Ives and Berowra was examined.

Meiosis in the species is normal (Text-figs. 61–64, Plate v, figs. 6, 7). Early separation of "rod" bivalents with single terminal chiasmata may give a false impression of univalents, but true univalents are at least rare. The type of chromosome arrangement at metaphase, as in *D. fascicularis*, suggests a lack of any secondary attractions (Table 3).

Pollen fertility in the Berowra material was found to be high (Table 7), except for one plant showing complete sterility suggestive of physiological rather than meiotic breakdown (Plate vi, figs. 4, 5).

(*d*) *D. taxifolia* var. *grandiflora* Bentham. n = 6.

A prostrate stoloniferous form most closely related to *D. intermedia*, but readily recognized by its unusual habit and the larger size of its leaves, floral parts and pollen. It occurs at moderate altitudes from Helensburgh southwards to Bulli and

TABLE 5.

Tetrad Formation in Darwinia taxifolia *var.* grandiflora.

No. of Microspores per Tetrad.	2	3	4	5	6	Total.
Plant No. 1	1	2	420	27	8	458
Plant No. 2	0	1	196	5	1	203
Total	1	3	616	32	9	661

West Dapto. Two populations, from Helensburgh and Bulli, have been studied. In the former locality considerable variation in pollen fertility was found (Table 7, Plate vi, fig. 7) and this variation may be correlated with the fact that the species not only co-exists with *D. fascicularis*, but also contacts, but does not overlap, the distribution of its close relative *D. intermedia*. In the Bulli locality pollen fertility is higher and meiosis is regular (Text-figs. 65–67). Table 5 shows the degree of regularity in tetrad formation in two plants from this locality, and the extent of pollen fertility is given in Table 7. In several plants "giant" pollen grains (Plate vi, fig. 6) occur with a frequency of as much as 1%, suggesting the occasional failure of meiosis.

(*e*) *D. intermedia* A. Cunn. n = 6.

A prostrate but not stoloniferous form with a fairly wide distribution extending from Helensburgh northwards to Kuringai. Material has been studied from several localities, chiefly at Loftus and at St. Ives. Material from Loftus showed reasonably

regular meiosis and a consequent high pollen fertility (Table 7), but the St. Ives population showed a very considerable variability in pollen fertility and an unusual frequency of abnormalities at meiosis. In this latter material the somatic chromosome number is 12 (Text-fig. 68), but owing to the considerable frequency of univalents at 1–M, and of divided univalents at 1–A and 2–M (Text-figs. 69–74) decisive meiotic counts were not easy to obtain.

In some plants of this material up to eight univalents per nucleus are found at 1–M, and in one case the mean frequency per nucleus was 5·4. Such univalents show the usual failure of congression to the metaphase spindle (Text-figs. 72, 73) and misdivision in the equatorial region of the spindle at 1–A (Text-fig. 74, Plate v, fig. 8). Apart from their abnormal behaviour, the univalents are not easily distinguished from bivalents, seen in polar view.

Neither bridges, multiple association nor other evidences of structural chromosomal changes were observed in any plants of this population, and although their presence cannot be excluded, it is possible that the partial failure of pairing is due to a reduction in chiasma frequency caused either by structural or genotypic factors. The formation of restitution nuclei has not been observed, but its occurrence is suggested by the presence of occasional "giant" pollen grains in some pollen samples.

TABLE 6.

Tetrad Formation in Darwinia intermedia. *A plant showing 48·9% bad pollen.*

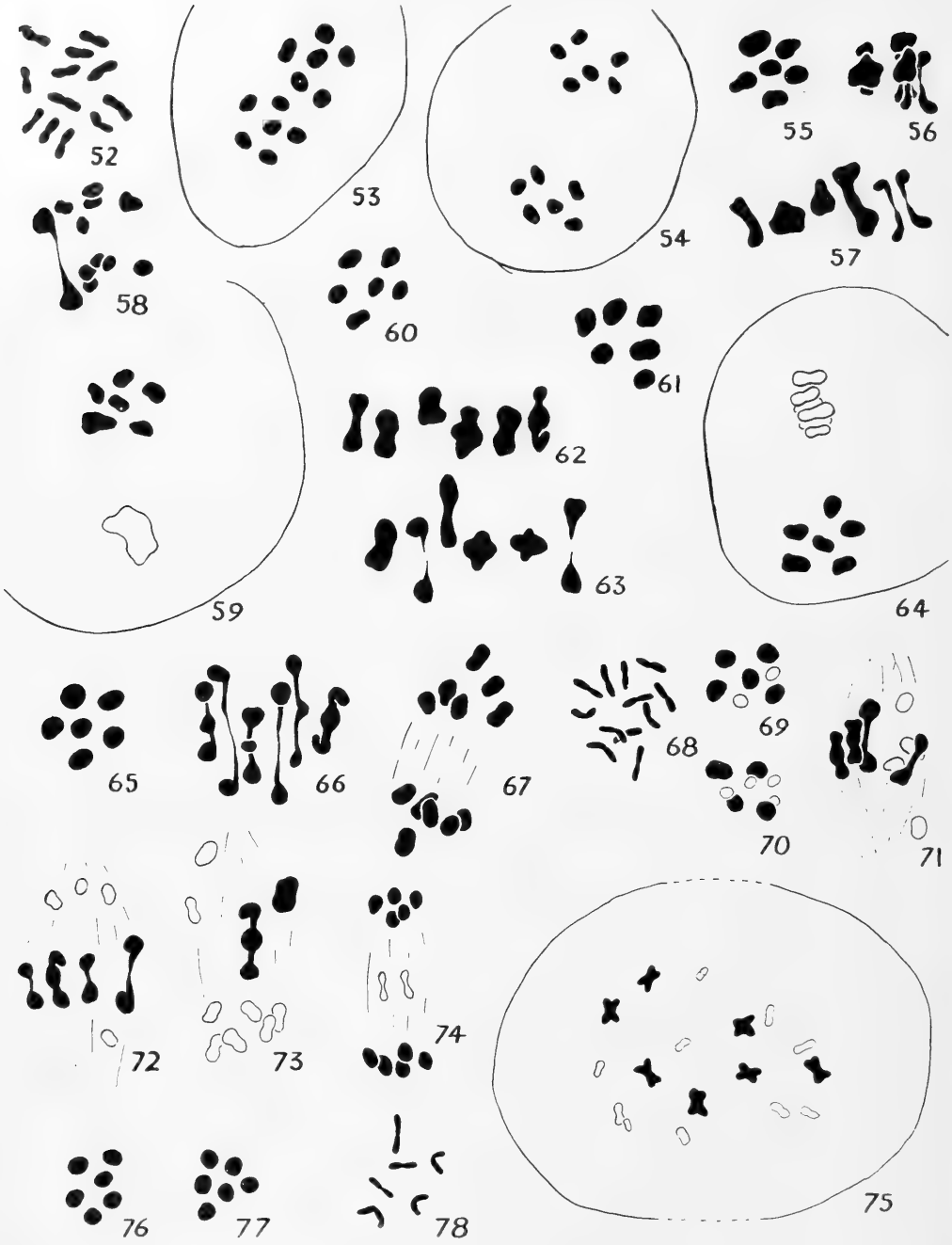
No. of Microspores per Tetrad.	Unequal 2	Unequal 3	Unequal 4	Equal 4	Equal 5	Equal 6	Equal 7	Total.
No. of tetrads observed	0	0	21	91	28	8	1	149
Total No. of microspores included	0	0	84	364	140	48	7	643

Bad pollen found in pollen counts .. .. 48·9%
Bad pollen expected from tetrad data (see text) .. .. .. 43·4%
χ^2=1·23 with 1 D.F. 5% level of χ^2=3·54.

At second metaphase unequal plates are frequent, and these may contain half-chromosomes and whole chromosomes. Text-figure 75 shows a late second prophase cell in which one half-chromosome has been lost in the cytoplasm. In the left-hand nucleus there are four whole chromosomes and five half-chromosomes, and in the nucleus on the right there are three whole chromosomes and four half-chromosomes. This condition could arise from a first metaphase with three bivalents and six univalents, followed by 1–A in which one univalent has passed without division to the left and the other five have divided at late 1–A, with one of the half-chromosomes failing to reach the right-hand group. Chromosomes lost in the cytoplasm may degenerate or may be organized into microcytes at the end of the first or second divisions.

Thus telophasic groups are formed which are grossly deficient or unbalanced. As a result of these irregularities, the number of microspores per tetrad is variable and the microspores are of variable size. In Table 6 the frequency of tetrads with different numbers of microspores is given for a plant showing 48·9% of sterile pollen. On the assumption that viable pollen will be produced only from tetrads with four visually equal microspores, a ratio of 279 non-viable to 364 viable pollen grains would be expected. Analysis shows this proportion to be in satisfactory agreement with the pollen viability actually found. Although it is obvious that some microspores from unequal tetrads may give viable pollen grains, the agreement is considered sufficiently close to indicate that meiotic irregularities could account for the pollen sterility found.

The considerable differences in pollen fertility found, not only between plants but also between populations, in this and other species of *Darwinia* and also within species of *Callistemon* and other genera of the Myrtaceae (Smith-White 1948) show that the inferences drawn by Lawson (1930) must be treated with reserve.



Text-figures 52-78. 52-54. *Actinodium Cunninghamii.* Mitotic metaphase in young anther tissue. 53. 1-A. 54. 2-M. Configurations are similar to those in *Darwinia.* 55-59. *Darwinia citriodora.* 55. 1-M. 56 and 57. The same division, in 57 the chromosomes being separated in drawing. 58. 1-A. The larger bivalent is late in disjunction. 59. 2-M. In all cases the larger size of one chromosome is evident and secondary association is absent. 60. *D. biflora,* 1-M. The bivalents are slightly smaller than in other species. 61-64. *D. grandiflora* B. & S. 61. 1-M. 62 and 63, early 1-A stages in side view. Early disjunction of some bivalents, as in 63, may give a false impression of the presence of univalents. 64. 2-M. 65-67. *D. taxifolia* var. *grandiflora* Benth. 65. 1-M. 66 and 67. Early and late 1-A stages. 68-75. *D. intermedia.*

(*f*) *D. taxifolia* f. *tetraflora*. n = 6.

This distinct form, or perhaps ectotype, is found in a windswept situation on King's Tableland, at the extreme western limit and greatest altitude for the species. It is a prostrate shrub which possesses foliage resembling *D. fascicularis*, but in some floral characters it approaches *D. intermedia*. Its characters are suggestive of a perhaps remote hybrid origin. The meiotic divisions, however, are quite regular, and pollen fertility is high (Text-fig. 77; Plate vi, fig. 2; Table 7).

Darwinia fascicularis Rudge. n = 6.

This species, which is the dominant one on the sandstone soils of the central coast of New South Wales, is relatively constant in morphological characters, but shows variability in characters such as pigmentation of the leaves and young shoots, in the essential oil in the leaves and to some extent in habit. Haviland (1884) and Brewster (1915) have shown that the species is dependent on small birds for pollination and that self-pollination is almost entirely precluded. This latter statement probably also applies to the other members of the genus studied and might account for the normally low seed fertility of all species even when pollen fertility is high.

Plants from a number of localities have been examined for meiotic irregularities and for the presence of pollen sterility (Table 7). Considerable variability in pollen fertility between plants and between populations occurs, and in some cases the character of the non-viable pollen suggests that it is caused by meiotic irregularities, whilst in others it appears to be physiologically or genetically controlled. In no cases, however, have meiotic irregularities comparable with those in *D. intermedia* been found.

The pollen grain mitosis has generally proved very difficult to find in the Myrtaceae. Text-figure 78 shows a metaphase of this division. The chromosomes are of similar length, ca. 1·5–2·0 microns, and have clearly marked median or submedian centromeres. One chromosome carries an indistinct secondary constriction which may represent the nucleolar organizer.

Pollen Fertility in Darwinia.

The data presented in Table 7 indicate that most, if not all, species of *Darwinia* examined show very considerable variability in pollen fertility. Since the work of Rosenberg on *Drosera* (1927) it has been usual to accept the occurrence of pollen sterility as indicative of interspecific hybridization (Allen 1929), and Lawson (1930) has made inferences on this basis concerning the importance of hybridization in the origin of the Australian flora. The present data show that in the Myrtaceae such inferences must be treated with reserve, at least until more is known of the breeding systems of the species concerned.

The distributions of the eastern Australian species of *Darwinia* show a number of peculiarities. All species tend to be divided into comparatively small communities, perhaps on the basis of ecological conditions. The degree of isolation between such populations seems to be of all degrees, from partial and slight to what might reasonably be considered absolute, and between the "species" of the *D. taxifolia* group this isolation has a geographical and probably a genetic basis. It would appear from casual inspection that the size of interbreeding populations is frequently small, sometimes very small, comprising in some cases the plants over an area of a few square chains, and that gene-migration between such populations may be very slow.

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68. Mitotic metaphase in young anther tissue. 69 and 70. 1-M plates in polar view. Univalents are in outline. 71 and 72. Early 1-A stages in side view. The univalents congress to the plate either erratically or not at all. 73. 1-A, showing eight univalents, the maximum number seen. 74. 1-A. Univalents left in the equator of the spindle divide into their chromatids. 75. Second prophase. Each nucleus contains a different number of whole and half-chromosomes, and one half-chromosome (stippled) has been lost in the cytoplasm. See text for further explanation. 76. *D. taxifolia* Cunn. 1-M. 77. *D. taxifolia* f. *tetraflora*. 1-M. 78. *D. fascicularis*. Mitotic metaphase of the pollen grain division. One chromosome carries a poorly defined secondary constriction. All text-figures are at × 2600 unless otherwise stated.

Wright (1940) has shown that such a condition within a species may lead to diversity by the operation of random selection, and even to the fixation of disadvantageous characters. It has been proved that extensive species usually contain many gene mutations and chromosomal changes, which may attain different frequency concentrations in different parts of the species range, and Huxley (1942) has termed such changes in gene concentration "clines". The existence of clines and of discontinuous changes in gene frequencies has been adequately established in *Drosophila* (Spencer 1947).

In the species of *Darwinia* it is suggested that the dissection of the total species population into small more or less isolated breeding groups has permitted the establishment of discontinuous changes in the frequency of structural changes involving small chromosome segments, and of different gene mutations, some of which might affect chiasma frequencies, pollen fertility, and similar characters. Perhaps gene migration between breeding groups is sufficient to prevent the final attainment of equilibrium. Under such conditions pollen sterility cannot be accepted as due to any recent or extensive interspecific hybridization.

TABLE 7.

Pollen Fertility in Darwinia *spp.*

Mean Percentage Fertile Pollen for Plants and Populations. Means of Four Determinations per Plant.

Species.	Population.	1	2	3	4	5	6	7	8	9	10	Population Means.	F.	P.
D. biflora	Epping.	99·5	98·2	96·8	98·5	97·7	98·5	98·1	98·3	96·7	95·8	98·0	1·38	n.s.
grandiflora B.&S.	Berowra.	—	96·5	97·7	97·1	97·7	97·8	92·3	94·9	95·2	93·4	96·0	2·41	<0·05
v. *grandiflora* Benth... ..	Bulli.	86·0	94·9	97·9	94·3	96·3	92·8	93·9	96·7	98·3	68·3	93·3	15·06	<0·001
Do.	Helensburgh.	49·1	78·5	26·5	93·7	86·6	45·0	98·1	91·7	88·1	75·5	75·4	9·88	<0·001
intermedia ..	St. Ives.	84·0	48·2	55·2	80·0	82·4	33·5	44·2	53·8	67·9	50·9	59·7	12·93	<0·001
Do.	Loftus.	86·0	95·7	91·4	92·1	93·8	83·6	88·1	82·8	91·7	96·0	90·6	1·13	n.s.
taxifolia tetraflora	King's Tableland.	97·6	98·5	78·0	81·9	73·9	65·2	92·2	98·6	95·8	99·0	91·0	3·26	<0·01
fascicularis ..	Kuring-gai.	84·2	97·5	95·6	76·6	94·4	84·3	74·9	52·0	92·6	—[1]	85·7	8·37	<0·001
Do.	Helensburgh.	—[1]	91·4	63·5	98·3	99·8	96·8	—[1]	99·6	99·6	—[1]	95·6	79·72	<0·001
Do.	National Park.	83·7	99·9	93·3	65·2	53·1	63·2	17·2	73·5	91·0	—[1]	74·9	71·44	<0·001

[1] Data for plants eliminated owing to their very low fertility, close to 0%, of a different type and not suggesting meiotic breakdown.

[2] All means and the analysis of variance have been obtained in terms of the angular transformation of the data.

[3] Test for variance between populations. $F_{9,283}=15{\cdot}74$. $P<0{\cdot}001$.

3. *Verticordia.*

The two sections of this genus are entirely western in distribution. All the material examined belongs to the section *Euverticordia*, which connects closely with *Darwinia*.

Verticordia sp. Collected and forwarded by Miss Baird. n = 8.

At 1–M there are regularly eight bivalents on the plate (Text-figs. 82, 83, Plate v, fig. 9). First anaphase and second metaphase are usually normal (Text-figs. 84, 85), but rarely true chromosome bridges are formed (Text-fig. 86), indicating that the plant examined was heterozygous for a small inversion. Tetrad formation is regular.

Verticordia plumosa Druce. n = 8.

Material identified and forwarded by Miss A. M. Baird and Mr. G. Smith, Perth. Very few meiotic stages were obtained. The haploid number determined at 1–M (Text-fig. 87) and 1–A was in agreement with the previous material.

Verticordia farnesiana (?). n = 11.

Material forwarded by Miss Eardley, of Adelaide, from cultivated plants. The specific name is given as synonymous with *V. plumosa.* Meiosis in the material was regular, with a haploid number of 11, determined at 1–M and 2–M. At both stages one chromosome or bivalent is of relatively large size (Text-fig. 88).

The disagreement in chromosome number found in the material available suggests that *Verticordia* may occupy a very interesting evolutionary position in the Chamaelaucoideae, but verification and detailed study of the genus are required before this position can be established.

4. *Chamaelaucium.*

Ch. uncinatum Schau. n = 11.

Details of meiosis in this species have already been described. Numerous cultivated plants and some material of a red-flowered variety forwarded by Miss Baird have been examined without detection of either meiotic irregularities or of any considerable pollen sterility. One young seedling purchased from a Gordon nurseryman, however, showed very considerable pollen sterility (Plate vi, fig. 9). Unfortunately this plant failed to survive and its meiotic behaviour was not studied.

II. Subtribe Calythriceae.

The two genera in this subtribe are chiefly western. In *Calythrix,* the larger of the genera, the widespread *C. tetragona* is polymorphic and might well be divided into several species.

Calythrix tetragona Labill. n = 11.

Material examined from several localities was found to be uniform in cytological constitution and behaviour. At diakinesis (Text-fig. 89) eleven bivalents are formed, and one at least is associated with the nucleolus. At 1–M, 1–A and 2–M (Text-figs. 90–94, Plate v, figs. 16, 17) one of the bivalents is of relatively large size, and this large bivalent may be late in disjunction at anaphase. When first observed, this large bivalent was tentatively assumed to indicate fusion, but the presence of large bivalents in *Darwinia citriodora* and in *Verticordia* removes its significance in this respect.

Secondary association in the species is very marked (Text-figs. 90, 94), reaching a maximum of five secondary pairs at both 1–M and 2–M. In side view of 1–M, the large bivalent is seen to remain unassociated. Rare abnormalities occur in this, as in most other species of the Chamaelaucoideae. Text-figure 95 illustrates a first telophase with a persistent bridge and two dividing univalents in the mid-region of the spindle. The frequency of such conditions is too low to cause any serious pollen sterility. Plate v, figures 18 and 19, illustrates the failure of unpaired chromosomes to congress to the 1–M plate, and the misdivision of univalents at 1–A.

Calythrix Fraseri A. Cunn. n = 11.

Material from Miss Baird, Perth.

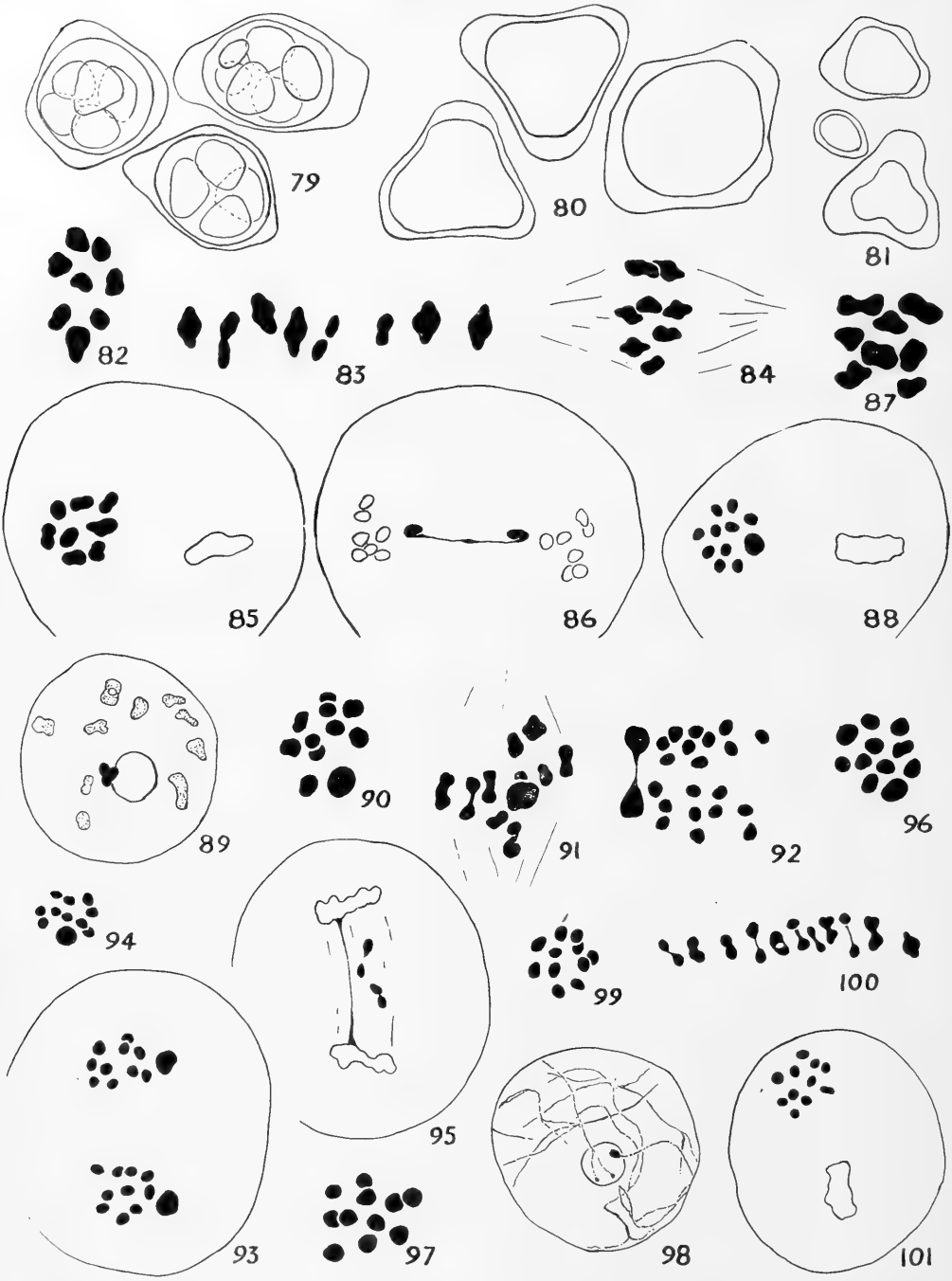
The count at 1–M (Text-fig. 96) confirms that made in *C. tetragona.* The size difference of one bivalent of the complement seems less marked.

III. Subtribe Thryptomeneae.

A subtribe of phylogenetic interest, since it contains genera such as *Scholtzia* which connect the Chamaelaucoideae with the Leptospermoideae.

Thryptomene.

Only one species has been examined.



Text-figures 79-101. 79-81. *D. intermedia.* 79. Tetrads with abnormal numbers of microspores (× 650). 80 and 81. Good and bad pollen grains (× ca. 650). 82-86. *Verticordia* sp. 82. 1-M. 83. 1-M or early 1-A in side view. The bivalents have been separated in drawing. 84. 1-A. 85. 2-M. 86. 2-A, showing an inversion bridge. The fragment could not be recognized. 87. *V. plumosa.* 1-M. 88. *V. farnesiana.* 2-M. One of the 11 chromosomes is distinctly larger than the others. 89-95. *Calythrix tetragona.* 89. Diakinesis. 90. 1-M, polar view. 91. 1-M in side view. 92. 1-A. In figures 90-92 the large bivalent is easily recognized and is often

Th. Mitchelliana F.v.M. n = 11.

The 1–M plate consists of eleven bivalents (Text-fig. 97). Secondary association is marked and reaches a maximum of five groups in approximately 10% of the pollen mother cells.

Micromyrtus.

This genus is closely allied to the preceding and is the only one represented in eastern New South Wales.

M. microphylla Benth. n = 11.

In the pollen mother cells of this species the meiotic process is closely similar to that in *Chamaelaucium*. A particularly good example of the association of two bivalent chromosomes with the nucleolus at zygotene-pachytene was found (Text-fig. 98), in which zygotene association of the chromosomes had not been completed, so that one bivalent appeared attached by two separate organizers. At 1–M and 2–M (Text-figs. 99, 101, Plate v, fig. 20) eleven bivalents are formed and show considerable secondary association. In side views of 1–M (Text-fig. 100) the bivalents are seen to be associated chiefly by terminal chiasmata and the secondary association of similar bivalents is also apparent.

Discussion.

(*a*) *Chromosome Arrangement and Secondary Association.*

Kuwada (1929) and his associates have discussed in detail the characteristics of chromosome arrangement on the metaphase plate at meiosis and have shown that stable configurations conform with those to be expected on the assumption that the determining factor is the repulsion between the chromosomes, acting in the limited space of the spindle. With equal repulsions the stable configuration will provide equal and maximum spacing.

Although secondary association was first observed by Kuwada in 1910, its significance was not appreciated until much later. Darlington (1928), Darlington and Moffett (1930), and Moffett (1931) drew attention to its significance as indicating ancestral homologies between the chromosomes concerned and made use of its occurrence to demonstrate the secondarily balanced nature of the Pomoideae. Lawrence (1931a, 1931*b*) has demonstrated its significance in *Dahlia*, and Catcheside (1934) has shown by a consideration of secondary association that secondarily balanced polyploidy occurs in *Brassica*. Since that time the occurrence of such secondary association has been used by many investigators for the inference of polyploidy or secondary polyploidy.

Secondary association is a variable phenomenon, and the maximum rather than the minimum degree occurring is of significance. It is likely to be most pronounced when the chromosomes are small and when, as in *Darwinia*, there is a marked prometaphase prior to the development of the spindle. Under such conditions the absence of secondary grouping of the chromosomes, and close conformity with the theoretically stable types of configuration may be accepted as evidence of lack of ancestral homology between the chromosomes. In all the species of *Darwinia* and in the related *Actinodium*, where the haploid number is 6, the frequency of the theoretically stable configuration 5 (1) (cf. Table 3) provides this evidence. In the 11-chromosome general *Chamaelaucium*, *Calythrix*, *Thryptomene* and *Micromyrtus* the theoretically stable configuration 8 (3) is rare, and maximum secondary association is given with the formation of five pairs, leaving six independent groups of one or two chromosomes. A similar condition has been reported in the Leptospermoideae (Smith-White 1948) and

late in disjunction. 93 and 94. 2-M, showing the large chromosome and different degrees of secondary association. 95. 1-A, showing a persistent chromosome bridge and laggard chromosomes which are dividing on the spindle. 96. *Calythrix Fraseri*. 1-M. 97. *Thryptomene Mitchelliana*. 1-M. 98-101. *Micromyrtus microphylla*. 98. Zygotene-pachytene, showing two nucleolar-associated bivalent chromosomes. 99. 1-M. 100. 1-M in side view. Chiasmata are terminalized. 101. 2-M. Secondary association is evident. All text-figures are at × 2600 unless otherwise stated.

there can be little doubt that the basic chromosome set for both tribes is six, the number now found in *Darwinia* and its allied genera.

(*b*) *Nucleoli and Attached Chromosomes.*

The occurrence of several nucleoli per nucleus is not infrequent, and DeMol in 1926 was perhaps the first to suggest a possible relation between nucleolar number and polyploidy. Since the discovery of satellited chromosomes by Navashin in 1912 their function as nucleolar organizers has also been established. Thus there is a close correspondence between the number of nucleolar chromosomes and the number of nucloli formed at telophase. Gates (1942) has reviewed this relationship between nucleoli and nucleolar chromosomes and their bearing on the inference of polyploidy. Although the number of nucleolar chromosomes and nucleoli may be increased either by polysomy or by translocation and reduplication of chromosome segments, Gates is of the opinion that truly basic diploids have only one pair of nucleolar chromosomes and that when the evidence of the number of nucleolar-associated chromosomes at prophase, and of the number of nucleoli formed at telophase supports the evidence of secondary association, the inference of secondary polyploidy is justified.

In the six-chromosome genera *Darwinia* and *Actinodium* only a single nucleolus has been observed in premeiotic pollen mother cell nuclei, and this has been presumably formed by the fusion of two initiated in the telophase of the previous diploid mitosis. At prophase of meiosis there is a single large and conspicuous heterochromatic body attached to the nucleolus, and at diakinesis a single bivalent is associated with the nucleolus. In interphase and recently formed microspore nuclei never more than a single nucleolus per nucleus has been observed. This evidence points to the inference that *Darwinia* has only a single nucleolar organizing chromosome in its haploid complement.

In *Chamaelaucium* and in *Micromyrtus* the presence of two separate nucleolar organizers associated with the nucleolus is frequently evident at pachytene of meiosis. At interphase and in recently formed microspore nuclei two nucleoli per nucleus are often found, but seem liable to early fusion. The evidence of nucleolar number and of the number of nucleolar associated chromosomes in the Chamaelaucoideae suggests that the number found in *Darwinia* is basic and the more frequent 11-chromosome genom is a derived one. The evidence fully supports the inference drawn from the study of secondary association.

(*c*) *The Basic Chromosome Number and the Phylogeny of the Myrtaceae.*

If the hypothesis that the basic number in the Myrtaceae is six and that the predominant number 11 has been derived from it by polyploidy and structural change is accepted on the basis of the evidence presented, several important questions need to be discussed.

(1) The actual method by which the 11-chromosome genom has originated is scarcely susceptible to experimental test, but polyploidy, followed by unequal translocations, permitting the loss of a centromere (Darlington 1937, Tobgy 1943) would seem to be the most probable method. The total loss of a pair of chromosomes, however, might not be impossible soon after or immediately consequent upon the occurrence of the tetraploid condition, particularly if the original tetraploid was subject to some meiotic irregularity.

(2) The implications of the hypothesis in regard to phylogenetic relationships in the family. The hypothesis put forward is incompatible with Andrew's view (1913) that the Myrtoideae is primitive, that the Leptospermoideae have been derived from the Myrtoideae, and that the Chamaelaucoideae represent an extreme specialization. The Chamaelaucoideae, and particularly the Euchamaelauceae, are shown to be primitive in respect to chromosome number, although they are certainly not so in some morphological features. It is possible, if features of geographical distribution are also considered, that the Myrtoideae in America and the Leptospermoideae-Chamaelaucoideae in Australia have evolved separately from primitive Myrtiflorae stock. Such an assumption would necessarily infer that the primitive stock possessed a haploid chromosome set

of six and that the derivation of a secondary number of 11 has occurred independently in the two groups. In view of the frequency of the number 11 in many families this independent origin is not unlikely. This assumption also means that the family as at present constituted is not a monophyletic one.

Data on chromosome number in the other families of the Myrtifloreae would be of value as indicating the basic number for the primitive stock of the order. These families are almost entirely tropical, and the Melastomaceae is the only family mentioned in Tischler's lists (1927, 1931, 1936, 1937, 1938) and in Darlington and Janaki (1944). The number of species in this family for which such data is recorded is small (Table 8). The haploid number of 28 reported by Suguira is too high to be basic, but the haploid number 12 reported for *Bertolonia* and three other genera is of interest, since it could be easily derived by polyploidy from a basic set of six. At least this evidence does not conflict with the present hypothesis.

TABLE 8.
Chromosome Numbers in the Melastomaceae.
(From Tischler's Lists.)

Species.	n	2n	Authority.
Centradenia floribunda	—	24–26	Heitz, 1926.
Bertolonia marmorata	12	—	Ruys, 1925.
B. aenea	—	28–32	Heitz, 1926.
Melastoma candidum	28	—	Sugiura, 1936.
M. sanguineum	—	56	Matsuura and Suto, 1935.
Miconia racemosa	12	—	Ruys, 1925.
Mouriria anomala	—	24	Ruys, 1925.
Memecylon floribundum	—	24	Ruys, 1925.
Triuranthera Winkleri	24	—	Ruys, 1925.

(3) The significance of the occurrence of secondary polyploidy in the evolution of the Myrtaceae. At the present time the 11-chromosome genom is almost universal throughout the Myrtaceae. The six-chromosome genom is found only in two genera of a single subtribe and must be regarded as a remnant. Obviously the 11-chromosome genom has proved more successful in the course of the evolutionary development of the group.

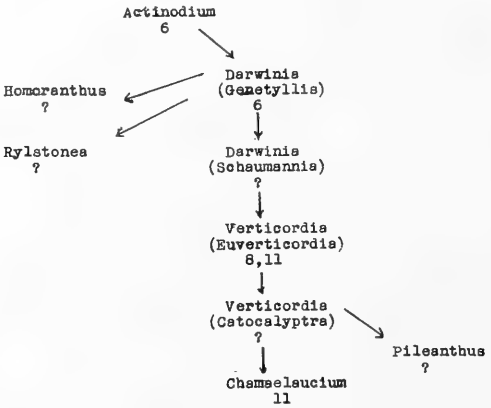
The importance of polyploidy in plant evolution and speciation has been discussed by many authors and has been reviewed by Muntzing (1936) and by Stebbins (1940, 1947). Babcock (1942, 1947) includes polyploidy as one of the important, but secondary, evolutionary processes, of lesser importance than gene mutation or chromosomal alterations, and Manton (1932) and Stebbins (1940) consider that polyploidy may lead to diversity of form and speciation, but that it is not of importance in the origin of major taxonomic groups. Stebbins points out that polyploidy leads to a reduction in the visible mutation rate and in the phenotypic expression of recessives, and would consequently lower the effect of selection; but Crane (1939) states that polyploids have a wider range of variation than diploids. It has been suggested by Aase (1935) that by the increase in the number of genes in the genom a greater total mutation rate is possible and that the presence of multiple sets of chromosomes provides protection against the immediate deleterious effect of most gene mutations. Polyploidy may thus allow greater polymorphism, which, as pointed out by Stebbins (1942), provides greater adaptability. Stebbins has more recently (1947) inclined to the view that the woody types of Angiosperms, in which chromosome numbers of 11 to 14 are common, have been derived from more ancient types with haploid numbers of 5, 6 or 7, a view supported by the data for the Leguminoseae (Senn 1938, 1942) and the Anonaceae (Bowden 1945), as well as by the present paper. The evolutionary dominance of higher chromosome numbers at the present time suggests that it has conferred some evolutionary advantage, either directly, as suggested by Muntzing (1936), or due to

the consequences of hybridization. Stebbins (1947) puts forward the view that not only is polyploidy a frequent consequence of hybridization, but that it may enable species previously inter-sterile to produce hybrids and so permit of an increase in the range of gene exchange.

Darlington (1937) points out that secondary polyploidy, by creating a new balance of genetic factors, is likely to cause more profound phenotypic changes than primary polyploidy. The frequency of secondary association in many plant groups with prime numbers of chromosomes in their haploid sets (e.g. 11, 13, 17) suggests that secondary polyploidy may be of considerable importance in the evolution of the Angiosperms.

Whilst gene mutation, small chromosomal alterations and segregation following hybridization have undoubtedly been responsible for nearly all speciation in the Australian Myrtaceae, the most important step in the origin of the group is considered to be the origin of the secondary haploid genom of 11 chromosomes. This step must rank as of equal importance with that concerned in the origin of the Pomoideae; the consequent increased variability has allowed an "explosive" speciation whilst the six-chromosome forms have been forced into a geographical corner, where they have suffered morphological specialization and specific restriction.

(4) The taxonomic and cytological sequence in the Euchamaelauceae. Within the Euchamaelauceae it is possible to arrange the genera in a more or less linear sequence on morphological grounds (Bentham 1866, Cheel 1922) with connecting species between the genera and sections of the larger genera. This sequence is illustrated diagrammatically below:



The cytological data presented in the present paper cover too few of the genera to support or deny this arrangement, but they are at least suggestive. A more detailed survey of the subtribe might well have important phylogenetic and taxonomic consequences.

Summary.

1. Seventeen species and varieties, representing seven genera and all three subtribes of the Chamaelaucoideae have been cytologically examined. Haploid numbers of 6, 8 and 11 have been found, the lower numbers being new for the Myrtaceae.

2. Evidence is produced that the haploid genom of six is primitive and basic for the tribe and family. This evidence concerns the occurrence of secondary association of chromosomes and the chromosome-nucleolar relationships.

3. The evolutionary and phylogenetic consequences of this hypothesis are considered. The origin of the 11-chromosome genom is considered to have begun an epoch in the evolution of the family.

4. The theory that the Chamaelaucoideae have been derived through the Leptospermoideae from the Myrtoideae is denied. The Chamaelaucoideae may be primitive to

the Leptospermoideae, but the Myrtoideae are more likely to have had a separate origin from primitive Myrtiflorae. The family is in this sense polyphyletic.

5. The data presented on pollen fertility in *Darwinia* reduce the significance of the inferences drawn by Lawson on the importance of interspecific hybridization as a cause of speciation in the family. It is suggested that variation in pollen fertility is a direct consequence of the dissection of the total species populations into small isolated breeding groups.

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DESCRIPTION OF PLATES V AND VI.

PLATE V.

(All photographs × 2000.)

Fig. 1. Pollen mother cells of *Actinodium Cunninghamii* in prophase. The heterochromatic nucleolar organizer is large and conspicuous. Acetocarmine.

Fig. 2. *Actinodium Cunninghamii*. 1-A in polar view. Acetocarmine.

Fig. 3. *Darwinia fascicularis*. 1-M, showing the 5 (1) configuration. Feulgen.

Fig. 4. *D. fascicularis*. 1-M. The 6 (0) configuration. Feulgen.

Fig. 5. *D. fascicularis*. 2-M. Feulgen.

Fig. 6. *D. grandiflora* B. & S. 1-M. Feulgen.

Fig. 7. *D. grandiflora* B. & S. 2-M. Feulgen.

Fig. 8. *D. intermedia*. 1-A, showing a dividing univalent. St. Ives population. Acetocarmine.

Fig. 9. *Verticordia* sp. 1-M. Acetocarmine.

Figs. 10-15. *Chamaelaucium uncinatum.* Feulgen-Light Green. Fig. 10. Resting pollen mother cell nucleus showing the heterochromatic nucleolar-associated chromatin. 11. The two nucleolar-associated bodies are shown attached to the single nucleus in a prophase nucleus. 12. 1-M. 13. Interphase, showing two nucleoli in each nucleus. 14. 2-M. 15. A pollen mother cell with microspore nuclei, one showing two nucleoli.

Figs. 16-19.—*Calythrix tetragona.* Acetocarmine. Fig. 16. 1-M. The large bivalent is conspicuous. 17. 1-M, side view. 18. 1-M, side view with two univalents near the poles. 19. 1-A, side view of the spindle showing misdivision of laggard chromosomes.

Fig. 20. *Micromyrtus microphylla.* 2-M. Acetocarmine.

Plate VI.

(All photographs × ca. 150.)

Fig. 1. *Darwinia fascicularis.* Kuringai. Plant No. 2.
Fig. 2. *D. taxifolia* f. *tetraflora.* King's Tableland No. 9.
Fig. 3. *D. intermedia.* St. Ives No. 6.
Fig. 4. *D. grandiflora.* Berowra No. 7.
Fig. 5. *D. grandiflora.* Berowra No. 1.
Fig. 6. *D. taxifolia* v. *grandiflora.* Bulli No. 10.
Fig. 7. *D. taxifolia* v. *grandiflora.* Helensburgh.
Fig. 8. *Chamaelaucium uncinatum.* Cult. National Park.
Fig. 9. *Chamaelaucium uncinatum.* Gordon seedling.

A REVISION OF THE AUSTRALIAN SPECIES OF THE GENUS LAGENOPHORA CASS.

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(Plate vii; thirteen Text-figures.)

[Read 31st May, 1950.]

Introduction.

Cassini (1816) erected the genus *Lagenifera* to accommodate specimens collected in Australia by Labillardière, and described by that author as a new species of *Bellis* (*B. stipitata*). In 1818 Cassini wrote "there are two corrections to make in my first number in the Bulletin of December, 1816: . . . and the name of the genus *Lagenifera* has been changed to that of *Lagenophora*". Although the earlier synonym has priority, it was never accepted in general usage, and in the opinion of the present writer, the later name should be retained.

The genus *Ixauchenus* was described by Cassini (1828) from a single specimen collected at Port Jackson by d'Urville, and which he considered was intermediate between *Lagenophora* and *Bellis*. This genus was listed as synonymous with *Lagenophora* by Bentham (1866).

A. Richard (1832) erected the genus *Microcalia* for Forster's *Calendula pumila* from New Zealand, but although he stated reasons for excluding this specimen from *Calendula*, he made no comparison with *Lagenophora*. De Candolle (1836) redescribed Richard's *Microcalia australis* as *Lagenophora Forsteri*.

Bentham, since he had at his disposal plants collected from the early days of Australian exploration, handled comparatively long series of specimens, where earlier workers had had to be content with one or two. As a result, he was able to note variations within species, and relegated many former names to synonymy. In the last fifty years collections in Australian herbaria have grown considerably so that as genera come under revision earlier workers' concepts of component species must sometimes be further modified when populations thought by them to be discrete, are shown to represent merely extremes in a continuously variable series.

The present writer is of the opinion that the species included by Cassini in his genus *Solenogyne* are generically distinct and that *Lagenophora*, in its revised sense, is represented in Australia by only two species.

Distribution of the Genus.

Lagenophora is widely distributed throughout the New World from Liukiu Island to the East Indies and Ceylon, throughout Australia, New Zealand and Subantarctic Islands, New Caledonia and Fiji, while seven species are recorded in Index Kewensis from South America. Since the original descriptions of the three species from the Sandwich Islands (*L. Maviensis* H. Mann, *L. Erici* C. N. Forbes and *L. Helena* C. N. Forbes) are devoted to vegetative characters, it cannot be determined whether they belong to this genus in its revised sense.

The original descriptions of the twenty-seven valid species of this genus are devoted almost exclusively to such vegetative character as would be most likely influenced by the habit, and variation within a species is not recorded. Such characters, unsupported by differences of a stable nature such as may be expected in the fruits, are insufficient for specific status and it is suggested that further work will show that a large proportion of these species are ecological variations.

Categories.

The general principles put forward in a previous paper (Davis, 1948) are applied also in this revision. On the material available the use of infraspecific categories is not

justified, and the very considerable vegetative variation shown by one species is seen to be continuous. The factors governing this variation have not yet been determined, but it is thought that they are ecological.

Evaluation of Taxonomic Characters.

A taxonomic character is one which is inherited as such and whose form is not influenced by the environment. It therefore remains constant under whatever conditions a particular plant may be growing, and is referred to as a primary taxonomic character. Habit, texture, shape and number of leaves are the result of the interaction between genetic factors and the environment and consequently should not in general be used as primary taxonomic characters. Secondary taxonomic characters are purely vegetative and are frequently, though not invariably, linked with primary ones. These are frequently of importance in identifying immature specimens in which the primary taxonomic characters are not yet developed.

In this revision a large number of specimens were critically examined and an attempt was made to evaluate the taxonomic importance of all characters whether previously used or not. The results of this comparison are set out below under appropriate headings.

Habit: Although *L. stipitata* shows very considerable variation in mode of growth it can be readily distinguished from *L. Huegelii*, which is usually a more robust plant with large bracts on the scapes. The two species can be separated on this character in the absence of fruits. The variation within *L. stipitata* was found to be continuous so no infraspecific categories were recognized.

Leaves show wide limits of continuous variation and are of no taxonomic value.

Indumentum of a septate-hairy nature is always present in some degree and consequently is to be regarded as a generic character.

Capitula are usually largest in *L. Huegelii*, but the lower limits of measurement in this species are overlapped by the upper limits of *L. stipitata*.

Involucral bracts are of little or no value in specific determination, their size depending on that of the capitulum as a whole.

Ray and disc florets are similar in both species, though they tend to be most numerous and smallest in *L. stipitata*.

Anthers bear a terminal appendage to the connective in both species and no variation was seen.

Stylar branches are dimorphic, and identical in similar florets in both species.

Receptacle varies in size in proportion to the size of the capitulum, consequently is usually largest in *L. Huegelii*.

Fruits are of primary taxonomic importance, though the difference between those of the two species is one of dimensions rather than character.

Specimens Examined.

Through the courtesy and co-operation of the Directors of the various Australian herbaria and several private collectors, a long series of specimens has been critically examined. These are all listed under the appropriate species, the source in each case being indicated as follows:

National Herbarium, Sydney (NSW).
National Herbarium, Melbourne (MEL).
Brisbane Herbarium (BRI).
University of Adelaide (AD).
State Herbarium, Perth (PERTH).
University of Tasmania (HO).
University of Florence (FLO).
J. M. Black (JMB).
J. B. Cleland (JBC).
F. A. Rodway (FAR).
N. A. Wakefield (NAW).

Specific Descriptions.

Both species have been redescribed from a longer series of specimens than was available to the original authors, and the specific limits have been extended to cover all variations. Marked departures from the usual condition are discussed separately in the text. The various measurements given are to be regarded more as a general indication of size than absolute criteria, since in all structures more or less considerable size variation is found on the same plant. All figures are camera lucida drawings.

Taxonomy.

COMPOSITAE, tribe ASTEROIDEA.

Lagenophora Cass., *Bull. Soc. Philom. Paris* (1818), 34.

Synonymy: *Lagenifera* Cass., *Bull. Soc. Philom. Paris* (1816), 199; *Ixauchenus* Cass., Dict. Sci. Nat., LVI (1826), 176; *Microcalia* A. Rich., Fl. Nova Zel., t. 30 (1832), 230.

Erect or ascending stoloniferous perennials from 2·8–39 cm. high, with a septate-hairy indumentum on all vegetative parts. Leaves up to 15 cm. long, 3·2 cm. broad, oblanceolate to elliptical in gross outline, acute to obtuse, with serrate, crenate or undulating margins, petiolate, basally clustered in erect plants, but commonly cauline as well when the plant has an ascending mode of growth. *Inflorescences* 1–15, 4–11 mm. diameter excluding the rays, borne on scapes or axillary peduncles which are provided with 3–11 linear to narrow elliptical bracts. *Involucral bracts* 35–54 in 3–4 rows, the innermost 2·2–5 mm. long, 0·5–1·2 mm. broad, linear to linear-lanceolate or obovate, obtuse or acuminate, with torn-ciliate margins and septate hairs on outer surface. Bracts become reflexed when fruits are shed. *Ray florets* 60–100, the rays 2·5–5 mm. long, 0·4–0·9 mm. broad in 3–4 rows, white, pale pink or bluish, rounded distally. *Disc florets* 12–20, staminate, sterile, corolla about 1 mm. long, tubular, 4–5 lobed distally. *Stamens* 4–5, present only in disc florets. *Anthers* united laterally forming a tube around the style, connective prolonged beyond the level of the pollen sacs to form a terminal appendage. *Stylar branches* dimorphic, those of the ray florets smooth, those of the disc papillose on outer surfaces. *Receptacle* 1·1–3·8 mm. broad, 0·5–1·9 mm. high, truncate hemispherical, bearing small elevations on the sides, corresponding to the points of attachment of the ray florets. The sterile disc florets are borne on the flattened summit of the receptacle. *Fruit* an inferior achene, 2·7–4·3 mm. long, 0·8–2·1 mm. broad, straw-coloured to dark brown, more or less crescentic to obovate, flattened, bearing a narrow beak curved away from the centre of the receptacle and surmounted by a paler collar.

Type species: *Lagenophora stipitata* (Labill.) Druce.

Key to the Species.

Erect or ascending plants with 3-6 linear or filiform bracts on scapes or peduncles. Fruits rather crescentic, tapering distally into a narrow curved microscopically glandular beak ..... 1. *L. stipitata*

*Erect plants whose scapes bear 5-11 narrow-elliptical sessile leaves. Fruits obovate, flattened, microscopically glandular distally passing abruptly into a curved neck ..... 2. *L. Huegelii*

1. Lagenophora stipitata (Labill.) Druce.

Rep. Bot. Exch. Cl. Brit. Isles, 1916 (1917), 630.

(Text-figs. 1–10; Plate vii.)

Synonymy: *Bellis stipitata* Labill., Pl. Nov. Holl. II (1806), 55; *Lagenophora Billardieri* Cass., Dict. Sci. Nat., XXV (1822), 111; *L. Billardieri* Cass. var. *α pusilla* DC., Prod. v (1836); 307; *L. Billardieri* Cass. var. *β media* DC., l.c.; *L. Billardieri* Cass var. *γ glabrata* DC., l.c.; *Ixauchenus sublyratus* Cass., Dict. Sci. Nat., LVI (1828), 176; *Lagenophora gracilis* Steetz in Lehmann, Pl. Preiss, 1 (1845), 428; *L. latifolia* W. J. Hooker, *Lond. Journ. Bot.*, VI (1847), 113; *L. montana* W. J. Hooker var. *α major* W. J. Hooker, l.c.; *L. montana* W. J. Hooker var. *β minor* W. J. Hooker, l.c.

Syntype series: Eleven specimens. "New Holland. Herbarium Webbianum ex herb. Labillardière" (FLO).

Erect or ascending stoloniferous perennials 2·8–39 cm. high, septate-hairy all over. Leaves usually petiolate, up to 15 cm. long, 2·2 cm. broad, oblanceolate to elliptical in gross outline, serrate, crenate or undulating margins, acute to obtuse, clustered basally,

cauline leaves present or absent. *Inflorescences* 1–15, 4–10 mm. diameter, borne on scapes or axillary unbranched peduncles bearing 3–6 linear or filiform acuminate bracts up to 5 mm. long. *Involucral bracts* 35–50, 2·2–4 mm. long, 0·5–1 mm. broad, in 3–4 rows, linear to linear-lanceolate, usually acuminate, torn-ciliate, septate-hairy on outer surface, reflexed when fruit are shed. *Ray florets* 60–100, 2·5–4 mm. long, 0·4–0·7 mm. broad, in 3–4 rows, female, white, pale pink or bluish. *Disc florets* 12–20, staminate, sterile, the corolla tube 4–5 lobed. *Receptacle* 1·1–2 mm. broad, 0·5–1·9 mm. high, truncate-hemispherical, with small elevations corresponding to the points of attachment of the florets. *Fruit* 2·7–4·3 mm. long, 0·8 mm. broad, straw-coloured to dark brown, more or less crescentic with a narrow curved microscopically glandular beak surmounted by a paler collar. The curve of the beak is directed away from the centre of the receptacle.

Habitat: Grassland and open forest.

Range: Coast and tablelands of eastern and southern Australia as far west as Spencer's Gulf, throughout Tasmania and islands of Bass Strait. Also recorded from tropical Asia.

Specimens examined: *Queensland*: Yarrabah, 7.1918, N. Michael, No. 414 (BRI); Atherton, 1.1919, C. T. White (BRI); Bellenden Ker Range, 1881, Karton (MEL); Bellenden Ker Range, 1891, S. Johnson (MEL); Herberton, 7.1.1912, F. H. Kenny (BRI); Mackay, A. Dietrich (MEL); Rockhampton, 10.2.1868, P. O'Shanesy (MEL); Rosedale, L. G. Dovey (BRI); Glasshouse Mts., sandy ground, 5.1910 (BRI); Virginia, near Brisbane, 16.9.1916, C. T. White and F. N. Evans (BRI); Pine River, I. W. Slatter (BRI); Ithaca Creek, F. M. Bailey (BRI); Helidon, J. Shirley (BRI); Moreton's Bay, Leichhardt (MEL); hills near Mt. Gravatt, "ray florets white", 14.3.1931, C. T. White, No. 7406 (BRI); Wellington Point, 11.1891 (BRI); Jimboomba, 5.1921, E. Cheel (NSW); Lockyer, 1875, Hartmann (MEL); Cunningham's Gap, 6.1892, F. M. Bailey (NSW); Inglewood, 3.1911, J. L. Boorman (NSW); McPherson Range, Easter, 1909, C. T. White (BRI).

New South Wales: Upper Barwan, 11.1882, B. Wilson (MEL); Tenterfield, 3.11.1886, E. Betche (NSW, MEL); Timbarra, New England, C. Stuart, No. 444 and 781 (MEL); Richmond River, 1877, C. Fawcett (MEL); Clarence River, 11.1875, Wilcox (MEL); Perretts (Tyringham) and Bald Hills station (Grafton-Armidale road), 12.1893, J. H. Maiden (NSW); Moona River, Walcha, 2.1884, A. R. Crawford (MEL); Port Macquarie, 2.1898, J. L. Boorman (NSW); Cundletown, Manning River, 12.1899, E. Cheel (NSW); Barrington Tops, in Swamp, 1.1934, L. Fraser and J. Vickery (NSW); Barrington Tops, in grassland, 7.1.1934, L. Fraser and J. Vickery (NSW); William's River, near Salisbury, 11.1.1934, L. Fraser and J. Vickery (NSW); Sandgate, Newcastle, 8.11.1902, "white flowers", R. H. Cambage (NSW); Christie's Gully, Mooney Mooney Creek, 9.1926, W. F. Blakely, G. P. Darnell-Smith and D. W. C. Shiress (NSW); Mt. Wilson, 4.1896, J. H. Maiden (NSW); Mt. Victoria, 1.1915, A. A. Hamilton (NSW); Jamieson Valley, 10.1894, J. J. Fletcher (NSW); Jenolan Caves, 11.1899, W. F. Blakely (NSW); Hawkesbury Agricultural College, Richmond, 23.10.1906 (NSW); Hornsby, chiefly in forest land, 4.1914, W. F. Blakely (NSW); Port Jackson, 7.1855, F. Mueller (MEL); Botany Bay, 7.1855, F. Mueller (MEL); Como, 6.3.1887, R. Collie (NSW); Mt. Kembla, 7.11.1891, J. J. Fletcher (NSW); Austinmer, 16.7.1933, 22.4.1934, G. Rodway (FAR); Seven Mile Beach, Berry, "in sand", 17.2.1930, F. A. Rodway (FAR); Kangaroo Valley, 30.4.1931, F. A. Rodway (FAR); Comerong Island, Shoalhaven River, 18.9.1932, F. A. Rodway (FAR); Roseby Park, Shoalhaven River, 10.1916, 30.3.1931, F. A. Rodway (FAR); Crookhaven Heads, 1.1.1941, F. A. Rodway (FAR); Huskisson, paddock, 29.3.1931, 2.4.1939, F. A. Rodway (FAR); Jerrawangala, 4 miles south of Wandandian, 11.3.1934, F. A. Rodway (FAR); Ulladulla, 1883, W. Bauerlen (MEL); Bateman's Bay district, 10.1890, W. Bauerlen (MEL), Sassafras, 26 miles s.w. of Nowra, about 2,000 ft., moorland, 14.6.1942, F. A. Rodway (FAR); Braidwood district, 3,000 ft., 10.1886, W. Bauerlen (MEL); Bimberi Peak, Queanbeyan, 5.1.1912, R. H. Cambage (NSW); the Peaks, Yarrangobilly, 24.1.1933, W. A. W. de Barzeville (NSW); Nimitybelle to Tantawonglo Mts., 12.1896, J. H. Maiden (NSW); Mt. Kosciusko, 5,500 ft., 11.1.1930, T. Harris (NSW).

Victoria: Hume River, 1886, S. Jephcott (MEL); Victorian Alps, 5,000 ft., 12.1921, A. J. Tadgell (MEL); around bogs at the Scout Hut, Middle Creek, Bogong High Plains, 5,400 ft., usually growing in the shade of Snow Gums, 4.2.1949, J. H. Willis (MEL); ranges near Omeo, metamorphic schist, 3,000 ft., Stirling (MEL); Snowy River, 1.1854, F. Mueller (MEL); near Mt. Ellery, 1886, E. Merrall (MEL); Nungatta, Genoa, Weatherhead (MEL); Genoa district, 2.1885, W. Bauerlen (MEL); moist sandy flats behind "Marshmead" homestead, N.E. portion of Mallacoota Inlet toward the Howe Range, growing with *Selaginella uliginosa*, *Caladenia carnea* var. *pygmaea* and other swamp vegetation, 24.10.1948, J. H. Willis (MEL); Cape Conran, N. A. Wakefield (NAW); Southern Croajingolong, N. A. Wakefield (NAW); Orbost, 3.1937, F. Robbins (MEL); Newmerella, near Orbost, 28.5.1902, Grove (MEL); Avon River, Gippsland, 1882, Howitt (MEL); Mt. Wellington, 3.1861, F. Mueller (MEL); McAllister River, F. Mueller (MEL); Mt. Baw Baw, 4-5,000 ft., 12.1860, F. Mueller (MEL); Red Jacket Creek, Victorian Alps, 1873, Gargurevich (MEL); Warburton rly. line between Killara and Woori-Yallock, 8.4.1904, P. R. H. St. John (MEL); Dandenong Range, 1.1853, F. Mueller (MEL); Dandenong Creek, 1.1853, F. Mueller (MEL); Anderson's Creek, between Doncaster and Warrandyte,

14.3.1902, P. R. H. St. John (MEL); Brighton, 10.1852, F. Mueller (MEL); Skye River, near Frankston, "in damp heath land scrub", 5.12.1904, P. R. H. St. John (MEL); Plenty Ranges, 11.1898, C. Walter (MEL, NSW); Upper Yarra, 10.1892, C. Walter (MEL); Maryborough, 1874, C. Maplestone (MEL); Bolmarra, near Ballarat, H. Wooster (MEL); near Ballarat, 1.1853, F. Mueller (MEL); Meredith, 1884, S. Johnson (MEL); near Geelong, 1885, J. B. Wilson (MEL); Geelong, H. B. Williamson (MEL); Curdie's River, 12.1873 (MEL); Hawkesdale, 11.1900, 11.1901, H. B. Williamson (MEL); Portland (MEL); Glenelg River, F. Mueller (MEL); Grampians, "wet flats", 11.1880, D. Sullivan (MEL).

Bass Strait: King Island, 1882, E. Spong (MEL); King Island, A. Neate (MEL); Flinders Island, 6.11.1844, Milligan, n.607 (MEL); Flinder's Island, 6.11.1844, R. C. Gunn, n.67 (HO).

Tasmania: Circular Head, 6.11.1837, Gunn, n.67 (NSW, HO); Woolworth, 30.3.1837, Gunn, n.833 (NSW); Emu Bay, 7.11.1841, Milligan, n.607 (MEL); Penquite, 10.12.1844, Gunn, n.67 (NSW, HO); Emu Bay and Hampshire Hills, 15.11.1841, Milligan (MEL); Hampshire Hills, Milligan, n.1024 (MEL); Surrey Hills and Macquarie Hbr., Milligan (MEL); Mt. Bischoff, 1884, F. Kayser (MEL); Waratah, 12.1924, A. H. S. Lucas (NSW); Mt. Barrow and foothills, Cc. Dorset, 1.1922, H. M. R. Rupp (NSW); Launceston, S. Hannaford (MEL); moist ground near Glendher, Launceston, 1.1862 (MEL); Wilmot, 12.1915, F. A. Rodway (FAR); South Esk River, 1886, S. Oakden (MEL); St. Marys, 11.1925, E. Rees (HO); Swanport, Storey (MEL); Somerset Co., "light bush", 14.2.1948, W. M. Curtis (HO); Arthur's Lakes, 18.2.1843, Gunn, n.833 (NSW); Interlaken, 3.1948, Liphott (HO); St. Peter's Pass, 24.1.1931, O. Rodway (HO); Mt. Dundas, 12.1893 (NSW); Margin of Lake St. Clair, 13.2.1845, Gunn, n. 1148 (NSW); National Park, about 200 ft., 27.2.1943, W. M. Curtis (HO); Russell Falls, 3.1910, E. Cheel (NSW); Mt. Dromedary, 2.1894, L. Rodway (HO); Mount Direction, 6.1922, F. A. Rodway (FAR); East Risdon, 13.11.1937 (HO); Bellerive, 2.1893, L. Rodway (NSW); Neck, marsh, 11.1.1837, Gunn, n.832 (NSW); Port Arthur, 1892, J. Bufton (MEL); near landslip, Glenorchy, 3.1.1881, Simpson (MEL); near Hobart, 12.1906, H.H.D.G. (JMB); Hobart, 11.1898, F. A. Rodway (FAR); Domain, Hobart, 1.1894, L. Rodway (HO); Huon Road, beyond Ferntree, dry heath, 1,000 ft., 2.2.1931, E. Atkinson (HO); Mt. Wellington, 1.1839, Gunn, n.1148 (NSW); Mt. Nelson, 11.1913, L. Rodway (HO); gully beside Mt. Nelson Road, 11.2.1937, Consett Davis (FAR); "Melaleuca", Blackman's Bay, 11.1935, F. A. Rodway (FAR); Southport, 1.1856, ? F. Mueller (MEL); Hartz Mts., 1.1901, A. H. S. Lucas (NSW); D'Entrecasteaux Rivulet, Recherche, 2.1852 (MEL); Van Diemen's Land, R. Brown (MEL).

South Australia: Mt. Remarkable, Campbell's Creek, "ray pink" (JMB); Lake Bonney, 1882, 1887, C. Wehl (MEL); Hindmarsh, 3.1.1924, J. B. Cleland (JBC); National Park, "burnt area, ligules bluish", 30.12.1939, J. B. Cleland (JBC); Mt. Lofty Ranges, 1.1894, O. E. Menzel (AD, NSW); Mt. Lofty, 1.4.1918, E. H. Ising (JMB); Aldgate, 12.1896, O. E. Menzel (NSW); Scott's Creek, 31.10.1906, H.H.D.G. (JMB); Coromandel Valley, 1850, Blondowsky (MEL); Clarendon, 12.1883, R. Tate (AD); Mt. Compass, 11.1930, J. B. Cleland (JBC); Square Waterhole, "pink", 22.2.1928 (JMB); Myponga, 26.1.1908, H.H.D.G. (JMB); Back Creek, Inman Valley, "pale pink", 5.11.1934, J. B. Cleland (JMB); Back Valley, Encounter Bay, 17.11.1930, 28.10.1934, J. B. Cleland (JBC, JMB); Hills between Inman River and Hindmarsh Tiers, 1.1926, J. B. Cleland (JBC); Hindmarsh Tiers, 22.5.1928, 13.1.1929, J. B. Cleland (JBC); Port Victor, Francis (AD); Encounter Bay, near Hall's Creek, 11.1.1934, J. B. Cleland (JBC); Davenport and Rivoli Bay, 10.1848, F. Mueller (MEL); Mt. Gambier, 1867, Wehl (MEL); Mt. Gambier, Wilhelmi (MEL); Port Macdonnell, 1.11.1941, J. B. Cleland (JBC).

Kangaroo Island: Rocky River, ray blue, 18.11.1924, J. B. Cleland (JBC, JMB); Middle River, 10.1905, E. Ashby (NSW); Peaty flats, 28.10.1924, T. G. B. Osborn (JMB); Rav. de Casvars, 3.2.1948, J. B. Cleland (JBC); Karatta, bare temporarily submerged flat near river, flowered purple, 9.11.1886 (MEL).

Through the courtesy of Professor Pichi-Sermolli, a photograph (Plate vii) was obtained of a sheet of eleven specimens in the herbarium of the University of Florence labelled "Herbarium Webbianum—ex herb. Labillardière—Nova Hollandia". The sheet bears the determination "*Bellis stipitata*", and is extensively annotated. Professor Pichi-Sermolli, in a personal communication, states: "all Labillardière's type specimens in our herbarium have extensive notes, like that of *Bellis stipitata*. In these notes the original description, or part of it, is quoted. Together with it, very often observations on the affinity of the species, further characters (not quoted in original description) of flowers and fruits and suggestions on systematical value of genera and species are added. Frequently in these notes some words are deleted and replaced with other ones, which are quoted in the original description of the book by Labillardière." Professor Pichi-

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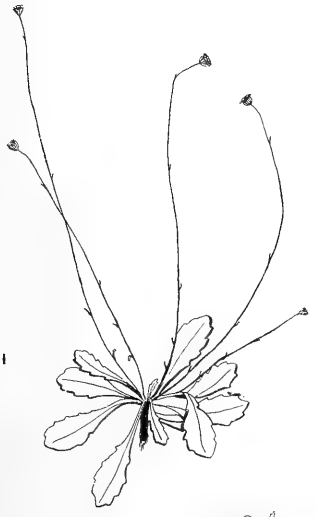
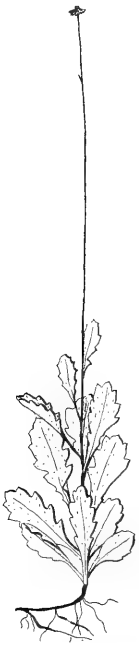
Text-figures 1-10, *L. stipitata*.

1-2, showing extremes of variation in habit, × ½; 3, capitulum, × 6; 4, receptacle, × 13; 5, ray floret, × 10; 6, disc floret, × 10; 7, stigma of disc floret, × 20; 8, anthers, × 10; 9, fruit from one of Labillardière's specimens at the University of Florence, × 10; 10, distribution.


1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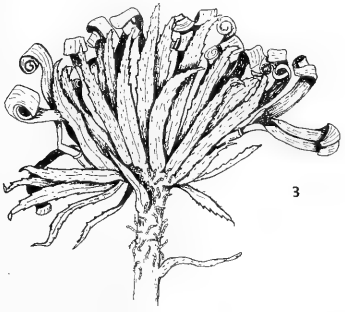
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10

Sermolli later wrote that he had sent a specimen of the handwriting in question to the British Museum, where it was identified as that of Labillardière. In habit, florets and fruits (Text-fig. 9) these specimens agree with the original description and accompanying figures, so have been nominated the syntype series, from which one specimen may be selected as lectotype.

It is of interest that in the National Herbarium, Melbourne, there are four mounted specimens from Steetz's collection labelled by Steetz "In insula Van Diemen leg. cl. Labillardière ipse, mecumque communicavit amicus cl. Sonder, 1843". The date, as in Steetz's other specimens, refers to the date of receipt by him and not that of collection. These specimens are very similar to those of the type series, of which they may be duplicates, and have been used as a basis of comparison in this work.

Cassini (1822) redescribed Labillardière's specimens as *Lagenophora Billardièri*, with the note "we have made this description from dry specimens collected by M. Labillardière at Cape Van-Diemen", consequently the specimens at Florence are also the type series of the later name. Cassini subsequently examined further material in both Labillardière's and Sieber's collections, and in 1826 erected three varieties based on small vegetative differences. His var. *media* corresponds with var. *typica* of modern usage, but the other two (var. *pusilla* and var. *glabrata*) are to be regarded as relatively small vegetative variations and were never accepted in general usage.

Ixauchenus sublyratus, a monotypic genus, was described by Cassini (1828) to accommodate a single dry specimen collected at Port Jackson by d'Urville, and which he distinguished from *Lagenophora* "by its disc composed of numerous flowers, perhaps hermaphrodite, or at the very least provided with a conspicuous false ovary, by its crown composed of numerous flowers arranged in two rows, by the involucral scales being homogeneous from one end to the other, and by the ovaries having a thick and sticky neck". Since these are characters of *Lagenophora* and not points of difference, *Ixauchenus sublyratus* was relegated to synonymy by later workers.

Bentham (1866), quoting Steetz (1845), listed *Brachycome pumila* in his synonymy of *L. Billardièri*, but, in view of the fact that in the original description Walpers (1843) stated "pappus shortly coronate", the present writer has omitted it from the synonymy, pending Walper's specimen ("Van Diemen's Land") being traced.

Lagenophora gracilis was reduced to synonymy by Bentham (1866). The type specimen, according to Steetz, was collected by Roë and deposited in the Court Herbarium of Vienna. In the National Herbarium, Melbourne, is a specimen (coll. "Thotsky, New Holland") labelled "*L. gracilis*" in Steetz's writing, and accompanied by an envelope containing three fruits. This envelope is inscribed by Steetz "mature achenes of *Lagenophora gracilis nobis*, from the herbarium of the Prince of Vienna", and suggests that these fruits were removed from the type specimen and retained by Steetz in his own collection. They are identical with those of *L. stipitata*.

Hooker's two species *L. latifolia* ("Mt. Wellington, Gunn") and *L. montana* are merely names given to vegetative variations and, as pointed out by Bentham (1866), pass gradually into the normal form. *L. montana* is divided by Hooker into two varieties, var. *α major* ("Marlborough and Woolnorth") and var. *β minor* ("Mt. Wellington, Gunn"). In the National Herbarium, Sydney, are specimens in Gunn's collection from Marlborough and Woolnorth which have been nominated *haptotypes* of the first variety, since they are almost certainly duplicates of those sent to Hooker. There is also a specimen collected by Gunn from Mt. Wellington, which is the type locality of both *L. latifolia* and *L. montana* var. *β minor*. Since this specimen agrees equally well with both descriptions, it is uncertain of which it is the haptotype.

Bentham (1866) drew attention to the wide variation in size of the inflorescences and rays of *L. Billardièri*, and on this established two varieties, *microcephala* with small inflorescences and short rays, the commonest form in tropical and subtropical regions, and *normalis*, with larger heads and rays, which is commonest in southern districts. He also pointed out that Labillardière's own specimens were almost intermediate between these two varieties. The present writer's observations have supported Bentham's state-

ments to the extent that the average diameter of the Queensland specimens examined was 5·5 mm. (excluding the rays), that of specimens from New South Wales 7·4 mm., while that of Victorian specimens was 8·6 mm. These measurements being taken from dried specimens are, probably, not in themselves accurate, but they do indicate a progressive increase in size from north to south of eastern Australia.

Variation in the habit of the plant (Text-figures 1, 2) as well as shape and arrangement of the leaves is considerable and the peaks of variation are in some instances far removed, but are connected by intermediate forms. As far as can be judged with only dried specimens, young plants possess a basal rosette of radical and clustered lower cauline leaves. As growth proceeds, and especially if dense herbage is present, a main stem bearing axillary peduncles and cauline leaves develops and the plant may assume an ascending type of growth. Unfortunately ecological notes are seldom available and how far vegetative features are influenced by environmental factors and age of the plant is a matter for speculation until some experiments being undertaken at the present time are completed. It is of interest that the single specimen examined from Lord Howe Island (28.8.1843, L. Leichhardt, MEL) was identical with mainland ones.

Variation in the fruits of *L. stipitata* is slight and confined to small size differences, the general shape and proportions remaining the same. The slight variation in the curvature of the neck noticed in some specimens did not appear constantly and it was thought to be influenced by drying condition. Bentham (1866) recorded "sometimes [the neck is] as long as the breadth of the achene, sometimes very short". By tracing the development of the fruit it has been found that the neck lengthens as the fruit matures, and in the young fruit is hardly apparent.

2. Lagenophora huegelii Benth., Enum. Pl. Hueg. (1837), 59.

(Text-figures 11–13.)

Synonymy: *Lagenophora Gunniana* Steetz in Lehmann. Pl. Preiss (1845), 431.

Perennial herbs 7–31·5 cm. high, the septate-hairy indumentum on scapes and leaves being visible macroscopically. Leaves radical, up to 14·3 cm. long, 3·2 cm. broad, oblanceolate, dentate to serrate, tapering proximally into a short or long petiole. *Scapes* 2–6, robust, bearing 5–11 narrow-elliptical, sessile, acuminate leaves up to 1·6 cm. long, 4 mm. broad, entire or distally and acutely toothed. These leaves are frequently confined to the lower half of the scape, but in some specimens are borne along the whole length, becoming smaller and finally filiform below the capitulum. *Inflorescences* 2–4, 8–11 mm. diameter excluding the rays. *Involucral bracts* 42–54, in several rows, the inner ones up to 5 mm. long, 1·2 mm. broad, narrow-obovate, usually obtuse to subacute, the margins torn-ciliate. Outer bracts narrower and shorter. *Ray florets* about 70, the rays 1–5 mm. long, 0·3–1 mm. broad, in about 4 rows, "white", "pink". *Disc florets* up to 3 mm. long, 1 mm. broad. *Receptacle*: 2–4 mm. broad, 1–1·8 mm. high, truncate hemispherical. *Fruits* 3·9–4 mm. long, 1·2–2·1 mm. broad, dark brown, obovate, flattened, microscopically glandular distally and usually bearing a few white straight hairs on each flat surface. Neck microscopically glandular.

Habitat: Forest land.

Range: Gippsland, Yarra River and Western Victoria, Hobart and Tasman Peninsula, in Tasmania, Yorke Peninsula, Kangaroo Island and south-east of South Australia, and south-west division of Western Australia.

Specimens examined:

Victoria: Gippsland (MEL); Yarra R., 10.1852, F. Mueller (MEL); Little R., Fullager (MEL); Wimmera, 1890, J. P. Eckert (MEL); West Wimmera, Walter (MEL); Shire of Dimboola, 2.10.1897, F. M. Reader (MEL); in dampish red gum forest about one mile east of Cherry Pool, towards the Black Range, upper Glenelg River area, 4.11.1948, J. H. Willis (MEL); Grampians, 10.1907, H. B. Williamson (MEL); Moyston, 10.1872, D. Sullivan (MEL); Wando R., forest, 2.10.1842, J. G. Robertson (NSW).

Tasmania: Hobart, 11.1898, F. A. Rodway (FAR); Hobart, 12.1923, A. H. S. Lucas (NSW); Domain, Hobart, 23.9.1943, W. M. Curtis (HO); Sandy Bay, 11.1894, L. Rodway (HO); Port Arthur, 1892, J. Bufton (MEL).

South Australia: Wirrabara forest, 10.1889, W. Gill (MEL); Yorke Peninsula, 1888, Beythieu (MEL); Tanunda, 9.1847, ? F. Mueller (MEL); South Para R., under trees,

21.10.1887 (MEL); Lyndoch, 8.10.1927, J. B. Cleland (JBC); Forest Range, "ligule very short", 14.10.1934, J. B. Cleland (JBC); National Park, Belair, 5.11.1905 (JBC); Lofty Ranges, F. Mueller (MEL); Mt. Lofty Range (JMB); Kuitpo, 23.9.1928, J. B. Cleland (JBC); Mt. Compass, "rays apparently very short", 14.10.1934, J. B. Cleland (JBC); Port Elliot, 9.1896, O. E. Menzel (NSW, AD); Hall's Creek, Encounter Bay, 23.5.1932, J. B. Cleland (JBC); Encounter Bay, 29.10.1934, J. B. Cleland (JBC); Mulgundawa, Lake Alexandrina, 3.10.1906 (JBC).

Text-figures 11-13, *L. Huegelii*.
11, habit, × ⅓; 12, fruit, × 10; 13, distribution.

Kangaroo Island: Karatta, "Grassy ground, riverside, flowers pink", 9.11.1886, O. Tepper (MEL).

Western Australia: South Hutt, Oldfield (MEL); Darling Ranges, "rays very short, white", 9.1907, M. Koch (MEL, PERTH); close to Perth, Preiss No. 118, "emi" 1843 (MEL); near Perth, 7.1911, F. Stoward (NSW); South Perth, 15.7.1923, W. M. Carne (PERTH); Midland Junction, 19.7.1897, R. Helms (NSW); Claremont, "in sandy soil, ray white", 6.1901, W. V. Fitzgerald (NSW); Cottesloe, 8.1908, J. B. Cleland (NSW); Swan R., Oldfield (MEL); North Fremantle, 10.7.1897, R. Helms (NSW); Armadale, 10.1911, F. Stoward (NSW); between Swan River and Geographe Bay, 1881, J. Forrest (MEL); Busselton, 1870, A. E. Pries (MEL); Blackwood River, 1875, Hester (MEL); Vasse R., Oldfield (MEL); Yallingup, 10.1909, J. H. Maiden (NSW); Lowdon, 8.1909, M. Koch (NSW); Big Brook, Warren District, M. Koch, No. 2584

(BRI); Gordon R., "wet places", Oldfield (PERTH); Tenterden, 23.9.1902, A. Morrison (PERTH); Stirling Range (MEL); Kalgan R., "grassy places", Oldfield (MEL); Mouliup, Albany, 3.10.1930, E. Elder (FAR); King George's Sound, 1889, Clarke (MEL).

The original specimens handled by Bentham were collected by Hügel from the Swan River and King George's Sound, but unfortunately are not available in Australia, and type selection has not been possible. In a redescription of this species (1866) Bentham lists a longer series of specimens, including "Wando R., J. G. Robertson", and three specimens from Robertson's collection bearing this locality label with the date (2.10.1842) are in the National Herbarium, Sydney. In the absence of the type, these specimens have been used as a basis of comparison.

Among specimens from Steetz's collection in the National Herbarium, Melbourne, is one bearing the type data of *Lagenophora Gunniana* ("In insula Van Diemen leg. cl. Gunn (Herb. Gunnian, No. 510)"), which has been nominated lectotype of this species, though the name was reduced to synonymy by Bentham (1866).

The relationship between *L. Huegelii* and *L. stipitata* is close and specimens of each species have been examined which tended towards the other. Typical plants of *L. Huegelii* are robust and rather coarse, with relatively large leaves on the scapes, but more slender specimens are sometimes seen which approach *L. stipitata* in appearance. In all cases, however, the broad scape leaves are diagnostic of *L. Huegelii* in the vegetative state. Certain specimens from the lower south-west of Western Australia (Big Brook, Gordon River, Yallingup, Lowdon) were much more slender than usual, and consequently were not unlike *L. stipitata*. In each case, however, well-developed bracts were present on the scapes and the fruits, though not mature, were more robust than in *L. stipitata*.

A number of specimens of *L. Huegelii* were examined in which the rays were extremely short, not exceeding 1 mm. in length. Although a discontinuity in this character appears to be present, breeding experiments and a much larger series of specimens are necessary before this variation can be more than recorded.

In view of their distribution, these two species might be regarded as subspecies, but the present writer is of the opinion that although both populations undoubtedly arose from a common ancestor comparatively recently, they have now diverged beyond subspecific status and are best regarded as distinct but closely related species. Text-figures 5–8, although drawn from *L. stipitata*, are equally applicable to *L. Huegelii*.

Acknowledgements.

In carrying out this work I am indebted to the Directors of various herbaria and private collectors who generously placed collections at my disposal. I would like particularly to acknowledge the co-operation in this respect of Mr. R. H. Anderson of the National Herbarium, Sydney, Mr. A. W. Jessep and Mr. J. H. Willis of the National Herbarium, Melbourne, Mr. C. T. White of Brisbane Herbarium, Professor J. G. Wood, Professor J. B. Cleland and Mr. J. M. Black of Adelaide University, Mr. C. A. Gardner of Perth Herbarium, Miss W. Curtis of the University of Tasmania, Dr. F. A. Rodway of Nowra, and Mr. N. A. Wakefield of Cann River, Victoria.

Finally, I am indebted to Professor R. Pichi-Sermolli of the University of Florence for sending photographs of Labillardière's specimens in that herbarium, one of which is reproduced in this paper, and carrying out examinations of these specimens on my behalf.

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DESCRIPTION OF PLATE VII.

Photograph of sheet of specimens in the herbarium of the University of Florence collected in Australia by Labillardière and named by him *Bellis stipitata*.

CORRIGENDUM TO REVISION OF THE GENUS BRACHYCOME CASS. PART III.

(PROC. LINN. SOC. N.S.W., lxxiv (1949), p. 151.)

The record of *Brachycome Mulleroides* from the Snowy River is erroneous. These specimens have since been shown to be *B. ptychocarpa*.

A REVIEW OF AND CONTRIBUTION TO KNOWLEDGE OF PHYLLOGLOSSUM DRUMMONDII KUNZE.*

By Frances M. V. Hackney, D.Sc.

(Twenty Text-figures.)

[Read 31st May, 1950.]

Introduction.

The genus *Phylloglossum* (Lycopodiaceae) is monotypic, being represented by the species *Phylloglossum Drummondii*. This species is limited in its distribution to parts of Western and South Australia, Tasmania and New Zealand. The sporophyte generation was first described by Kunze in 1843, and has received much attention from Bower, Jeffrey, Osborn, Sampson and several other investigators. The gametophyte generation is imperfectly known, the only original descriptions being those of Thomas, Sampson and Holloway.

However, despite the fact that so much has been done to elucidate the morphology and anatomy of the sporophyte, the literature on the subject is scattered and somewhat fragmentary. There is need of a comprehensive and connected account of *Phylloglossum* in its various aspects. Furthermore, the investigations carried out by the writer throw a little more light on certain controversial aspects of the morphology and anatomy. It was for these reasons that the present paper was prepared.

The sporophyte generation will be described and discussed first. The literature dealing with the gametophyte generation and the embryo sporophyte will then be reviewed.

The final section of the paper will be devoted to a discussion of the phylogenetic status and affinities of *Phylloglossum*.

Materials and Methods.

Most of the material was obtained from Adelaide, South Australia. Some of this material was germinated at Sydney University by Dr. P. Brough, who prepared from it slides showing the development of the "dropper" and young tuber (see later). A few of the plants examined were collected from swampy ground near Cannington, a suburb of Perth, Western Australia, by Miss A. M. Baird, University of Western Australia. The plants from South Australia were all examined in the preserved condition, but some specimens from Western Australia were still living while under examination.

Method of Preparation for Microscopic Study.

The material was fixed in 1% chromoacetic acid, washed thoroughly in water and subsequently passed through successive concentrations of alcohol from 10% to 100%. It was then passed through mixtures of alcohol and chloroform, and thence to pure chloroform; it was finally embedded in pure paraffin wax (melting point 51°–52°C.) and serial microtome sections were prepared.

The Sporophyte Generation.

Gross morphology of the plant.

Phylloglossum Drummondii is a small tuberous perennial which attains a total length of 1·5 to 5·0 cm. during the growing season (autumn and early spring, i.e., May to October, approximately). The general features of a typical well-developed fertile plant are shown in Text-figure 1*a*. Above ground level appears a crown of simple quill-like leaves, generally not more than 3 cm. in length. Poorly developed plants

* The investigations described in this paper were carried out while the writer was a fourth-year honours student in Botany at the University of Sydney.

bear only one or two leaves, but well-developed plants may have as many as twenty. A young plant in its first year produces only one leaf, and the number is usually increased in the second year. However, the number of leaves produced does not depend entirely on the age of the plant, since some young plants complete their first three years of growth without producing more than one leaf in each growing season, while others produce several leaves in their second year.

In the mature fertile plant (Text-figure 1*a*) the axis terminates in a comparatively long peduncle, bearing a small cone. Measurements taken from various plants show that the length of the cone varies from 0·4 cm. to 1·0 cm., approximately, according to the size of the plant. The peduncle is nearly always unbranched, but rare specimens have been reported in which the peduncle bifurcates into equally well-developed portions. Thomas (1901) states that "the branching always takes place above the lowest sporophyll, sometimes quite at the base of the spike, near the lowest leaf (sporophyll), sometimes further up, or even close to the apex of the strobilus". However, Bower (1908, p. 297) presents a figure in which the peduncle is branched below the lowest sporophyll, the two branches being unequally developed.

The cone bears short, rather fleshy sporophylls, each of which gives rise to a single reniform, homosporous sporangium on its adaxial surface. On dehiscence the valves gape, allowing the yellow spores to be shed gradually.

From the region of the stem at the base of the crown of leaves arise from one to three relatively long, thick, unbranched roots, which run horizontally just below the surface of the ground. These roots are covered throughout their length with root hairs. Also embedded in the soil are two stalked tuberous bodies; one of these, referred to as the "current tuber", has given rise to the present year's growth; the other is the "young tuber", by means of which the plant is enabled to perennate. The stalk, or "dropper", of the young tuber arises from the stem of the plant which has been produced by the growth of the current tuber.

All vegetative parts of the plant except the young tuber die at the end of the growing season. The young tuber lies dormant in the soil until the following autumn, when growth is initiated. Germination of tubers to produce either sterile or fertile plants has been described by Bower (1885) and will be referred to later in this paper.

The Relation of External Morphology to Ecological Conditions.

Osborn (1919) has made some observations on the ecology of *Phylloglossum* growing near Adelaide, South Australia. He points out that there are two sets of climatic conditions which tend to alter the ground level in the area where *Phylloglossum* has been studied by him. During the dry summer months the surface of the soil may become desiccated and wind erosion may take place. During the wet winter months, or even following occasional heavy summer rains, there may be a flow of water over the surface of the soil. This results in erosion of soil from some areas and subsequent deposition of soil over other areas. Alterations in ground level due to these climatic factors would not amount to more than a few millimetres rise or fall, but these variations could influence the growth of a plant as small as *Phylloglossum*.

Osborn has paid special attention to the behaviour of the tuber as an organ of perennation. The developing "dropper" or young tuber stalk usually attains such a length as to cause the young tuber and the current tuber to lie side by side in the soil. However, the young tuber is sometimes formed several millimetres above or below the current tuber. Osborn considers that "the depth at which the new tuber is formed in the soil in relation to ground level is not a matter of chance, but is the result of definite growth of the plant producing it. If for any reason the current tuber is buried by deposition of more soil above it, the burial is compensated for by the development of a short stalk bearing the new tuber. Conversely, if, by removal of the surface soil, the current tuber is brought near the surface, the new tuber is sunken to a greater depth." Statistical analysis of data showed that over 80% of the plants examined formed their new tubers at depths of 9 to 12 mm.

The existence of *Phylloglossum* in some areas is very precarious, being threatened alternately by burial and desiccation, as a result of the ecological conditions under which it grows. In plants where considerable adjustment for depth of tuber has to be made, or where the early onset of the dry season halts growth, the new tuber may be smaller than that produced in the previous growing season. Thus the plant does not always develop steadily from season to season towards its maximum size.

From the number of old tuber coats in the immediate vicinity of the plant it is sometimes possible to assess its age. Osborn gives figures showing a single-leaved plant entering on its fourth year, a two-leaved plant in its fifth year, and a three-leaved plant entering on at least the sixth year of its existence.

Vegetative Multiplication in Phylloglossum.

The tuber of *Phylloglossum* is generally regarded as an organ of perennation rather than of vegetative multiplication. As a rule, only one tuber is formed by each plant at the end of the growing season. Occasionally, however, two tubers are formed. Thomas (1901) was the first to observe double tuber formation in *Phylloglossum*. He recorded that this phenomenon occurred most frequently in larger plants, but sometimes occurred in smaller plants. One plant possessing only a single leaf was observed by Thomas to be forming two new tubers. The two tubers may arise on the same side or on opposite sides of the plant, simultaneously or in succession. The present writer observed only one small plant (three leaves) having two new tubers. In this specimen the presence of two tubers appeared to have been due to the dichotomous branching of a single dropper (see Text-figure 1*b*).

The infrequency with which *Phylloglossum* forms more than one new tuber indicates that double tuber formation is not of much significance as a method of vegetative multiplication. Sampson (1916) mentions five plants, out of a collection of one hundred, which possessed two new tubers apiece. The present writer, after examining over one hundred plants, has found only one possessing two new tubers.

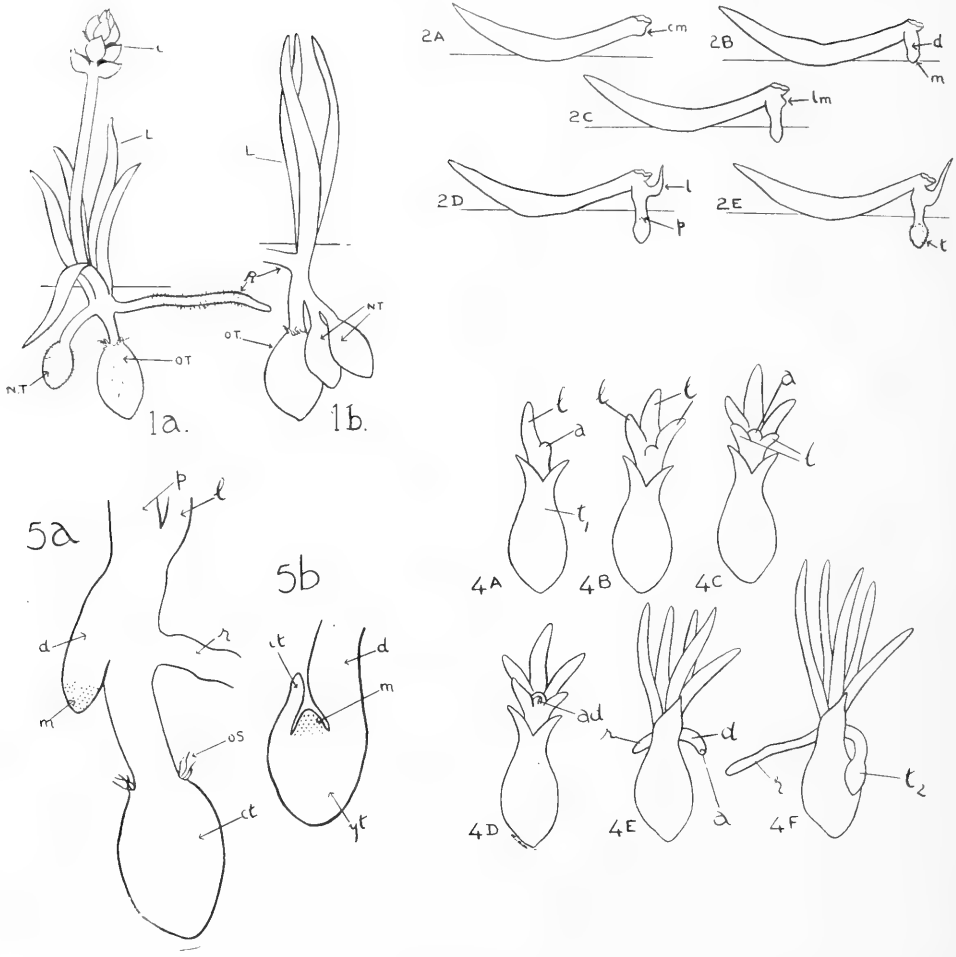
Osborn (1919) has recorded and described the regeneration of *Phylloglossum* plants from detached leaves. This method of vegetative reproduction may be compared with that of *Lycopodium ramulosum*, in which gemmae are produced from old roots and detached leaves (Holloway, 1916). Osborn first observed vegetative reproduction of *Phyloglossum* in the field and later succeeded in promoting it experimentally in the laboratory. Detached leaves kept on moist soil under favourable conditions may retain turgidity and greenness for two or three months. Curvature of the leaves in a vertical plane is a frequent phenomenon preliminary to the initiation of vegetative propagation. The first step in vegetative propagation is the development of one or more irregular, greenish-yellow cell masses at or near the proximal end of the leaf. From a growing point differentiated on the lower surface of the cell mass a dropper and tuber develop which resemble those of a mature plant. Occasionally more than one dropper and tuber develop from one cell mass, due to the initiation of more than one meristematic zone. After the development of the tuber one or more minute "leaflets" may be developed from the cell mass. The young plant, consisting of dropper, tuber and "leaflets", is then complete and capable of carrying on an independent existence. Text-figure 2 shows diagrams indicating stages in this method of reproduction (drawn by the writer from Osborn's description).

Osborn was unable to give any estimate of the frequency with which new plants were formed from detached leaves under field conditions. When plants of *Phylloglossum* are turgid the leaves are very brittle. In Osborn's opinion, a blow from a falling twig or a wind-swept leaf might be sufficient to break them. Under suitable conditions production of new plants from detached leaves might be of considerable importance in the field.

Detailed Morphology and Anatomy of the Sporophyte Generation.

THE ROOT.

It is unusual for a root to be formed during the first year or two of the life of the plant. The function of absorption during this time is presumably carried on directly



Text-figures 1, 2, 4, 5.*

Figure 1, *a* and *b*. Two mature plants of *Phylloglossum*. Figure 1*a* represents a typical well-developed fertile plant, while Figure 1*b* represents a small sterile plant bearing two new tubers (abnormal). Figure 1*a*, × $3\frac{2}{3}$; Figure 1*b*, × $5\frac{1}{2}$. n.t. = new tuber; o.t. = older tuber; l = leaf; r = root; c = cone; horizontal line represents soil level.

Figure 2, Diagrams A to E. Vegetative reproduction of *Phylloglossum* from detached leaves (see text), × $3\frac{2}{3}$. cm. = cell mass; d = dropper or stalk of tuber; m = meristematic zone; lm = "leaflet" meristem; l = "leaflet"; p = invaginated growing point; t = tuber. Drawn from description by Osborn (1919).

Figure 4, Diagrams A to F. Diagrams illustrating Bower's description of germination of the tuber at the onset of the growing season (see text), × $4\frac{1}{2}$. A = one-leaved stage; B = three-leaved stage; C = five-leaved stage; D = apical growing point depressed; E = root and dropper of tuber developing; F = almost fully developed sterile plant; a = apical growing point; l = leaf; t_1 = tuber; ad = depressed apical growing point; r = root; d = dropper of new tuber; t_2 = new tuber.

Figure 5, *a* and *b*. Development of the young tuber in a fertile plant (× 9) (from a slide belonging to Dr. P. Brough, University of Sydney). Figure 5*a* shows the dropper beginning to develop, with the meristematic apex directed downwards. Figure 5*b* shows the young tuber at a later stage in longitudinal section, with the meristematic apex directed upwards and enveloped in the invaginating tissue. See text for details. ct = current tuber; os = remains of dropper of ct; m = meristematic apex of new tuber; d = dropper; r = root; p = peduncle; l = leaf; yt = young tuber; it = invaginating tissue.

* For Text-figure 3 see page 139.

through the tuber wall, which bears many rhizoid-like outgrowths. Specimens past the second growing season usually bear one to three long, straight, unbranched roots, covered with numerous root hairs. Bower (1885), describing the germination of the tuber at the onset of the growing season, showed that the root is of exogenous origin. It originates as an excrescence on the surface of the main axis. Furthermore, the superficial cells of the very young root undergo both periclinal and anticlinal divisions, so that there is no clearly marked dermatogen. As development proceeds, periclinal divisions become more and more frequent at the growing tip; this results in the formation of a root cap.

The internal structure of the mature root is very simple (see Text-figure 3). The epidermal cells give rise to unicellular root hairs and enclose a broad cortex with large intercellular spaces. In the material examined by the writer the outer cortex had a distinctly lacunar appearance in both longitudinal and transverse section. This feature was noted by Wernham (1910), but other writers do not mention it. It is too definite to be an artifact due to the method of fixation of the tissues. It has probably been developed in response to the high moisture content of the habitat and consequently will vary in degree in material from different localities. The presence of lacunae in the lower (subterranean) regions of the stem (see later) and of large air spaces in the aerial parts of the plant may also be due to the same cause.

The cortex is bounded internally by the endodermis. In *Phylloglossum* the root is the only organ in which an endodermis has been recognized. The stele consists of xylem elements surrounded by small, closely packed parenchymatous cells. In the root figured above (Text-figure 3) the xylem was diarch, but in other roots examined it was monarch. Bower refers to the small parenchymatous cells surrounding the xylem as phloem. They differ from the cortical cells in having smaller dimensions and lacking intercellular spaces, so probably represent a rudimentary or reduced type of phloem. In longitudinal section they were observed to be elongated, with oblique end walls. They were similar in all respects to the "phloem" cells shown in Text-figures 15, *a* and *b*, occurring in the stem and leaf.

THE TUBER.

For many years the morphology of the tuber was a puzzle to botanists. Bower (1885) observed and described in detail the germination of a number of tubers of *Phylloglossum*. A brief résumé of his observations will be of interest at this stage. At the time of Bower's work the structure of the tuber was not understood. The tuber was misinterpreted as being homologous with the protocorm of *Lycopodium* (see later section). Consequently, parts of Bower's original description of its germination are presented in terms which are now known to be inapplicable. However, the actual observations recorded are accurate, and, in more up-to-date terms, can be summarized as follows. At the onset of germination the growing point sunken in the apex of the tuber elongates to give rise to the new axis of the plant. The first leaf then makes its appearance as a lateral outgrowth upon the axis. Other leaves are then produced to form a crown. According to Bower, these later leaves are usually produced in pairs "with some irregularities of arrangement". However, later and more detailed work indicates that the arrangement of the leaves is more generally a much-compressed spiral (see later section). Bower records that "in plants which do not produce cones, the apex of the plant becomes depressed after the production of the leaves and the young tuber begins to develop from a growing point in the centre of this depression. As a result of specially localized intercalary growth, the growing point of the tuber gradually becomes inverted; the tuber stalk elongates and enters the soil. Subsequently the meristematic apex of the tuber is turned once again into the vertical position as a result of increased growth on one side. During this period of growth the first root arises from the side of the plant opposite to that on which the new tuber is being formed."

Text-figure 4 shows a series of diagrams drawn by the writer from Bower's description of germination as above.

In those plants which produce cones the apex does not become depressed, but grows up in an erect position and produces the sporophylls. In such plants the young tuber is recorded by Bower as developing from a growing point in a depression near the base of the peduncle.

Bower described the mode of germination of *Phylloglossum* as "strikingly similar to the embryonic stages of *Lycopodium*". Believing the tuber of *Phylloglossum* to be homologous with the protocorm of *Lycopodium cernuum*, he was of the opinion that "we may regard *Phylloglossum* as a form which retains, and repeats in its sporophore generation, the more prominent characteristics of the embryo as seen in *Lycopodium cernuum*". He concluded that *Phylloglosum* was "a permanently embryonic form of Lycopod", regarding it as the most primitive in structure of all living Pteridophytes. However, detailed anatomical studies reveal striking differences between the tuber of *Phylloglossum* and the protocorm of *Lycopodium*. The resemblances between the two structures are superficial. The phylogenetic status and affinities of *Phylloglossum* will be discussed in detail later in this paper.

The tuber, whether that of a sterile or a fertile plant, develops exogenously. Text-figure 5 shows the young tuber developing in a fertile plant. During development the orientation of the growing point is gradually inverted, owing to increased meristematic activity on one side of the distal region of the dropper. A continuation of this growth gradually invaginates the organic apex, which consequently is no longer visible externally. This development finally leaves the growing point at the base of a long narrow canal.

The mature tuber consists of a stalk or dropper and a swollen basal storage region (the tuber proper). Text-figure 6 shows the young tuber in longitudinal section. The bulk of the tuber proper consists of thin-walled storage parenchyma. This tissue is composed of rather large isodiametric cells with very few and very small intercellular spaces. Each cell has a prominent nucleus and is packed with minute starch grains. The density of the cell contents here is in marked contrast with those of the corresponding cells of the current tuber, which are almost exhausted of carbohydrate at the end of the growing season. The storage tissue is surrounded by several layers of elongated, transparent cells with scarcely any contents. There is a well-defined epidermis, consisting of cells which are elongate in longitudinal section and have pits and peculiar thickenings on their radial walls (cf. Bower, 1885). There is a nucleus in each epidermal cell. Some of the epidermal cells give rise to prolonged root-hairlike outgrowths which may be cut off from the parent cells by walls.

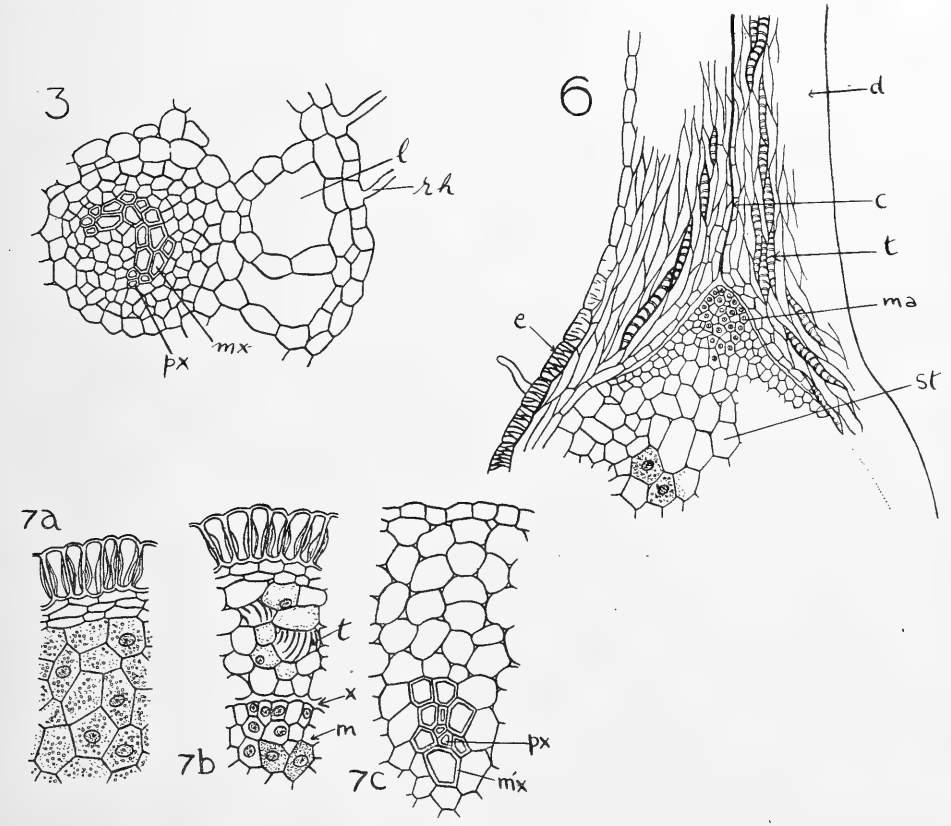
The meristematic apex appears as a cone of tissue above the storage region. The cells have large nuclei, each with one nucleolus, and dense cytoplasmic contents.

Above and around the meristematic apex is the narrow canal formed by the growth of the invaginating tissue. This canal has been traced from the region immediately above the organic apex to the top of the mature dropper.

Unlike those of the tuber proper, the epidermal cells of the dropper are not thickened. The main tissue of the dropper is composed of narrow, elongated parenchymatous cells with pointed ends. The vascular strand is not in the centre of the dropper, but on one side of the central canal, from which it is separated by some parenchymatous cells. It is composed of weakly lignified scalariform tracheids, similar in shape to the parenchymatous cells. In the region encircling and enclosing the meristematic apex of the tuber is an inverted funnel of tracheidal tissue. This has been described by both Wernham and Osborn as an expansion of the vascular strand of the tuber stalk. Such an interpretation may be true, but, on the other hand, the tissue in question may have been formed by the convergence of several small tracheidal strands developed independently of one another in the invaginating tissue.

There is apparently no highly developed phloem in the tuber stalk. Around the tracheids of the tuber strand are a number of elongated parenchymatous cells which may represent a rudimentary type of phloem (cf. those already described in the root).

The anatomy of the tuber has been further studied from transverse sections cut at various levels. The storage tissue appears much the same in transverse section as in longitudinal section (see Text-figure 7*a*). It is surrounded by parenchymatous cells of the inner wall, with no sign of vascular tissue. The epidermal cells are oblong in transverse section and the thickenings on their radial walls are plainly seen. These thickenings are well developed and markedly striated. Bower (1885) reported that the



Text-figures 3, 6, 7.*

Figure 3. Transverse section of root, × 100. px = protoxylem; mx = metaxylem; l = lacuna: rh = root hair.

Figure 6. Longitudinal section of young tuber, × 40. d = dropper; c = canal; t = tracheids: ma = meristematic apex; st = storage tissue; e = epidermis.

Figure 7, *a*, *b* and *c*. Figure 7*a*: epidermis and storage tissue as they appear in transverse section of the new tuber, × 80. Figure 7*b*: tissues seen in transverse section of young tuber at level of meristematic apex; m = meristematic tissue; x = canal; t = tracheidal cell. Figure 7*c*: tissues seen in transverse section of dropper; px = protoxylem; mx = metaxylem.

thickenings gave a mauve colour with Schulze's solution and were not stained with aniline sulphate; he concluded that they were not lignified. In some of the sections cut by the writer the thickenings stained red with safranin, suggesting lignification, but in other sections they remained unstained. When sections from mature tubers were treated with sulphuric acid and iodine the thickenings remained colourless, indicating that they did not consist of cellulose.

* For Text-figures 4 and 5 see page 136.

In a transverse section of the tuber at the level of the apical growing point the latter appears as a central circular mass of meristematic cells, separated from the surrounding tissue by the canal (see Text-figure 7*b*). The parenchymatous cells of the invaginating tissue are roughly isodiametric in transverse section and their contents are scanty. There is a small nucleus in each cell. The epidermal cells are thickened as described above. In transverse section the extent of the vascular tissue in the invaginating tissue is noteworthy. In some specimens it is represented by a few degenerate tracheids grouped on one side of the canal. This type of vascular system appears to be confined to the smaller tubers. In the larger ones a more interesting development is seen, inasmuch as a more or less complete ring of degenerate tracheids appears in the invaginating tissue. This ring corresponds to the rim of the funnel of tracheids seen in longitudinal sections of a large tuber (Text-figure 6). In transverse sections of the tuber at the level of the meristematic apex these tracheids are seen in oblique section. The thickenings on their walls are not pronounced and do not stain with safranin. Those in hand-cut sections of a mature tuber gave the cellulose reaction with sulphuric acid and iodine. Evidently lignification does not take place here. Text-figure 7*c* shows the dropper of the tuber in transverse section. The mesarch nature of the vascular strand is clearly evident.

The diagrams shown in Text-figure 8 represent the vascular tissue seen in a series of transverse sections at various levels of a large tuber and its dropper (from a fertile plant). Text-figure 8 A shows the groups of degenerate tracheids arranged in a ring in the invaginating tissue around the meristematic apex. The circular arrangement does not persist for a great distance up the dropper; in a section cut at a level slightly higher than 8 A it has given place to a horseshoe-shaped arrangement, 8 B. Text-figure 8 C (at a higher level than B) shows the tracheidal strands arranged in a semicircle. In the succeeding diagrams the vascular strand of the tuber stalk appears as a broken arc of xylem with its concave side directed away from the main body of the plant. The shape and orientation of similar bundles have been noted by Sampson (1916), who pointed out that in several well-developed plants examined the form of the stele changes as it passes down the dropper. When it is first separated from the stele of the main stem the tuber stele is convex inwards; by the time it enters the dropper the curvature is in the opposite direction. In well-developed plants the tuber stele sometimes makes a sharp upward bend after passing out from the main stele and before going down the dropper. Sampson's observations of stelar behaviour in well-developed plants have been confirmed by the present writer.

In the droppers of small tubers examined by the writer the vascular strand is not horseshoe-shaped, but small and compact in transverse section (see Text-figure 8 D). The protoxylem of all vascular strands is mesarch and there is no definite bundle sheath.

THE STEM.

The vascular system of the stem of *Phylloglossum* is extremely variable. A number of stems of fertile plants of all sizes were examined by means of serial transverse sections. The extent of development of vascular tissue is greater in larger plants than in smaller ones.

Below the departure of the root traces there is no vascular tissue in the stem. The root traces are continuous with the stele of the main axis before they pass out into the roots. The series of diagrams in Text-figure 9 demonstrate this in a plant bearing a single root. The young tuber and its vascular strand are also included in these diagrams. Text-figure 9 A represents a transverse section of the axis of the plant cut below the point of origin of the root. The dotted regions are lacunae. The dropper of the young tuber is seen in section alongside the main axis. In 9 B the root trace is seen in oblique section, moving out from the main axis. Note the lacunar tissue in the root. Text-figure 9 C represents a transverse section cut at a higher level than 9 B. The stele of the main axis is now recognizable as such, the root trace having lost its identity. The dropper of the young tuber is now shown

joined to the main axis; the tuber stele is still seen in transverse section. In 9 D the main stele, with perforations, is again plainly shown. The tuber stele is seen in oblique section, moving through the cortex of the main axis.

The diagrams shown in Text-figure 10 were made from sections of a plant bearing two roots. In this specimen two root traces are continuous with the stele of the main axis.

Behaviour of the Stele of the Stem in Fertile Plants.

(A) Medium-Sized Fertile Plants.

Text-figure 11 shows the distribution of vascular tissue in portion of the stem of a typical medium-sized plant. Text-figure 11 A represents a cross section cut below the departure of the tuber strand, the young tuber being included in the diagram. The stem stele in the base of this specimen was a solid protostele of irregular shape. In some specimens the protostele is medullated and the xylem is broken up into a few irregularly anastomosing strands. Text-figure 11 B represents a section at a slightly higher level than 11 A, at the junction of the dropper and the main axis. The stem stele is as before, but the tuber trace is seen in oblique section as it moves across to pass into the dropper. Text-figure 11 C shows a section a little higher up the stem. The stele appears here to be a medullated protostele with two arcs of xylem, the smaller of which is the tuber trace. The next diagram (11 D) shows the stele just above the departure of the tuber trace. There is a distinct gap in the xylem of the main stele, apparently due to the departure of the tuber trace. This gap is a constant feature of the stele in this region, and in larger plants is even more pronounced than here. In the present specimen the first leaf trace has already separated from the right-hand side of the main stele in 11 D. In 11 E the leaf trace has moved into a position directly opposite the centre of the main stele. At first sight and without a knowledge of 11 D, the leaf trace would appear to be responsible for the gap in the main stele, but this, of course, is not so. The shift in position of the leaf trace was observed in several specimens. Jeffrey (1908) observed similar instances of leaf traces occupying misleading positions opposite stelar gaps for which they were not responsible. In Text-figure 11 F another leaf trace has been given off, leaving no gap. In 11 G the third leaf trace has been given off, and a second gap has appeared in the central stele, opposite the second leaf trace. Since it makes its appearance after the leaf trace has been given off, this gap is merely a perforation and not a leaf gap. In 11 H two leaves have separated off and the base of the third is shown still attached to the stem. At this level the leaf trace is not in the centre of the leaf, as it is higher up. The stem stele has now passed into the base of the peduncle. Its irregular behaviour is obvious from 11 G and H. It is a medullated protostele in which perforations appear which form no particular pattern.

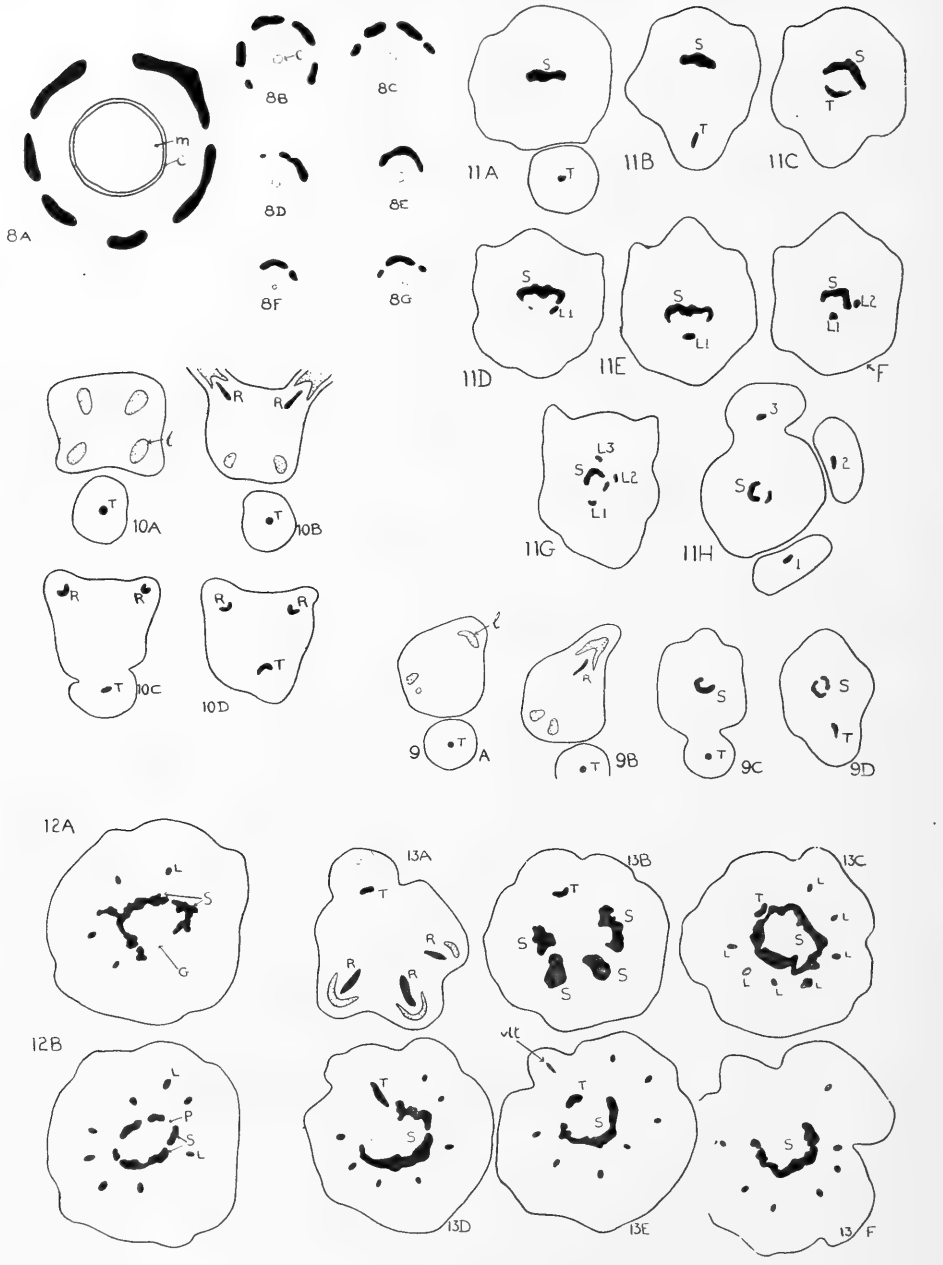
(B) Stems of Large Fertile Plants.

The behaviour of the stele in a fertile plant much larger than the above is shown in Text-figure 12. The section represented by the first diagram (12 A) was cut at a level comparable with that at which that shown in Text-figure 11 D was cut. The stele in this region is a comparatively large, horseshoe-shaped protostele, from which four leaf traces have already been given off. The gap where the tuber stele has been given off is large and distinct.

Text-figure 12 B represents a section cut a short distance above that shown in the preceding diagram. The stele is now clearly a medullated protostele with irregular perforations in the xylem. In many specimens the tuber gap persists as far as the base of the peduncle, but in this specimen it has been bridged by tracheids a short distance above the departure of the tuber strand.

Vestigeal Leaf on Tuber Stalk.

The diagrams in Text-figure 13 show the arrangement of the vascular tissue in another large fertile plant. This plant is of special interest because it exemplifies the occurrence of what is probably a vestigeal leaf arising from the tuber stalk.



Text-figures 8-13.

Figure 8, Diagrams A to G. Diagrammatic representation of arrangement of vascular tissue in transverse sections of a large tuber and its dropper. Vascular tissue is indicated by black masses; m = apical meristem of tuber; c = canal. See text for details.

Figure 9, Diagrams A to D. Diagrammatic representation of arrangement of vascular tissue (black) in a series of transverse sections of the base of the stem in a plant bearing one root (× 12). l = lacuna; T = vascular strand of tuber; R = vascular strand of root; S = vascular strand of stem. See text for details.

Figure 10, Diagrams A to D. Diagrams showing the distribution of vascular tissue (black) in a series of transverse sections of the base of the stem in a plant bearing two roots (× 12). Lettering as in Figure 9. See text for details.

Mettenius (1867) observed a small ligulate piece of tissue, frequently found above the new tuber. He regarded this as an atrophied leaf, since he observed a series of transitional forms between it and the normal leaves of the plant. Bertrand observed a similar body and named it the "organe de Mettenius". His material showed no transitional leaves. Bower (1886) noticed that in several specimens examined by him a leaf placed above the new tuber was smaller than the other leaves. He referred to this as the "supernumerary leaf".

In several large specimens, Sampson (1916) observed a small hump of tissue borne in the angle between the new tuber and the peduncle of the cone. In some specimens the hump of tissue was visible only in a microscopic examination; in others it was visible to the naked eye. Occasionally a small but otherwise normal leaf was found in its place. Sampson observed a complete series of transitional forms between the microscopic outgrowths of tissue and the small normal leaves.

Text-figure 13 A represents a section just above the point of attachment of the young tuber to the base of a large fertile plant examined by the writer. Three root traces and the vascular strand of the young tuber appear at this level, in oblique section, entering the stem. In Text-figure 13 B the root traces have converged to form an irregular ring of tracheids in the centre of the stem. The young tuber strand is moving through the cortex. The next development is of interest (13 C). The stem stele becomes a complete ring of xylem, distinctly medullated, from which seven leaf traces are given off almost simultaneously; meanwhile the young tuber trace (T) occupies a position very close to the central stele. In 13 D perforations have appeared in the xylem cylinder; the tuber trace is still distinctly visible (in oblique section at this level). Text-figure 13 E shows the tuber trace connected with the main stele. Of particular interest is the presence of a tiny group of tracheids, running from the tuber trace into the small hump of tissue just opposite the tuber trace. This hump probably represents a vestigeal leaf and corresponds to those described by Sampson (1916). The group of tracheids may be interpreted as the vascular trace of this vestigeal leaf.

Just above the region where the young tuber strand joins the main stele, a gap similar to that previously described for another plant (Text-figure 11) becomes apparent (Text-figure 13 F). As in the specimen previously described, this gap seems to be definitely associated with the exit of the tuber trace. In ascending the main stem further, the stele, with its associated leaf traces, repeats the type of behaviour shown in Text-figure 11. It is worth noting that, in some large fertile plants, Sampson observed that the tuber stele made a sharp upward bend as it passed out through the cortex of the main stem towards the dropper. A similar bend was observed in one plant by the present writer.

Behaviour of the Stele in Sterile Plants.

In some plants the roots and the characteristic crown of leaves are present and a new tuber is formed, but no cone is produced. Text-figure 14 presents a diagrammatic reconstruction of the vascular system of a typical sterile plant, built up from a series of longitudinal sections. In this specimen a single root trace enters the base of the short stem to join the main stele. Four leaf traces are given off from the main stele. The latter assumes what can only be described as a broken-up, diffuse appearance just below the point at which the leaf bases separate from the very short

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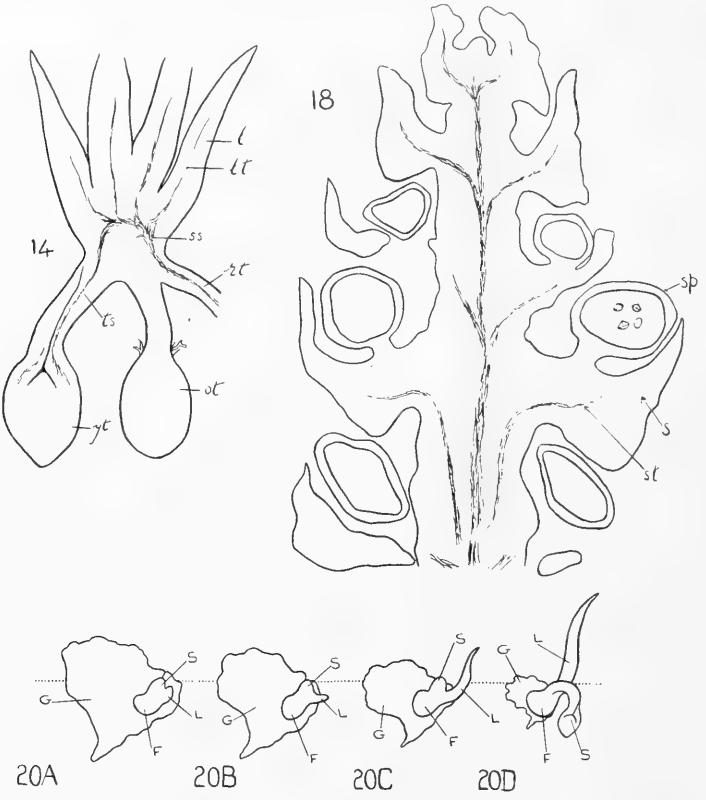
Figure 11, Diagrams A to H. Distribution of vascular tissue (black) in portion of the stem of a typical medium-sized plant (× 12). See text for details. S = vascular strand of stem; T = vascular strand of tuber; L = leaf trace.

Figure 12, Diagrams A and B. Behaviour of the stele in a large fertile plant (× 12). See text. S = stele of stem; L = leaf trace; P = typical perforation in xylem; G = gap in stele.

Figure 13, Diagrams A to F. Arrangement of vascular tissue (black) in a large fertile plant (× 12). See text for details. T = tuber strand; R = vascular strand of root; S = stele of stem; L = leaf trace; vlt = probable vascular strand of vestigeal leaf.

stem. In transverse sections of sterile plants the stele appears in oblique section in this region. Except for the leaf traces there is a complete absence of vascular tissue from the upper part of the stem. There is no sign of vascular tissue in the stalk of the current tuber. It must be remembered that this is not the original dropper of the current tuber, corresponding to that seen in connection with the new tuber, but a new structure produced during germination of the tuber by growth of its apical



Text-figures 14, 18, 20.*

Figure 14. Diagrammatic representation of the vascular system of a typical sterile plant, built up from a series of longitudinal sections (× 6). l = leaf; lt = leaf trace; ss = stele of main stem; ts = tuber strand; yt = young tuber; ot = old tuber. See text for details.

Figure 18. Longitudinal section of a mature cone (× 30). S = sporophyll; sp = sporangium; st = sporophyll trace.

Figure 20, A, B, C and D. Diagrams illustrating the development of the embryo as described by Thomas. See text for full details. G = gametophyte, or prothallus; F = foot; L = leaf; S = stem apex. The dotted line represents soil level.

meristem. Traces of vascular tissue may be seen in the remains of the original dropper, which is now represented by some torn tissue around the top of the current tuber.

There is usually no medulla in the stele of a sterile plant. The most striking feature of the anatomy of sterile plants is the apparent disappearance of the stele from the upper part of the stem. Sampson (1916) has also commented on this feature and offers the explanation that it may be due to a sharp bend in the axis (cf. the bend already mentioned as occurring in the tuber steles of some fertile plants). The stele of the main axis may have bent over to pass down the dropper of the young tuber. If this be so, the plant would consist of an unbranched axis bending over to

* For Text-figures 15, 16, 17, 19, see page 146.

form the annual storage tuber. This explanation is all the more feasible in view of the fact, already mentioned, that, in sterile plants, Bower (1885) has identified the growing point of the new tuber with the stem apex.

Although in most of the sterile plants examined by the present writer the young tuber is probably derived from the main stem apex, this is not true of all sterile plants. Sampson describes one sterile plant (the largest examined) in which the tuber was apparently a lateral structure, as in fertile plants. The stele of the stem in this plant was medullated and showed the tuber gap characteristic of large fertile plants.

It seems probable that there are two types of sterile plants; one type consists of a simple unbranched axis bending over to form the annual storage tuber, the other consisting of a branched axis, one branch of which forms the tuber while the other is arrested in development before the formation of the peduncle.

Tissues Present in Stem and Leaf.

Text-figure 15*a* shows a transverse section of the stem of a large fertile plant above the departure of the leaf traces. Text-figure 16*a* depicts a longitudinal section of the same region. The stele is a medullated protostele in which the ring of xylem tracheids is interrupted by perforations and surrounded by small closely packed parenchymatous cells. One of the most striking features of the xylem is the presence of cavities in the centres of the groups of tracheids. The writer observed a few small mesarch protoxylem elements at the sides of these cavities (cf. Wernham, 1910). In the transverse sections cut by the writer no tissue was discernible which could be definitely identified as phloem. In longitudinal sections, however, certain large, elongated cells were observed outside the xylem. These probably represent a rudimentary or reduced type of phloem (cf. those seen in the root and tuber). Each of these cells contains a prominent nucleus and some cytoplasm. No sieve areas are seen on their walls, and they resemble the surrounding cells of the cortex in many respects, including their staining properties. Bower (1885) mentions similar cells, pointing out that they would probably serve the purpose of phloem, but both Wernham and Sampson report failure to find any sign of phloem in the stem. No definite bundle sheath can be identified in either stem or leaf.

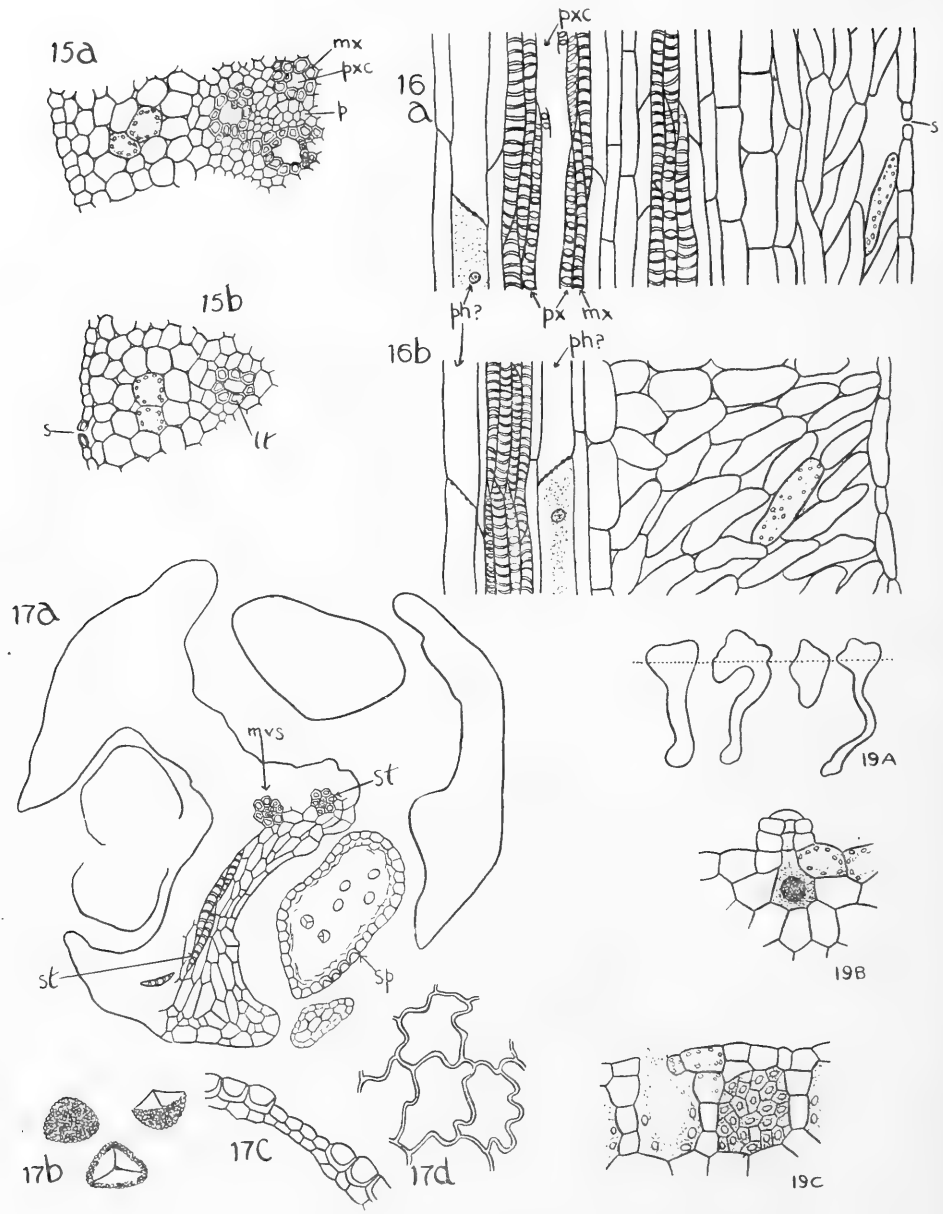
Nothing unusual has been observed about the epidermis and cortex, except that the intercellular spaces in the cortex are often very large. The large intercellular spaces are best seen in Text-figure 16*a*.

Text-figure 15*b* shows a transverse section of a leaf; Text-figure 16*b* shows a longitudinal section of the same tissues. There is a single leaf strand, usually with a cavity in the centre. The cavities seen in *Phylloglossum* may aptly be compared with the protoxylem cavities of such a type as Equisetum. Where cavities are absent their positions are occupied by protoxylem elements. The probable rudimentary or reduced phloem, the cortex and the epidermis of the leaf are similar to those already described for the stem.

THE PEDUNCLE AND THE CONE.

The peduncle is comparatively long and bare of leaves except for the sporophylls arranged in a compact cone at the top. It is usually unbranched (see section on gross morphology of the whole plant). No transitional forms occur between the vegetative leaves and the sporophylls. The number of sporophylls varies from four to twelve or more, according to the size of the cone, the average number in a well-developed cone being eight.

At the base of the peduncle the stele consists of a number of irregular, anastomosing, mesarch strands, arranged in the central region. Eames (1936) refers to this as a dictyostele. He also refers to the medullated protostele of the lower part of the stem as a siphonostele. The use of these terms in this connection is very confusing, as they do not apply in their generally accepted meanings. Strictly speaking, a siphonostele is one in which the xylem forms a ring around the medulla and is



Text-figures 15, 16, 17, 19.*

Figure 15, *a* and *b*. Figure 15*a* shows a transverse section of the stem of a large fertile plant above the departure of the leaf traces (× 40). Figure 15*b* shows a transverse section of a leaf (× 40). See text. mx = metaxylem; px = protoxylem; p = perforation in xylem; lt = leaf trace; s = stamate; pxc = protoxylem cavity.

Figure 16, *a* and *b*. Figure 16*a* shows a longitudinal section of the stem of a large fertile plant above the departure of the leaf traces (× 60). Figure 16*b* shows a longitudinal section of a leaf (× 60). See text. Lettering as in Figure 15. ph? = probable rudimentary or reduced phloem.

Figure 17, *a*, *b* and *c*. Figure 17*a* represents the mature cone in transverse section (× 40). sp = sporangium; st = sporophyll trace; mvs = main vascular strand of peduncle. Figure 17*b* shows details of spores viewed from three angles. Figure 17*c* shows the thickened epidermis

* For Text-figure 18 see page 144.

broken at intervals by leaf gaps which do not overlap; a dictyostele is a further development of the siphonostele in which the leaf gaps occur closely enough for two or more to appear in the same cross-section. As there are no leaf gaps in *Phylloglossum* there can be neither siphonostele nor dictyostele in the true sense of these terms. Eames also mentions an amphiphloic siphonostele in the peduncle. In view of the facts that the extent of the phloem is difficult to determine and that there is no reference to possible rudimentary or reduced phloem cells having been found anywhere but outside the xylem, the stele cannot be regarded as amphiphloic.

Throughout the region between the base of the peduncle and the base of the cone the xylem cylinder is usually medullated and perforated by gaps, but at the base of the cone it becomes a solid mesarch protostele. In many cone-bearing plants the cone axis appears to be a conservative region, retaining ancestral features (cf. the retention of centripetal metaxylem in the cone axes of certain cycads). In *Phylloglossum* the sporophyll traces are given off from the main stele close together in spiral succession. In most cones a few cauline tracheids are left at the top of the cone axis after the departure of the last sporophyll trace.

Text-figure 17*a* shows the mature cone in transverse section. The mesarch protostelic structure of the axial vascular strand is clearly seen. The lower portions of three sporophylls and the sporangia borne by them are included in the section. The section was cut above the departure of the first sporophyll trace; the second sporophyll trace is shown in oblique section as it passes into the base of its sporophyll. The third sporophyll trace is visible in transverse section, the material having been cut below the point of attachment of the third sporophyll.

The sporangia are reniform in transverse section and contain numerous uniform tetrahedral spores with pitted sculpturings on their curved bases. Text-figure 17*b* shows details of spores viewed from three angles. No developmental stages were available, but formation of the sporangium presumably occurs in the same manner as in *Lycopodium*. In the sporangia examined only the outer thickened wall cells were intact, so that tapetum and inner wall cells were not observed.

The epidermal cells are thickened on the radial and inner tangential walls (Text-figure 17*c*). They have peculiar wavy outlines when seen in surface view (Text-figure 17*d*).

In a longitudinal section of the cone (Text-figure 18) the sporangia are seen to possess short stalks and to be foliar in origin. Each sporangium is protected by the downwardly directed dorsal (abaxial) lobes of the sporophylls above it and by the upturned tip of its own sporophyll. There are no ligules in *Phylloglossum*.

The Gametophyte Generation.

The germination of the spores and the early stages of the development of the gametophyte have never been observed. The only descriptions of the gametophyte are those of Thomas (1901), Sampson (1916*b*) and Holloway (1935). Of these writers Thomas alone had access to more than one specimen. Thomas, collecting in the vicinity of Auckland, New Zealand (see Holloway, 1935), obtained prothalli of *Phylloglossum* growing naturally among parent sporophytes. He mentioned the fact that they were only discoverable in three of the areas searched, although sporophyte plants were abundant in all these areas. Sampson recorded failure of all attempts to raise prothalli from spores, and found only one badly preserved specimen in some

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of the sporangium in section, with the stomium consisting of thin-walled cells. Figure 17*d* represents a surface view of the epidermis of the sporangium, the cells having peculiar wavy outlines.

Figure 19, A, B and C. Figure 19 A: diagrammatic representation of shapes of prothalli. The dotted line indicates soil level. Figure 19 B: an archegonium in which the egg is ready for fertilization. Figure 19 C: two antheridia. The one on the right-hand side contains sperm mother cells; the one on the left-hand side is almost empty, the sperms having matured and the lid having been lost, allowing their escape. Drawings made from descriptions by Thomas and by Holloway. 19 A, × 7; 19 B and 19 C, × 272.

Australian material to which she had access. Holloway was also unsuccessful in his attempts to produce prothalli from spores, and was able to obtain only one specimen in the field, near Auckland.

Apparently very special conditions are necessary for the germination of the spores. These are probably not of regular annual occurrence, even where sporophytes are numerous. Thomas suggests that perhaps the most important of these conditions is the presence of a fungus with which the prothallus may live symbiotically (cf. the symbiotic fungi found in all species of *Lycopodium* whose developmental stages are known). Thomas adds that it is not impossible that the prothallus may begin life as a saprophyte, dependent upon an endophytic fungus. Among his specimens he found one young prothallus which was quite colourless except for a faint yellow tinge at the upper end, as well as two others, without sexual organs, showing only very few chloroplasts. When the young prothallus is prevented from forming chlorophyll by being buried in the soil it will doubtless be colourless.

All fully developed (sexually mature) prothalli recorded had green tissue above the soil.

The prothalli examined by Thomas varied greatly in external form. The variations are apparently not entirely due to differences in age, but may be due in part to obstacles encountered in the soil during growth, or to differences in depths at which development of the prothalli commenced in the soil. The length of the prothalli varied between 6 mm. and less than 2 mm.

General Features of the Prothallus.

The general features of the prothallus as described by Thomas may be summarized as follows. The subterranean parts of the body consist of an oval "tubercle" and a cylindrical shaft of varying length and thickness. The "tubercle" is probably the first part to be formed on germination of the spore. The length of the shaft is probably related to the depth at which germination occurs below the surface of the soil. At the level of the soil surface there is a slight expansion of the top of the shaft to form a crown, on which the sexual organs are developed. With the exception of the archegonial necks, the whole of the upper part of the prothallus is green. Chloroplasts are most abundant in the part just below the crown. The upper cells of the shaft may be green, but the colour fades out as the shaft passes into the soil. In some specimens there is a region of conspicuous meristematic activity at one side of the shaft, just below the crown.

There is little internal differentiation of the tissues. The cells of the "tubercle" of the mature gametophyte are of rounded polygonal form with scanty protoplasmic contents; those of the shaft are elongated, rectangular at the surface, longer and more pointed in the centre. Starch is often abundant in these cells. Thomas observed very fine hyphae of an endophytic fungus in the cells of the lower half of the prothallus. These hyphae pass in through the rhizoids and often form a dense mass on the surface of the "tubercle".

Male and female sex organs develop on the same prothallus and the archegonial necks are plainly noticeable on the crown. On each young prothallus Thomas found only two or three archegonia, but on older prothalli there may be ten or twenty. They appear to be formed in basipetal succession. The necks project from the surface of the prothallus, usually in two tiers of four cells each. The venter, with the large egg cell, lies at a short depth below the surface. The antheridia lie sunken in the crown, covered by a single layer of cells. There are no multicellular paraphyses among the sexual organs, but on some parts of the crown the surface cells are slightly papillose.

Thomas' description of the prothallus was made from a number of specimens but no text-figures were published with it. Thomas obviously intended to follow his first paper with a second, which was never published. Holloway (1935) gives a description of a single specimen, together with a set of text-figures. No archegonia are present in this specimen, but antheridia occur in considerable numbers, sunken

in the tissue of the crown. So closely are the antheridia packed that usually only a single layer of cells separates each from its neighbour. They are covered by a single layer of cells, in which a definite cap cell is present. Most of the antheridia in the specimen are empty, but a few are still packed with sperms.

The drawings of gametophyte organs shown in Text-figure 19 were made by the writer to illustrate the descriptions by Thomas and by Holloway.

An endophytic fungus was present in Holloway's specimen (cf. Thomas' observation). Sampson (1916*b*) also observed fungal hyphae in and between the cells of the prothallus, but her specimen was too poorly preserved to show other details.

The Embryo Sporophyte.

Information concerning the embryogeny of *Phylloglossum* is scanty. Thomas (1901) gives a description of the development of the embryo. This description has since been rendered somewhat less valuable by the fact that Thomas, like Bower, believed the tuber of *Phylloglossum* to be homologus with the protocorm of *Lycopodium*. In some places where Thomas speaks of the "protocorm" he was evidently referring to the young tuber. The single prothallus examined by Sampson (1916*b*) carried a young sporeling which was forming a storage tuber at an early stage of its life (see later). Thomas also refers to the first leaf as a protophyll, but this interpretation is clearly erroneous. The organ in question is not a protophyll but a true leaf, because a clearly defined vascular strand is present when development is complete.

Thomas' description of the development of the embryo emphasizes its similarity to that of the embryo of *Lycopodium cernuum*. However, the comparison appears to depend for its aptness on the interpretation of the tuber as the protocorm. If there be no protocorm the development of the embryo of *Phylloglossum* will resemble that seen in *Lycopodium clavatum* rather than that seen in *Lycopodium cernuum*.

Text-figure 20 shows a series of diagrams drawn by the writer to illustrate the facts recorded in Thomas' description.

Early in development the embryo grows obliquely downwards and outwards; the lowest part forms the foot, while the stem apex and the first leaf are formed at the opposite end. The tip of the leaf is the first part to burst through the prothallus and its emergence causes a split to occur down the side of the prothallus; at this stage the embryo "is a short, cylindrical body, bluntly tapering at either end and connected with the prothallus by the foot, which now appears to be lateral in position; the ends of the embryo represent the stem apex and the first leaf respectively". No root formation has occurred in any of Thomas' specimens, but a root is shown in Sampson's specimen (see later). According to Thomas, "the first leaf grows up and attains a height of 2–3 cm. above the ground. It becomes green even before it emerges from the prothallus. Soon after its emergence stomata are formed and a thin strand of tracheids appears in the centre. The first leaf has the same structure as any other small leaf formed in later years." Thomas states that the stem apex grows downwards into the soil and "forms a protocorm, apparently in the same manner that the adult plant forms its annual storage tuber". From his description of this "protocorm" and from later work by Sampson (1916*b*) it is clearly not a protocorm but a tuber.

Sampson describes a single specimen of a sporeling at a fairly advanced stage of development. It consisted of a downwardly directed stem apex, the first leaf, a single root and an "embryonic swelling" partly embedded in the prothallus. Sampson debates whether the swelling is similar to the foot found in an embryo of the *Lycopodium clavatum* type or to the protocorm of *Lycopodium cernuum*. Her main reasons for considering the second possibility were: (1) the description given by Thomas, discussed above, and (2) the fact that the swelling was partly extra-prothallial. However, Sampson remarks that the extra-prothallial appearance may have been merely due to shrinkage of the poorly preserved gametophyte tissue. As Thomas' belief in the existence of a protocorm is based on a misunderstanding of the nature of the tuber, and as he clearly observed the presence of a foot, there seems to

be no reason why the embryonic swelling observed by Sampson should not be interpreted as a foot.

The specimen examined by Sampson demonstrates that, although root formation is not common in the first year of growth (see Thomas, 1901), it does occur in some instances and probably arises in the embryonic region between the points of origin of the leaf and the foot.

General Discussion of the Phylogenetic Status and Affinities of Phylloglossum.

The simplicity of the plant body and the superficial similarity of the tuber to the protocorm (the presence of which in some Lycopodian embryos was once regarded as a primitive feature) have misled many of the early morphologists (e.g., Bower, 1885, Thomas, 1901). Subsequent research has shown that *Phylloglossum* must no longer be regarded as "the most primitive living Pteridophyte", but as a highly specialized plant adapted to a geophilous habit. Its tuber is not homologous with the protocorm. The available data is against the view that *Phylloglossum* forms a protocorm at any stage of its life.

The Morphology of the Tuber.

The morphology of the tuber was not understood for many years after the first descriptions of the plant were published. Bower (1885) believed that the tuber was homologus with the protocorm of *Lycopodium cernuum*, and this belief was shared by several of his successors.

In 1908 Jeffrey suggested that the gap frequently observed in the vascular tissue at the base of the stem might be due to the passing out of the tuber strand, but he did not discuss the morphology of the tuber. Wernham (1910) was of the opinion that *Phylloglossum* was an extremely specialized type. He considered that the gap in the stele of the stem was "an ancestral character, and one of considerable importance". He was of the opinion that it might represent the leaf gap of a megaphyllous leaf which had been suppressed by specialization. *Phylloglossum*, according to Wernham, might resemble *Tmesipteris*, which he described as "microphyllous" in its lower portion and "megaphyllous" in its upper portion. This opinion was never widely accepted. However, Wernham's paper is one of the earliest published attempts to prove that *Phylloglossum*, "far from being a primitive form, is highly specialized".

Sampson (1916*a*) considers that the occurrence of the stelar gap is a strong piece of evidence in favour of the interpretation of the tuber as a modified branch. The full evidence for this view, presented first by Sampson and later verified by the present writer (with the exception of observation 2), may be summarized as follows:

(1) In large fertile plants a gap is left in the medullated protostele of the main axis by the exit of the vascular strand of the tuber.

(2) Where two tubers are produced on opposite sides of the plant two gaps are present in the main stele.

(3) The stele of the tuber is frequently concave inwards as it passes out through the cortex of the main axis; i.e., it shows a gap corresponding to that left in the main stele.

(4) The dropper of the tuber sometimes bears leaves, usually reduced.

(5) In some specimens there is evidence, from the bend in the vascular strand of the tuber, that the direction of growth of the tuber was not originally downwards.

(6) In sterile plants the tuber is usually derived from the apical growing point.

An additional piece of evidence is contained in the study of the embryology (see above). Early in the life of the sporophyte a storage tuber is formed from the stem region of the embryo.

In view of these facts there can be little doubt that the tuber of *Phylloglossum*, far from being a primitive organ, as was once generally believed, is in reality a

relatively recent structure developed to meet the peculiar climatic (edaphic) conditions to which *Phylloglossum* became exposed.

Evidences of Reduction in Phylloglossum.

Evidences that *Phylloglossum* is a highly specialized form which has undergone reduction, rather than an example of extreme primitiveness, are as follows:

(1) The highly developed cone and tuber are advanced features. They are more advanced, for instance, than the reproductive structures seen in the more primitive species of *Lycopodium* and *Isoëtes*.

(2) The presence of a medulla in the stele is not generally regarded as a primitive feature.

(3) The presence of vestigeal leaves (on the tuber stalk) indicates reduction.

(4) The protoxylem cavities and the perforations indicate reduction in the xylem; reduction may also account for the occurrence of a solid protostele in small specimens.

(5) The rudimentary nature of the phloem may be a secondary development due to reduction. This point is, of course, open to argument.

(6) The root is absent during the early life of most plants, in spite of the fact that roots are produced in some sporelings.

(7) In most plants branching occurs only once a year, that is, when the new tuber is initiated. Occasionally, however, two new tubers may be formed, or branching may occur in the cone axis. It is at present impossible to tell whether this branching is due to the retention of an ancestral character (palingenetic) or the manifestation of a new feature (cenogenetic). As very primitive Pteridophytes (e.g., the Psilophytales) branch freely, the former alternative is the more probable.

The conception of *Phylloglossum* as an extremely primitive form is of historical interest rather than of phylogenetic significance.

AFFINITIES OF PHYLLOGLOSSUM.

The Lycopodian affinities of *Phylloglossum* are indicated by many morphological and anatomical features.

(1) The roots are exogenous and adventitious in origin. Their internal structure recalls that of Stigmarian rootlets and the roots of *Isoëtes*.

(2) The tuber appears to be homologus with the method of perennation seen in *Lycopodium inundatum*, where the whole plant dies down except the tip of the rhizome, which persists as a resting bud (see Holloway, 1916).

(3) The anatomy of the stem shows several features characteristic of the Lycopodiales. The stele is a mesarch protostele; in large specimens medullation and perforation of the xylem cylinder frequently occur, but true leaf gaps are never formed.

(4) Dichotomous branching sometimes occurs.

(5) The leaves are very simple in structure, with no definite palisade tissue, and each is supplied by a single mesarch strand which dies out before reaching the tip.

(6) The cone, with its spirally arranged sporophylls, is very like that of *Lycopodium*. Each sporophyll bears a single sporangium which resembles that of *Lycopodium* in structure, position and mode of dehiscence. There are no ligules.

(7) The spores are of one type only.

(8) The gametophyte appears to be similar to that of *Lycopodium cernuum*.

(9) From the available evidence the development of the embryo appears to be similar to that of *L. clavatum*. The occurrence of a protocorm has not been demonstrated.

SUMMARY.

The external morphology of the sporophyte is described briefly and its relation to ecological conditions is discussed, and the methods of vegetative reproduction are considered.

The morphology and anatomy of the sporophyte are detailed under the following headings: Root; Tuber; Stem (including leaves); and Peduncle and the Cone.

The literature describing the gametophyte and the embryo is critically reviewed. The phylogenetic status and affinities of *Phylloglossum* are discussed. It is shown that, far from being an extremely primitive form, *Phylloglossum* is probably a type in which apparent simplicity has been achieved as a result of modification and reduction through specialization.

ACKNOWLEDGEMENTS.

The writer expresses her gratitude to Dr. P. Brough, Botany Department, University of Sydney, for his guidance in this work, for the use of his collection of material, and for his helpful criticism of the manuscript. She also wishes to express her thanks to Professor E. Ashby, who was Professor of Botany at Sydney University at the time when this work was carried out, for the use of his laboratories and facilities.

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NOTES ON THE APHODIINAE OF AUSTRALIA (COLEOPTERA, SCARABAEIDAE).

THE APHODIUS TASMANIAE, HOWITTI, YORKENSIS, ANDERSONI COMPLEX.

By B. B. Given, Entomologist, Department of Scientific and Industrial Research, New Zealand.

(*Communicated by P. B. Carne.*)

(Ten Text-figures.)

[Read 28th June, 1950.]

Introduction.

The species *tasmaniae* Hope and *howitti* Hope of the genus *Aphodius*, have long been the cause of considerable controversy. The species *andersoni* Blackburn and *yorkensis* Blackburn have not previously been carefully considered with the first two species, as they are (if valid species) relatively rare and of no known economic importance. What we have long accepted as *A. howitti* is a major pasture pest in Victoria, South Australia, and parts of New South Wales, and it is this fact which now makes it necessary that the systematic status of this and other closely allied species should be carefully examined.

This, the first of what is hoped to be a series of papers on the Aphodiinae, is mainly a collection of extracts from official and personal files, and published descriptions and opinions. Little personal research has been done by the writer, and the only really original contribution is a series of line drawings.

Aphodius howitti Hope.

Proc. Ent. Soc. Lond., 1846, p. 147.

tasmaniae Hope, *Proc. Ent. Soc. Lond.*, 1846, p. 147.
australasiae Blanchard, Voy. Pole Sud 4, 1853, p. 101.
andersoni Blackburn, *Proc. Roy. Soc. Vic.*, xvii, Pt. 1, 1904, p. 154.
longitarsis Redtenbacher, Reise der . . . Fregatte Novara um die Erde, 1867, p. 58.

This is a rather variable species as regards structure, colour, and size. Its distribution is wide (from Eyre Peninsula through south-eastern South Australia and Victoria to south-eastern New South Wales), and it is often extremely common.

The following description is greatly condensed, as detailed descriptions have frequently been published in the past.

Colour normally black, but sometimes brownish or, rarely, reddish. Ventral surface and coxae brownish. Lateral and basal borders of pronotum usually reddish brown, anterior angles testaceous. Entire surface nitid.

Head broad and finely punctured, clypeal margin narrowly reflexed, anterior clypeal margin evenly curved, or straight and somewhat angled laterally. Medially on the frons, behind the clypeal suture, is a distinct but small tubercle, while laterally, in front of the eyes and on the suture, are raised areas. Eyes black, not prominent. Antennae rather small, and not highly distinctive.

Pronotum glabrous except for elongate marginal bristles; evenly convex, narrowly margined, finely punctured, and with distinct though not prominent anterior angles. Posterior angles almost completely rounded. The pronotum of the male is much larger and somewhat more convex than in the female, although in both sexes (particularly males) size is very variable.

Elytra strongly convex, elongate, and with nine sharply impressed, narrow striae on each elytron; inter-strial spaces strongly convex, with a rather irregular row of punctures on either side, each puncture bearing a minute hair.

Fore-tibiae strongly toothed, with the teeth more slender in the female. Spaces between teeth crenulate.

Length, 8·5–12 mm.; breadth, 3·8–5·3 mm.

Explanation of Synonymy, etc.

Harold (*Berl. Zeit.*, 1859) appears to have been the first to doubt the validity of Hope's species *howitti*, and at that time reported the two species *tasmaniae* and *howitti* to be identical. In 1861 (loc. cit.) he reversed his opinion as a result of an examination of further material. An account of this is to be found in Blackburn's paper (*Proc. Roy. Soc. Vic.*, 1904, pp. 153–4). However, in this paper Blackburn makes an error in saying that *tasmaniae* was described a year later than *howitti*. Actually, *howitti* was described on the same page as *tasmaniae*, but below it. Blackburn considers that Harold's reversal of opinion was due to the fact that in the first case he was examining specimens of one sex, and in the second, specimens of opposite sex.

Junk (Cat. Coleopt., 1910) does not consider *howitti* and *tasmaniae* as being synonymous, but places *australasiae* Blanch. as a synonym of *howitti*, and *longitarsis* Redt. as a synonym of *tasmaniae*. *Andersoni* Blackb. is left valid, and a second species, *australasiae* (Boheman, Res. Eugen., 1858), is also left. This last species should be renamed (Blanchard's species had priority), but whether or not it is synonymous within this group is not herein determined. However, Blackburn apparently considers it to be close to *A. frenchi* Blackb., which relationship would exclude it from the group under discussion.

Schmidt (Genera Insectorum) considers the two species *howitti* and *tasmaniae* to be both valid.

Resulting from correspondence with Miss W. Kent Hughes (Division of Entomology, C.S.I.R.O., Canberra) in 1931, Mr. G. J. Arrow, of the British Museum, examined types of *andersoni*, *howitti* and *tasmaniae*, and concluded that the last two species were the same, and that a specimen sent by Miss Hughes, named *howitti* by Mr. A. Lea, agreed with the type of *andersoni*. Miss Hughes, in her reply, writes:

"You mention in your letter that *A. andersoni* Blackb. had smooth elytral intervals and more punctured head and thorax. Some of my specimens agree with *A. andersoni* Blackburn, as regards the puncturation of the head and thorax, but seem to have the elytral structure of *A. howitti* Hope. Would it be possible for these all to belong to one variable species, or for one or other of them to be a variety or sub-species? When looking through the series one seems to get a gradual change from one to the other."

In 1939, correspondence was conducted between Mr. A. L. Tonnoir, Division of Economic Entomology, Canberra, and Mr. H. J. Carter on the subject. In his first letter Mr. Tonnoir mentions the tubercles on the head of *howitti*, stating that his information was obtained from J. W. Evans, who obtained it from Renaud Paulian of Paris. Carter's final opinion was that the three species *howitti*, *tasmaniae*, and *andersoni*, were the same, and that all should be called *A. tasmaniae* Hope.

In the *Tasmanian Journal of Agriculture* (Feb. 1), 1941, p. 29, J. W. Evans places on record his findings resultant on correspondence with M. Paulian of the Paris Natural History Museum. The presence of the three tubercles on the head (vid. sup.) are mentioned and illustrated, and Evans also considers the anterior outline of the head (clypeus) to be distinctive. He also makes an interesting and very important statement as follows:

"With regard to their distribution, *A. tasmaniae* occurs in southern Tasmania and has been bred only from larvae collected at Gretna and Huon Island. *A. howitti* has been reared from larvae collected at several places on the north-west coast and Flinders Island, and is the injurious species in Victoria and South Australia, and probably New South Wales." (*Note.*—The present writer has not yet seen specimens of the typical mainland form from Tasmania.)

Finally, type material was again examined at the request of Mr. P. B. Carne of the Division of Entomology, C.S.I.R.O., Canberra. This examination was carried out by Mr.

E. B. Britton of the British Museum, who makes the following observations on the Hope material and Blackburn's type of *andersoni*, in a letter dated 5th June, 1948:

"I am quite certain that they belong to the same species. The only difference is that the type of *tasmaniae* is 10 mm. long while that of *howittii* is only 8 mm. Hope's descriptions differ only in the matter of size and colour.

"The type of *A. andersoni* is in our collections here. The length is 9·5 mm. and colour yellowish brown. The outline of the clypeus differs slightly from that of *tasmaniae* and the tubercles of the frons are less prominent, but I do not hesitate to consider this also synonymous with *howitti*.

"The series of specimens labelled *tasmaniae* in our collections is mixed. The majority of the specimens are the true *howitti* but there are five examples of a species with hairy elytra apparently restricted to Tasmania. This is, I suppose, the species which Blackburn and others have identified as *tasmaniae*.

"There are two other species, *australasiae* Blanchard and *longitarsis* Redt., recorded by Schmidt as synonyms of *howitti* and *tasmaniae* respectively. The descriptions of these species are fairly detailed and yet make no mention of hairs on the elytral intervals. It seems certain that these are *howitti* and not the '*tasmaniae*' of Blackburn. This latter species is, therefore, left in need of a name."

Britton also gives differences of the hind tarsi, sides of pronotum, and posterior pronotal angles, as separating characters for the species *tasmaniae* (= *howitti*, = *andersoni*, etc.) and "*tasmaniae*" as erroneously accepted in Australia.

On Hope's type of *A. howitti*, the label reads "*howettii*". However, as "howitti" was employed in Hope's published description, it being stated that the species was named after its collector, a Mr. Howitt, it is safe to assume that the specimen label is incorrect.

In a later letter, dated 6th July, 1948, Britton replies to queries regarding *A. yorkensis*, and gives the results of a comparison between types and specimens of "*howitti*" and "*tasmaniae*" (*howitti* and *pseudotasmaniae* respectively in the nomenclature introduced in this paper). He also examined the genitalia of the two species and declares them to be quite distinct in this regard. All points mentioned in this comparison bear out Britton's earlier observations and conclusions; that is, that the so-called species *andersoni*, *howitti* and *tasmaniae* are actually all *howitti* and that what had previously been considered to be *tasmaniae* is an undescribed species (herein described as *pseudotasmaniae*, n. sp.).

From the above extracts and summaries, it is obvious that Hope's species *tasmaniae* and Blackburn's *andersoni* must be reduced to synonymy under *howitti* Hope. The two species actually occurring, *howitti* and *pseudotasmaniae*, have been carefully examined, and certain characters found to be useful in separating them. Seven males and fifteen females from Tasmania, and eighteen males and sixteen females from the mainland were studied, and the following characters were found to occur:

Tubercles on head.—All specimens from the mainland with the exception of one female from Canberra had well-developed, or at least definite, tubercles. In two Tasmanian males, head tubercles (median in particular) were faintly developed.

Clypeal outline.—This character was found to be extremely variable, and diagnostically useless.

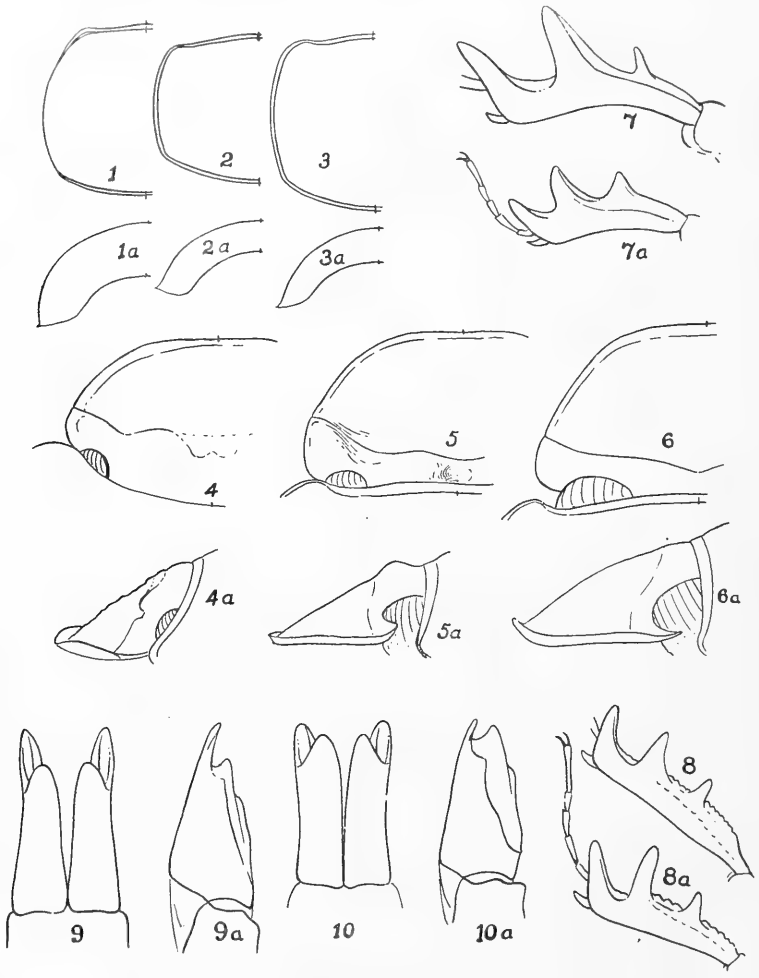
Hairiness of elytra.—Distinctly hairy elytra were present on all males from Tasmania, but on no other specimens.

Genitalia.—From three males from Tasmania and three from the mainland, genitalia were dissected and examined. Constant differences were again found and these are illustrated in Text-figs. 9 and 10. These figures are somewhat idealized to illustrate points of difference, but no actual distortion of proportion occurs. Male genital characters are not suitable for general use on account of the extreme fragility of some parts of the structure, and examination must be made before mounting in any clearing mountant as certain important parts are liable to become practically invisible.

Aphodius yorkensis Blackburn.

Trans. Roy. Soc. S.A., 1892, p. 209.

In characters of colour (rufo-ferrugineous), coarse puncturing of head, truncate clypeus, puncturing of elytra, and proportions of the male pronotum, the pair before me agree very well with Blackburn's description, and the only characters mentioned below are those not mentioned by Blackburn, but which appear to be useful for separation from *howitti* and *pseudotasmaniae*.



Text-figures 1-10.

Figs. 1, 1*a*.—Pronotum, dorsal and anterior, *A. yorkensis*.
Figs. 2, 2*a*, 3, 3*a*.—Pronotum showing range for *howitti* or *pseudotasmaniae*.
Figs. 4, 4*a*.—Head, *yorkensis*.
Figs. 5, 5*a*.—Head, *howitti*.
Figs. 6, 6*a*.—Head, *pseudotasmaniae*.
Figs. 7, 7*a*.—Fore-tibiae, male and female, *yorkensis*.
Figs. 8, 8*a*.—Fore-tibiae, male and female, *howitti*.
Figs. 9, 9*a*.—Male genitalia, *howitti*.
Figs. 10, 10*a*.—Male genitalia, *pseudotasmaniae*.

Head very broad, clypeus strongly reflected anterolaterally, clypeal suture of male (in specimen examined) apparently deeply arcuate in its median part. Lateral raised areas on suture as well-developed as in *howitti*.

Pronotum of male very large, and so strongly convex as to obscure portion of lateral margins when viewed from above. (Text-fig. 1.)

Fore tibia somewhat curved, and with the margins between the teeth smooth. Fore tarsus with apical segment shorter than preceding two together (Text-figs. 7, 7*a*; cf. Text-figs. 8, 8*a*).

Ventral surface more densely haired than in *howitti*. The sizes of the specimens before me are as follows:

Male.—Length, 10·8 mm.; breadth, 4·8 mm.

Female.—Length 8·9 mm., breadth 3·8 mm.

Note.—Mr. E. B. Britton, of the British Museum, has kindly examined the type of this species. He describes the colour as being a uniform cherry red, and confirms the smoothness of the fore-tibial outline between the teeth, the fore-tibial curvature or anterior concavity, and the male pronotal characters. Mr. Britton's letter is dated 6th July, 1948.

Aphodius pseudotasmaniae, n. sp.

This species has been long known as *A. tasmaniae* in error. An analysis of the confusion which has given rise to this error appears earlier in this paper, under *A. howitti*.

Very close to *A. howitti*, but differing in being devoid of tubercles on the head, having conspicuous rows of hairs on the elytra of the male, and in details of the male genital terminalia.

Material examined:

Holotype male, collected by Key, Kerr and Carne, 11 m. S.E. of Waddamana, Tasmania, January, 1948. *Allotype* female, collected by Mr. J. R. Cunningham, Kingston, Tasmania, February, 1948; C.S.I.R.O. Collection, Canberra.

Two *paratype* males, three *paratype* females, collected by J. R. Cunningham, Kingston, Tasmania, February, 1948; pair in collection of Entomology Division, Dept. S. & I.R., New Zealand; male and two females in collection of Dept. of Agriculture, Hobart, Tasmania.

Paratype male, *paratype* female collected by E. Nye, Glenora, Tasmania, February, 1939; Collection F. E. Wilson, Melbourne.

Paratype male, *paratype* female, collected by J. W. Evans, Glenelg, Gretna, Tasmania, emerged 2.2.39 (reared?); collection of Dept. of Agriculture, Hobart.

Two *paratype* females, collected by J. W. Evans, Glenelg, Gretna, Tasmania, emerged 2.2.39 (reared?), one paratype male, five paratype females, collected by Messrs. Key, Kerr and Carne at Broadmarsh, Bothwell, Scamander, and Waddamana in Tasmania, January, 1948; C.S.I.R.O. Collection, Canberra.

Summary.

On evidence submitted by Mr. E. B. Britton of the British Museum and others, the species *tasmaniae* Hope is herein reduced to synonymy under the species *howitti* Hope. Material from Tasmania, previously considered to be *tasmaniae*, is described as a new species, *pseudotasmaniae*. The species *andersoni* Blackburn is also reduced to synonymy under *howitti* Hope, and *yorkensis* Blackburn retains its status.

Acknowledgments.

I wish to thank Mr. P. B. Carne for material and information from the files and collections of the Division of Entomology, Commonwealth Scientific and Industrial Research Organization, Canberra, A.C.T. I am also indebted to Mr. E. B. Britton, of the British Museum, for making comparisons with type material, and to Mr. F. E. Wilson of Melbourne for the loan of his collection of the Aphodiinae, for the loan of literature, and for his valued personal opinions on the group. To the South Australian Museum, I owe thanks for the loan of a pair of *Aphodius yorkensis* Blackburn. Finally, I am indebted to the Tasmanian Department of Agriculture for the loan of specimens of Tasmanian forms.

THE MORPHOLOGY OF THE IMMATURE STAGES OF APHODIUS HOWITTI HOPE (COLEOPTERA, SCARABAEIDAE, APHODIINAE).

By P. B. Carne, B.Agr.Sc.*

(Plate viii, thirteen Text-figures.)

[Read 28th June, 1950.]

Introduction.

Aphodius howitti Hope in the larval stage is a pest of pastures, occurring in economic densities in the south-eastern portion of Australia, including Tasmania.

This paper describes the external morphology of the immature stages, no detailed account of which has been published previously. The author had the opportunity to consult an unpublished thesis prepared by Miss D. M. Cumpston in 1939, in which the raster and epipharynx of the larva are described briefly, the terminology introduced by Hayes (1929) being employed. However, the more recent terminology originated by Böving (1942) appears preferable, although primarily designed for use in the description of larval *Phyllophaga* (Melolonthinae). Recently, this terminology was extended for use in the description of larval Coprinae (Ritcher, 1945). It is possible to describe Aphodiine larvae almost completely in the terms used in these publications, as the morphology of the Aphodiinae is intermediate between that of the Coprinae and that of the Melolonthinae.

The method of description used is patterned to some extent on that employed by Ritcher in describing the species *Anomala innuba* Fab. (Ritcher, 1943), the writer being of the opinion that the excellent descriptions published by this author (Ritcher, 1943, 1945*a*, 1945*b* and 1945*c*) provide models which can profitably be paralleled in the description of Australian larval Scarabaeidae.

The larvae were collected in the vicinity of Canberra and Weetangera (A.C.T.); Queanbeyan, Burra, Crookwell, Tumbarumba (N.S.W.); Ballarat, Hamilton, Melbourne (Victoria); and Gretna (Tasmania).

The majority of the figures are composite: the slide preparations of the mouthparts employed in making the figures, together with the series of larvae from the above localities, have been deposited in the Museum of the Division of Entomology, C.S.I.R.O., Canberra, A.C.T.

Larvae were fixed for ten minutes in Carls' fixative maintained at approximately 70° C., and preserved in 70 per cent. alcohol. Slide preparations were made in polyvinyl alcohol mounting medium.

The larva of *Aphodius howitti* Hope is distinctive among other pasture scarabaeids in that it has a brownish-black head capsule, raster pattern as illustrated (Text-fig. 12), and a mode of life which involves the making of a vertical burrow in the soil from which it emerges at night to feed on dung or vegetation.

The only larvae with which it might be confused are those of *Aphodius pseudotasmaniae* Given and *A. yorkensis* Blackb. In neither of these species is the larva known, but in view of the similarity of the three species in the adult stage, it seems probable that their larvae would exhibit little macroscopic differentiation. Given (1950) observes that *A. pseudotasmaniae* is restricted to Tasmania, and *A. yorkensis* to the Yorke Peninsula of South Australia.

Description of the Late (Prepupal) Third Instar Larva.

The body is cylindrical and typically scarabaeiform (Text-fig. 1); when at rest, the mouthparts approximate to the anus. The length of twenty prepupae ranged from 13 mm. to 18 mm. with a mean length of 15·0 mm.

* An officer of the Division of Entomology, C.S.I.R.O.

Head (Text-fig. 2).

The *cranium** is equal in width to the prothorax, surface shining, dark brown to black. The mean maximum width of cranium of 100 prepupae taken from seven sites was 2·97 mm., with a S.D. (Standard Deviation) of 0·11 mm. The clypeofrontal suture (CS) is distinct, and is bounded laterally by the precoilae (PCL). The frontal sutures (FS) are fine, and meet well in front of the hind margin of the head, forming an angle of approximately 60°. The epicranial suture (ES) is about one-half the length of one of the frontal sutures. The frons (F) bears a pair of prominent anterior frontal setae (AFS), between which lie a pair of paramedian pigmented depressions. Each anterior frontal angle (AA) carries a single seta.

Epicranium (Text-fig. 2, E): The epicranium possesses four pairs of dorso-epicranial setae (DES). Of each group of four setae, three lie in a transverse row, while the fourth lies slightly cephalad of the junction of the frontal sutures. Five setae occur on each side of the epicranium, and a sixth lies caudad of each anterior frontal angle.

Antenna (Text-figs. 2, A, and 3): The antennae are approximately equal in length to the cranium, 4-segmented and borne on a basal piece fused to the epicranium. The first segment is longer than the second, and the second longer than the third. The ultimate segment is reduced to a small conical structure terminated by four or five sensory pegs (SP) and a long terminal hair (TH). A very similar structure is found in the larvae of the Coprinae (Ritcher, 1945*c*). The second segment carries a single microsensillum (MIS) dorsally, the third bears a ring of four or five short setae distad, and an oval convex sensorial spot (SS). The fourth segment possesses a small, apparently concave, sensorial spot.

Clypeus (Text-fig. 2, CY): The clypeus is trapezoidal and is not divided into post- and preclypeus. A pair of short paramedian setae are present, laterad of which are two pairs of exterior clypeal setae (ECS), the more median pair being the longer. These setae all arise from an approximate mid-transverse line.

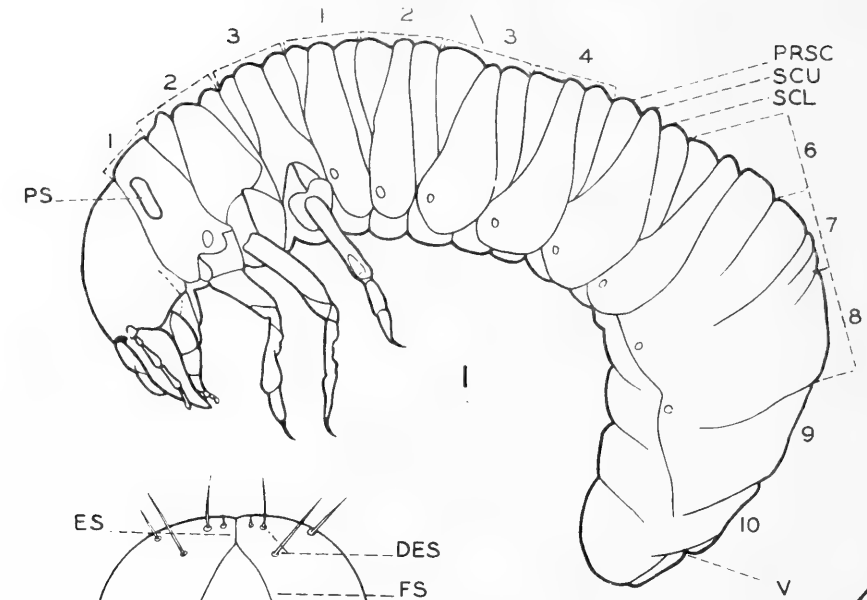
Labrum (Text-fig. 2, L): The labrum is asymmetric, trilobed, in length approximately equal to the clypeus, and in width to the clypeus at its distal margin. Two pairs of setae lie across the mid-transverse line, a paramedian pair, and a pair near the lateral edges of the labrum. The lateral lobes bear three setae set close together, the central seta of each triad being the longest. The apical lobe carries a pair of short setae.

Epipharynx (Text-fig. 4): The epipharynx is broader than long and weakly trilobed. The acanthopariae each bear six or seven setae (ACP), the posterior four or five being short and curved slightly cephalad; the anterior two setae, which are about twice as long, are set in slightly from the edge. Plegmata are absent.

The acroparia carries two rows, each of four setae (ACR), one row along the edge of the epipharynx, the other set in from it. Between the acroparia and the acanthopariae lie the clithra (CA). The haptomerum (H) is a hump bearing two large macrosensilla (MAS). The pedium (PE), which is raised above the general level of the epipharynx, is bare except for a row of fine non-articulated processes which runs obliquely across the right side. The pedium is bounded cephalad by the protophoba (PPH), which consists of a series of small appressed processes, at the bases of which are about 19 sensilla. Laterad, the pedium is bounded by the dexiophoba (DPH) and the laeophoba (LPH), and caudad by the mesophoba (MPH), all of which consist of non-articulated processes, those of the laeophoba being finer than those of the dexiophoba. Caudad, the latter is modified to form a plate-like structure which is fringed with processes similar to those of the dexiophoba proper, and which is borne perpendicular to the plane of the epipharynx as a whole. Between the lateral phobae and the acanthopariae lie extensive gymnopariae (GP). Chaetopariae are absent.

A pair of stout setae are present near the lateral margins of the epipharynx, adjacent to the clypeolabral condyles. A dexiotorma and laeotorma (DX, LT) are present, fused mesad and produced cephalad to form a long anterior epitorma (ETA).

* "Cranium" is here used to denote that part of the head capsule consisting of the epicranium and the frons, i.e., the immovable part of the head.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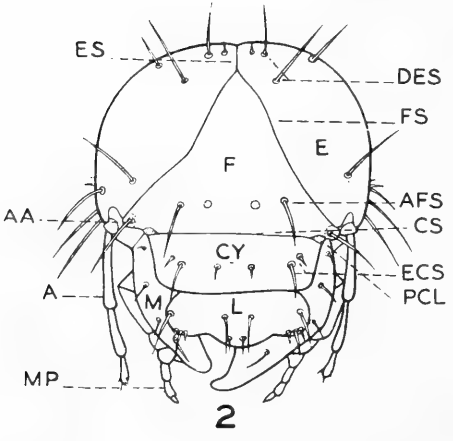
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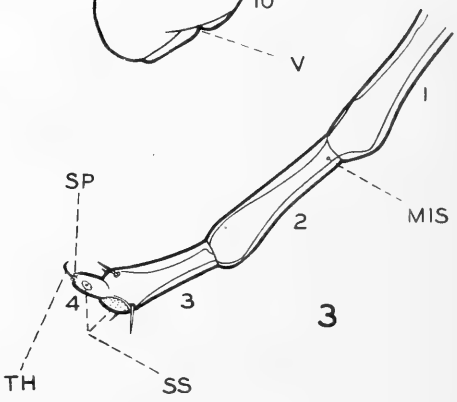
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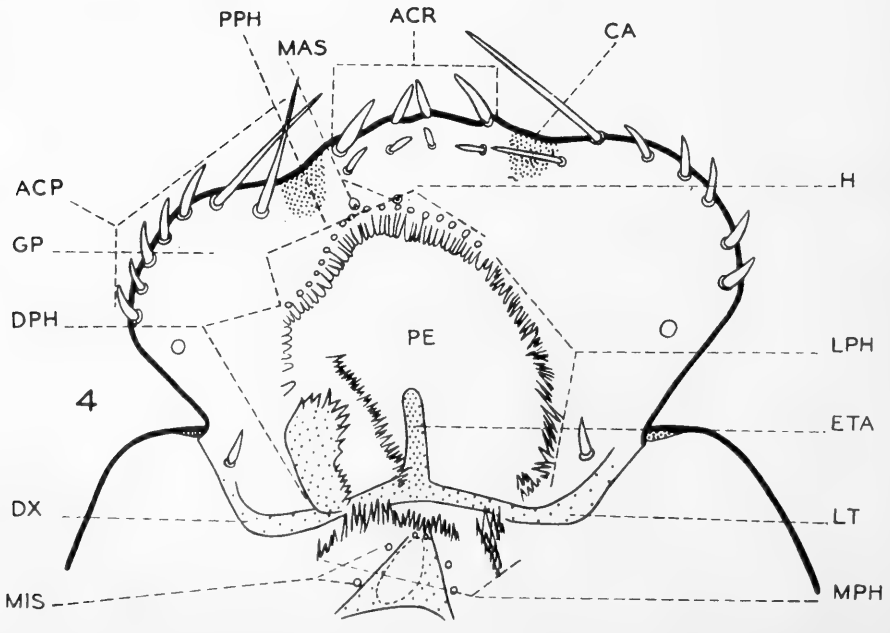
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Caudad of the mesophoba is a variable, faintly chitinized structure, subtriangular in shape, adjacent to which lie four to six sensilla. The affinities of this structure are doubtful. Somewhat similar structures occurring in Coprine larvae are termed crepides by Ritcher (1945*c*).

Mandibles (Text-figs. 2, M, and 5–6): These are asymmetric, the scissorial area (SA) reddish-black and stout, cutting edge notched, with a tooth proximad of the notch. The left mandible is longer than the right and overlaps the latter (viewed dorsally).

Dorsad (Text-fig. 5), each mandible bears three setae near its exterolateral margin, and a fourth smaller seta, often obscured, adjacent to the preartis (PA). A brustia (BR) is present on each mandible, while an acia (AC) is present on the left mandible only.

Ventrad (Text-fig. 6), on each mandible, is borne the postartis (PTA) and the ventral process (VP). The right mandible bears two or three small groups of bristles. Of these, only one group is paired on the left mandible. The central portion of each mandible is a pale yellow in colour, the molar structures, and the edges generally, grading red to black. No oval stridulating area is present, as it is in the Melolonthinae. The surface bears a large number of minute asperities, not shown in the figures. These are particularly noticeable about the postartis.

Maxilla (Text-figs. 7 and 8): ventrad (Fig. 7), the maxilla is composed of cardo (CAR), stipes (ST), galea (G), and lacinia (LAC). The cardo is longer than wide, also the stipes. The lacinia and galea are entirely separate. The cardo bears a variable number of setae, usually four. The stipes bears two setae: one long proximal seta and a somewhat shorter distal seta. The galea terminates in a simple uncus (U) from which extends caudad a row of 10 to 12 short setae. A number of larger setae are grouped about the uncus, only one arising from the ventral surface of the galea. The lacinia ends in a notched uncus, proximad of which is a very stout seta. The lacinia carries a single seta proximad.

Dorsad (Text-fig. 8), the stipes bears two setae, proximad of which lies a row of 11 to 15 stridulating teeth (SD), the apices of which are directed cephalad. The galea possesses distad a group of five or six stout setae. The lacinia carries a row of 10 to 11 setae, longer than those in the row on the ventral surface of the galea. The maxillary palpus (MP) is 4-segmented, and arises from a palpifer (PF) which bears a singlet seta on its ventral surface. The third segment bears a pair of setae; the fourth ends in a group of sensory pegs.

Labium (Text-figs. 7 and 8): Ventrad (Text-fig. 7), the labium consists of a large subtrapezoidal postmentum (PMP) and a terminal prementum (PRM), which carries a pair of labial palpi (LP). The postmentum is bare except for a pair of setae, one in each proximolateral corner. Adjacent to each seta is a single sensillum. The prementum bears proximad a pair of rather long setae, laterad of which are one or two pairs of very small setae; and distad, four pairs of setae. The latter consist of a paramedian pair, a pair of very long setae, each situated directly proximad of a labial palp, and, laterad of these, a third pair of shorter setae close to the edge of the prementum. The fourth pair

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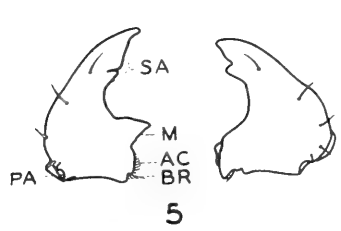
Text-figures 1-4.

Fig. 1.—Third instar larva, left view. PRSC, prescutum; PS, prothoracic shield; SCL, scutellum; SCU, scutum; V, venter.

Fig. 2.—Third instar larva, cranium, front view. A, antenna; AA, anterior frontal angle; AFS, anterior frontal setae; CS, clypeal suture; CY, clypeus; DES, dorsoepicranial setae; E, epicranium; ECS, exterior clypeal setae; ES, epicranial suture; F, frons; FS, frontal suture; L, labrum; M, mandible; PCL, precoila.

Fig. 3.—Third instar larva, right antenna. MIS, microsensillum; SS, sensorial spot; SP, sensory pegs; TH, terminal hair.

Fig. 4.—Third instar larva, epipharynx. ACP, acanthoparia; ACR, acroparia; CA, clithrum; DPH, dexiophoba; DX, dexiotorma; ETA, epitorma; GP, gymnoparia; H, haptomerum; LPH, laeophoba; LT, laeotorma; MAS, macrosensillum; MIS, microsensillum; MPH, mesophoba; PE, pedium; PPH, protophoba.



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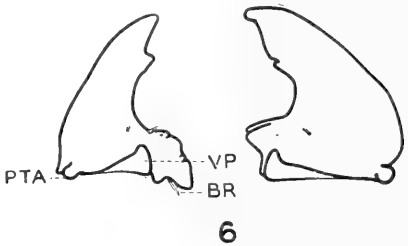

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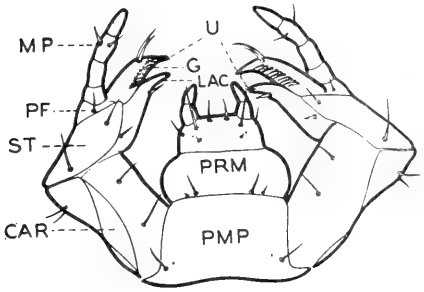

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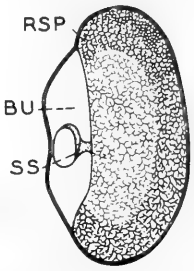

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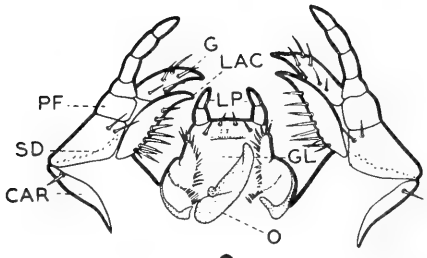

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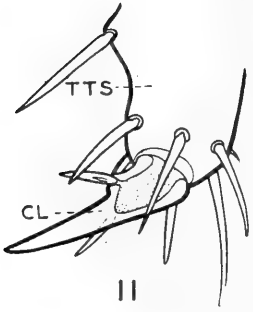

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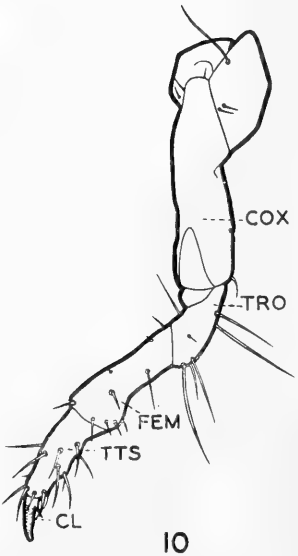

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Text-figures 5-11.

Fig. 5.—Third instar larva, mandibles, dorsal aspect. AC, acia; BR, brustia; M, molar lobe; PA, preartis; SA, scissorial area.

Fig. 6.—Third instar larva, mandibles, ventral aspect. PTA, postartis; VP, ventral process.

Fig. 7.—Third instar larva, maxillae and labium, ventral aspect. CAR, cardo; G, galea; LAC, lacinia; MP, maxillary palpus; PF, palpifer; PMP, postmentum; PRM, prementum; U, uncus.

are very small and lie proximad of the labial palps, caudad of the very long setae. Adjacent to each is a sensillum.

Dorsad (Text-fig. 8), is located the glossa (GL), which bears a complex of sclerotized masses, the oncyli (O). A longitudinal row of short setae lies proximad of each labial palp, the row curving laterad near the base of the palps; proximad of this row of setae, and running obliquely, is a row of non-articulated spines, which, towards the sides of the glossa, are much reduced and triangular in shape. A transverse row of very small, closely appressed processes occurs on the glossa distad of the oncyli. At the base of each process (which is directed caudad) is a microsensillum. The glossa bears two pairs of setae near its distal edge; between the more proximal pair is a group of four sensilla.

Thorax.

The thorax consists of pro-, meso-, and metathoracic segments. The prothoracic segment bears a pair of sub-oval heavily chitinized areas, the prothoracic shields (Text-fig. 1, PS). Ventrad of these are the prothoracic spiracles. The meso- and metathoracic segments are divided dorsally into three annulets, the prescutum (PRSC), the scutum (SCU), and the scutellum (SCL). Each annulet bears a transverse row of long setae.

Spiracles (Text-figs. 1, 9): Only the prothoracic segment bears spiracles. Each consists of a respiratory plate (RSP) which measures 0·26 mm. along its major axis. There is a spiracular slit (SST) and a bulla (BU). The concavity of each spiracle faces caudad.

Legs (Text-figs. 1, 10, 11): These are well developed, the larva being capable of far more rapid movement than most scarab larvae. The prothoracic legs are slightly shorter than the mesothoracic, and these slightly shorter than the metathoracic pair.

Each leg consists of a long cylindrical coxa (COX), a short trochanter (TRO), a markedly clavate femur (FEM), and a fused tibiotarsus (TTS), which ends in a single claw (CL). Setation is sparse, there being about seven setae on the coxa, and about six on the trochanter (which bears ventrally the longest setae on the legs). The tibiotarsus is more plentifully supplied with setae. The claws of the prothoracic and mesothoracic legs are slightly longer than those of the metathoracic legs (ratio 2·0 : 1·9 : 1·6). The distal portion of each claw (Text-fig. 11) is a reddish-brown; the proximal portion is a pale yellow and bears two minute setae.

Abdomen.

The abdomen consists of ten segments, of which only the first eight bear spiracles. The first six segments are dorsally divided into prescutum, scutum and scutellum, as in the meso- and metathoracic segments. Rows of short setae are present on all the annulets of the abdominal segments from the scutellum of the first to the scutellum of the fifth segment. Long setae are present on the prescutum of the first segment, and are interspersed among the short setae caudad. Setae are fewer on the scutum of the sixth segment, while the scutellum, and subsequent segments caudad, which are undivided dorsally, carry only sparse long setae. The tenth segment bears, dorsad, the venter (V), which has a transverse anal slit.

Each of the abdominal spiracle-bearing sclerites carries two long setae. The sternum of each segment bears a transverse row of six to eight mixed long and short setae. The venter is bare. Ventrad, the tenth segment bears the raster.

Spiracles: There are eight abdominal spiracles, the concavities of which face cephalad. Measurements made along the major axis of the respiratory plate show that

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Fig. 8.—Third instar larva, maxillae and labium, dorsal aspect. CAR, cardo; G, galea; GL, glossa; O, oncylus; PF, palpifer; SD, stridulating teeth.

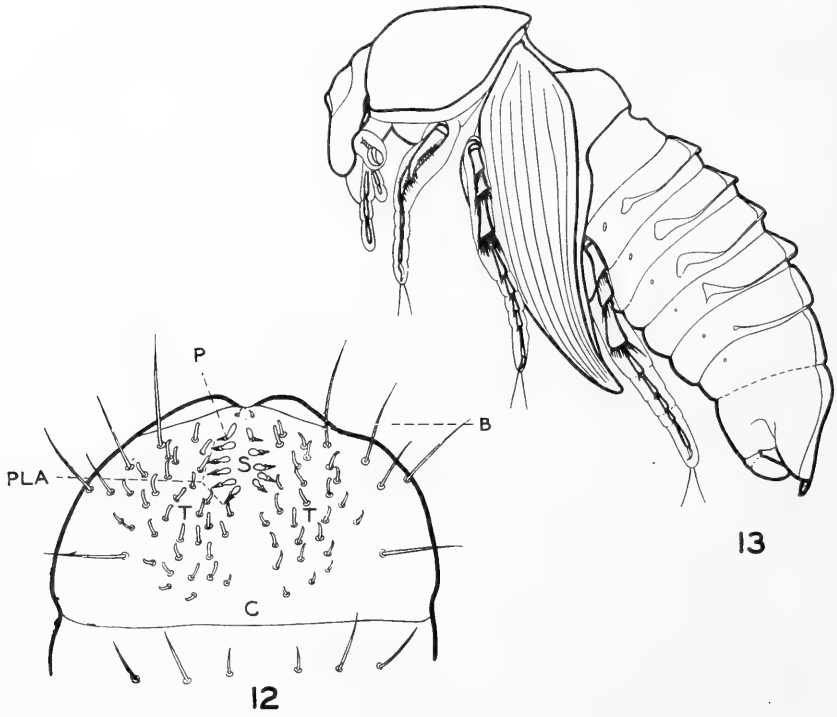
Fig. 9.—Third instar larva, left prothoracic spiracle. BU, bulla; RSP, respiratory plate; SS, spiracular slit.

Fig. 10.—Third instar larva, left mesothoracic leg. CL, claw; COX, coxa; FEM, femur; TRO, trochanter; TTS, tibiotarsus.

Fig. 11.—Third instar larva, left mesothoracic leg, distal portion. CL, claw; TTS, tibiotarsus.

all the abdominal spiracles are smaller than the prothoracic pair, with the exception of the pair on the sixth abdominal segment. Those of the eighth abdominal segment are the smallest. The following are measurements made on a single specimen: Prothoracic spiracle 0·26 mm. Abdominal spiracles 1–8: 0·14, 0·20, 0·22, 0·22, 0·24, 0·26, 0·18, 0·12 mm.

Raster (Text-fig. 12): The raster is composed of a septula, a pair of palidia, a pair of tegillae, and a campus. The septula (S) is ovoid and extends from close to the lower anal lip to a point somewhat short of the middle of the tenth abdominal segment. Each palidium (PLA) is composed of from five to seven blunt translucent pali (P). Each



Text-figures 12, 13.

Fig. 12.—Third instar larva, raster. B, barbula; C, campus; P, palus; PLA, palidium; S, septula; T, tegilla.

Fig. 13.—Pupa, left view.

palus is about twice as long as wide and has a very short constricted stem. At its point of articulation there is a chitinized structure embedded in the tissues laterad. Böving describes four distinct classes of pali (Böving, 1942), but the pali of *Aphodius* are unlike any described by him.

The tegillae (T) extend from the barbulae (B) to the palidia, and are not united anterior to the septula. The setae composing the tegillae are hamate and curved caudad. The barbulae consist each of a group of usually five prominent setae. The campus (C) is narrow and bare although sometimes possessing one or two very small non-hamate setae mesad. Two very small hamate setae are borne caudad of the septula.

Range of Variation.

Epipharynx: The acanthopariae consist each of five to seven setae; about 50 per cent. of the larvae studied had seven setae on each side. Frequently the number was reduced to six on the right side, less frequently on the left. The haptomeral macrosensilla in rare cases numbered three instead of two.

Maxillae: The number of setae in the row upon the lacinia varied from 10 to 11 with an average of 10·6 (16 specimens examined). The number of setae in the row on

the galea varied from 10 to 12 with an average of 11·5 (15 specimens). The number of stridulating teeth on the stipes varied from 11 to 15 with an average of 12·3 (13 specimens).

Raster: The palidia consist of five to seven pali, the most frequent arrangement being five on one side and six on the other, with a tendency for the lower number to be found on the left side. A variable number of non-hamate setae (rarely more than four) were found either in the campus or among the setae of the tegillae.

In the course of examination of larval mouthparts, a series of second instar larvae from Crookwell, New South Wales, was found to contain a considerable proportion of individuals in which the epipharynx and raster exhibited a large number of supernumerary setae. It was at first thought that these setae were those of the third instar, showing through the second instar tissues just prior to ecdysis. However, this phenomenon was not observed in specimens from any other locality, and moreover, there existed a complete gradient from normal to "fully aberrant" in the Crookwell series, this latter fact removing any question of a second species of *Aphodius* being involved.

A microphotograph of the epipharynx of an aberrant specimen is reproduced in Plate VIII, which should be compared with Text-figure 4. The maxillae are similarly possessed of many additional setae, while in the raster (but not in every case) each seta is paired with another rather less pigmented seta. The larvae were active and of normal size.

DISTINGUISHING FEATURES OF INSTARS.

The instar of a specimen may be determined with ease from a number of points of difference:

1. Cranium: The maximum width of cranium is useful as an indicator of instar. The following figures (Table 1) are derived from a series of measurements of over 1,000 specimens, all of which were collected at Dickson Experiment Station, A.C.T., during the winters of 1946 and 1947.

It will be noted that there is neither contact nor overlap between the ranges characteristic of each instar.

TABLE 1.

Widths of crania of Aphodius howitti *larvae from Dickson Experiment Station, Canberra, A.C.T.*

Instar.	Number of Specimens.	Mean Width of Cranium (mm.).	Range (mm.).	S.D.
1st	40	1·05	0·81 – 1·14	0·0625
2nd	97	1·82	1·67 – 1·95	0·0619
3rd	1,031	2·83	2·29 – 3·29	0·1407

2. Epipharynx: The clithra are absent in the first instar, and present in a reduced state in the second instar.

3. Raster: The first instar raster is characterized by barbulae, the setae of which are relatively extremely long, and by the absence of palidia. In the second instar, the barbulae are relatively much shorter. Palidia are not present, although the setae towards the inner edge of each tegilla possess pigmented supporting structures.

PUPA.

The exarate pupa is a light yellow in colour and specimens collected at Dickson Station measured 1·0 to 1·2 cm. in length. It possesses a tough semi-transparent skin, a large mounded tubercle on the head, and in pupae from which male beetles will emerge, a finger-like projection caudad (Text-fig. 13).

The sheaths enveloping the legs terminate in pairs of fine hairs. In older pupae, when the adult structures are well developed and clearly visible as in Text-fig. 13, it may be seen that the tibiae of the meso- and metathoracic legs each carry two sharp spines, and that the abdominal spiracles are borne on the pleural membrane, dorsad of which, on the

pupal skin, are irregularly shaped areas of thickening. The antennae may be seen to be 9-segmented. These observations enable the pupa to be identified immediately as one of the larger Aphodiinae. The relatively large size of the pupa would prevent confusion with that of any other species of the subfamily known in Australia, again with the exception of the two species mentioned above, i.e., *A. pseudotasmaniae* Given and *A. yorkensis* Blackb.

EGG.

The eggs are found in the soil in batches of 20 to 50, the average being about 35. Eggs collected just prior to hatching are pale yellow, ovoid, and measure approximately 1 mm. along their major axis. The membrane is smooth and devoid of ornamentation.

SUMMARY.

Series of third instar larvae collected from a wide range of sites in south-eastern Australia have been examined: the present paper describes in detail the external morphology of the larva and the variations observed. Emphasis has been laid on those structures of taxonomic importance in other sub-families of the Scarabaeidae.

Larvae of the two earlier instars have been studied and the distinguishing features of the three larval instars are listed. The pupa and egg are described briefly.

ACKNOWLEDGEMENTS.

The writer wishes to thank Dr. K. H. L. Key of the Division of Entomology, C.S.I.R.O., for his criticism of the draft of this paper, Mrs. M. Spencer for reference to her thesis, and the many persons who have supplied him with larval material. Miss B. M. Rothery provided technical assistance, and Mr. T. Pickard the microphotograph for Plate viii.

The work described in this paper was carried out as part of the research programme of the Division of Entomology, C.S.I.R.O.

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EXPLANATION OF PLATE VIII.

Microphotograph of the epipharynx of a second instar larva of *Aphodius howitti* Hope, taken at Crookwell, New South Wales. See explanation in text.

NOTES ON AUSTRALASIAN SIMULIIDAE (DIPTERA). II.

By M. J. Mackerras and I. M. Mackerras,
Queensland Institute of Medical Research, Brisbane.

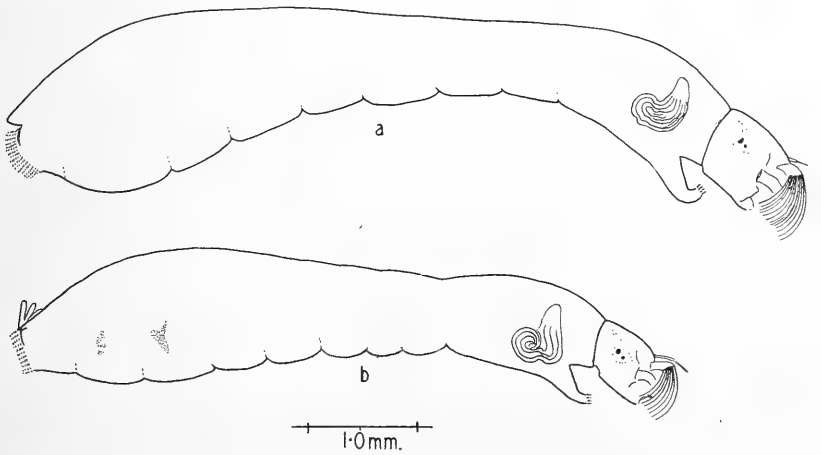
(Fifteen Text-figures.)

[Read 28th June, 1950.]

Introduction.

Since our previous paper (1949), we have accumulated a considerable quantity of new material, much of which was obtained during a tour of Cape York and north-eastern Queensland. The present notes include observations on the *aurantiacum* group of *Cnephia*, descriptions of five new species (one *Cnephia*, three *Simulium*, one *Austrosimulium*), a new subspecies of *Cnephia tonnoiri* Drum., the early stages and male of *Simulium faheyi* Tayl., and records extending the known distribution of previously described species. The Queensland fauna has been increased from nine to seventeen species.

References listed in our earlier papers are not repeated here.



Text-fig. 1.—Lateral view of larvae of (*a*) *Cnephia tonnoiri orientalis*, and (*b*) *Simulium ornatipes*, showing form of abdomen.

The Genus Cnephia End.

We were previously unable to give characters separating the larvae from *Simulium*. We find that they may be recognized by the truncated posterior end of the abdomen, the maximum width being at the seventh segment, as compared with the more fusiform abdomen of *Simulium* (Text-fig. 1). They are also more waxy and opaque in appearance, and the ventral incisure of the head capsule is shallow, contrasting with the deep incisure of all known Australasian species of *Simulium* (Text-fig. 2). The incisure is shallow in several species of *Austrosimulium* also, but that genus is distinguished by the anal sclerite, and the body is fusiform like *Simulium*.

Although its early stages are still unknown, we now consider that *C. umbratorum* (Tonn.) should be placed in the *aurantiacum* group.

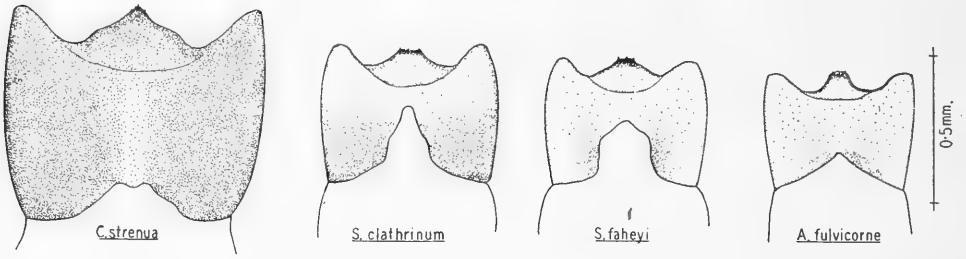
Keys to species of *aurantiacum* group.

Adults.

Orange to reddish brown flies; Cu_1 gently curved; claws of ♀ with powerful basal tooth.

1. Wing with dark marking at fork of R 2
   Wing without dark marking 3
2. Scutum brown scaled, with median and dorsocentral vittae of golden scales .. *strenua* n. sp.
   Scutum uniformly covered with golden scales ..... *tonnoiri* (Drum.)*
3. Large orange species; propleural hairs present ..... *aurantiacum* (Tonn.)
   Small to medium reddish brown species; propleural hairs absent ..... *umbratorum* (Tonn.)

* All subspecies.



Text-fig. 2.—Head capsules of larvae, showing ventral incisure.

Pupae.

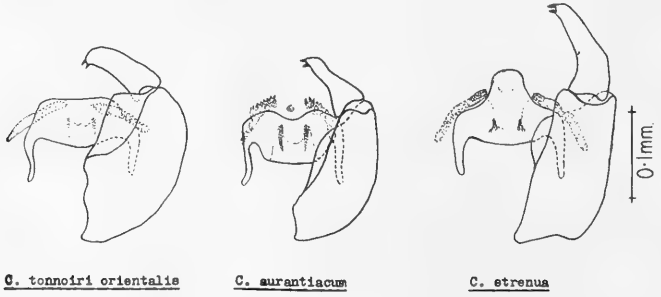
Abdomen with strong terminal hooks, chaetotaxy as in Text-fig. 4; gill filaments many-branched, arborescent.

1. Pleural membrane of abdominal segments 5-7 without chitinous plates .... *aurantiacum* (Tonn.) *strenua* n. sp.
   Pleural membrane of abdominal segments 5-7 with rounded chitinous plates 2
2. Gill filaments antler-like, about 20-40 ..... *tonnoiri tonnoiri* (Drum.)
   Gill filaments slender, in sweeping curves, about 50-70 ..... *tonnoiri orientalis* n. subsp.
   Gill filaments stout, about 15-20 ..... *tonnoiri fuscoflava* M. & M.

Larvae.

Abdomen truncate, widest at 7th segment; cuticle waxy, opaque; antennae short (Text-fig. 11); ventral incisure of head capsule shallow (Text-fig. 2); anal sclerite without backwardly directed strut; rectal gills simple; ventral papillae absent.

1. Proleg with prominent palp-like processes at either side of apical segment; arms of anal sclerite reduplicated ..... *strenua* n. sp.
   Proleg without such prominent processes; arms of anal sclerite single 2
2. Posterior circlet broad, rows closely placed, teeth light brown, small and numerous, difficult to see individually at 50 diameters ..... *aurantiacum* (Tonn.)
   Circlet narrower, rows not so closely placed, teeth dark, larger, and can be seen individually at less than 50 diameters 3
3. Circlet with 18-24 teeth per row .... *tonnoiri tonnoiri* (Drum.) *tonnoiri orientalis* n. subsp.
   Circlet with about 15 teeth per row ..... *tonnoiri fuscoflava* M. & M.



Text-fig. 3.—Hypopygia of males of *Cnephia* spp.

CNEPHIA AURANTIACUM (Tonn.).

The characters of adults and larvae previously reported have been confirmed. In addition, the upper facets of the male eyes are smaller and more numerous than in other

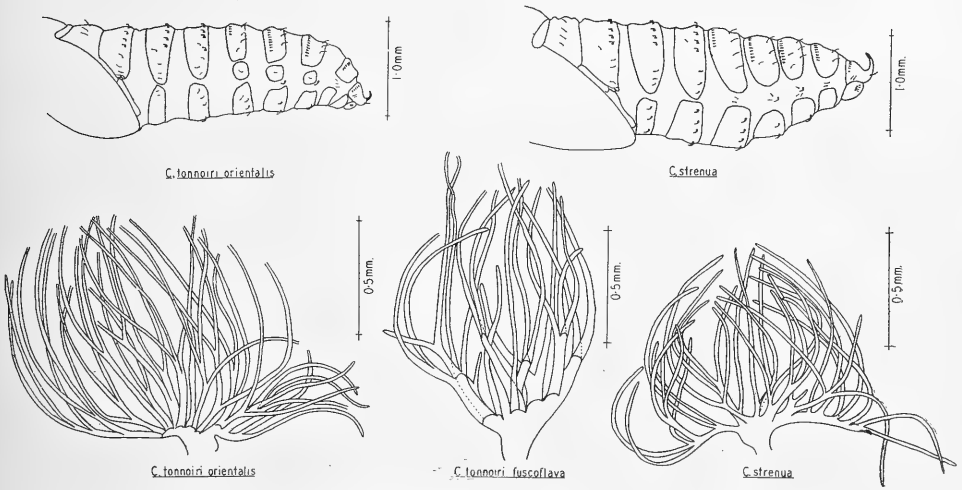
species of the genus, being about 0·04 mm. in diameter and in more than 20 rows vertically and across. The abdomen, in specimens we have seen, is less hairy than in *C. tonnoiri*. The hypopygium (Text-fig. 3) has the distal end of the anterior part of the phallosome distinctly concave and the distal end of the apodemes more heavily armed than in *C. tonnoiri*.

The posterior part of the cocoon is of more definitely "wall-pocket" shape than in the other species, but is loosely constructed anteriorly, so that the head and thorax of the pupa often hang freely from the opening.

Pupae are best separated from *C. tonnoiri* by the absence of pleural plates on abdominal segments 5–7. The gill filaments number about 30 to 40, and are stouter than in *C. tonnoiri orientalis*, with broadly angled branches, and a stiff, antler-like form; they cannot be distinguished readily from those of *C. strenua* and *C. tonnoiri tonnoiri*.

The posterior circlet of the larva has a more distinctive appearance than can be indicated in words, and may be seen quite easily in unmounted specimens at the magnification indicated. The median notch in the anal sclerite is closed posteriorly, so that the body of the sclerite appears to have a hole in it (Text-fig. 5).

New distribution.—Queensland: South coast district. Little Nerang Creek, elevation 300 ft., August. Early stages on reeds in fairly fast, cold, clear, turbulent water; adults bred from the pupae.



Text-fig. 4.—Pupae of *Cnephia* spp. Top, abdominal chaetotaxy; below, respiratory horns.

Cnephia tonnoiri (Drum.).

All subspecies are to be distinguished from *C. aurantiacum*: as *adults* by the dark spot on the wing, the almost invariable absence of propleural hairs, the more extensively darkened mid and hind legs, the larger upper facets of the ♂ eyes (about 0·06 mm. in diameter and in about 15 rows vertically, 12 across), and by the ♂ hypopygium (Text-fig. 3), the distal end of the anterior part of the phallosome being gently sinuous and the apodemes relatively weak; as *pupae* by possessing well defined, rounded chitinous plates on the pleural membrane of abdominal segments 5 to 7 (Text-fig. 4); and as *larvae* by the coarser posterior circlet and open median notch in the anal sclerite (Text-fig. 5).

The three subspecies now recognized are distinguished from each other primarily on the pupae.

Cnephia tonnoiri tonnoiri (Drum.).

The respiratory filaments of the pupa number about 20 to 40, the main branches are a little stouter than in the eastern race, relatively stiff, with wide angle of branching

and antler-like appearance similar to *C. strenua* in Text-fig. 4 and also very like the filaments of *C. aurantiacum*.

Distribution.—Western Australia (localities given in previous paper).

Cnephia tonnoiri orientalis, n. subsp.

Types: Holotype ♀, allotype ♂, morphotype pupa and larva, from Little Nerang Creek, south coastal Queensland, September, in the Division of Entomology, C.S.I.R.O., Canberra, A.C.T.

Adults.

Indistinguishable from *C. tonnoiri fuscoflava* as originally described (Mackerras and Mackerras, 1948, p. 238). They may be darker than the typical subspecies, but the only specimens we have seen from Western Australia are rather old, and we did not previously give sufficient weight to the possibility that they may have faded. We have searched for propleural hairs on a considerable series of specimens, and found two or three weak hairs near the lower margin of the sclerite on one side only in two females. As these hairs form a well-defined group on the surface of the sclerite in *C. aurantiacum*, this character remains substantially reliable. Hypopygium of male as in Text-fig. 3.

Cocoon.

A shapeless bag, as in other subspecies.

Pupa.

Chaetotaxy and terminal spines as in other subspecies. Gill filaments about 50 to 70 in number, rather slender, branching mostly close to the base with narrow fork, more flexible than in the typical subspecies and sweeping forward in even curves (Text-fig. 4). Specimens from the type and southern localities have about 50 filaments, those from Springbrook 50 to 70, but are otherwise indistinguishable.

Larva.

As typical subspecies. The posterior circlet is composed of well-spaced rows of eighteen to twenty-four medium-sized teeth.

Distribution.—Tasmania: Rheban (Griffiths R., Sandspit R.), January. A.C.T.: Canberra, November (Tonnoir); Coree Creek, November, January (Tonnoir); Cotter R. and Paddy's R., November (Mackerras). Queensland: Little Nerang Creek (300 ft.), August, September; Purling Brook (Springbrook area), 2,000 ft., December.

Biology.

The early stages occur in clear, moderate to fast, turbulent streams, generally adjacent to, rather than in, the line of fastest flow. They are nearly always attached to vegetation, rarely to stones. Adults have not been collected in the field.

Cnephia tonnoiri fuscoflava M. & M.

The pupal gill filaments are stouter and fewer than in the other subspecies (Text-fig. 4), and there are fewer teeth in the posterior circlet of the larva. Adults are indistinguishable from *C. tonnoiri orientalis*.

Distribution.—Queensland: Still only known from the type locality on Stradbroke Is., our (1949) record of it from Little Nerang Creek being in error.

CNEPHIA STRENUA n. sp.

Types.—Holotype ♀, allotype ♂, morphotype pupa and larva, from the Cascades, Freshwater Creek, Cairns district, north Queensland, September, in the Division of Entomology, C.S.I.R.O., Canberra, A.C.T.

Distinctive features.

A large species. Adults resemble *C. tonnoiri*, but are distinguished by the dark antennae, scutal pattern, black hairs at sides of scutellum, differently marked legs, and less hairy abdomen. Pupae similar to *C. aurantiacum*. Larvae gigantic when full grown, with very dark head and very broad posterior circlet of very fine teeth; on each side of the distal segment of the proleg there is a conspicuous, palp-like process, which has not been seen in any other species we have examined.

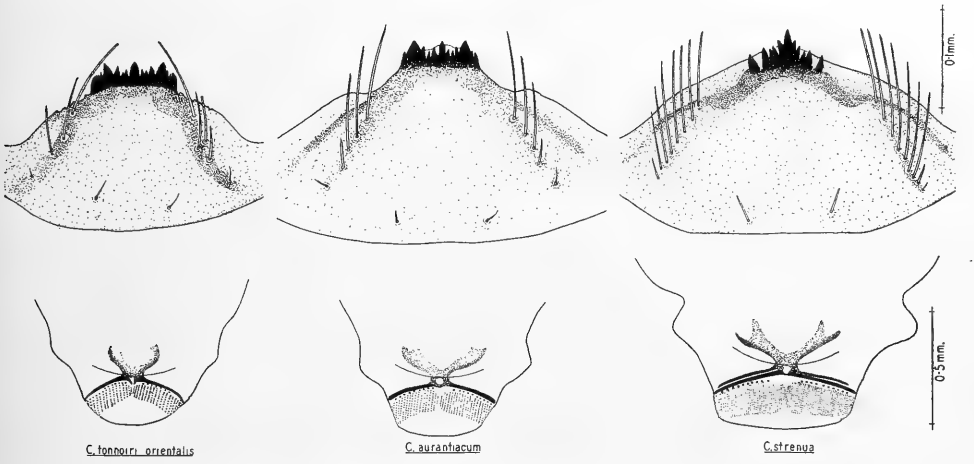
Female.

Length: Body 3·5 to 4 mm., wing about the same.

Head.—Frons about one-eleventh of head width, dark greyish brown, with fine golden hairs. Antennae with basal three segments light brown, remainder dark brown. Face and palpi brown.

Thorax.—Scutum rich brown, covered with brown scales showing golden reflections anteriorly and posteriorly in certain lights, and with narrow but distinct median and dorsocentral vittae of golden scales. Scutellum with conspicuous long black hairs laterally and short golden ones centrally. Pleurae yellowish brown, darker on lower part of sternopleuron. A few propleural hairs are present in all specimens.

Legs.—Coxae and femora yellowish, but with dark brown tips to femora. Basal quarter of tibiae yellowish, remainder dark brown. Hind metatarsus similar; other tarsal segments brown, with narrow yellow zone at base. Calcipala large, of typical form of the group; pedisulcus shallow, corrugated. Claws with strong basal tooth, as in other species of the group.



Text-fig. 5.—Submenta (above) and anal sclerites (below) of larvae of *Cnephia* spp.

Wings.—Hyaline, with small but distinct dark marks at base and at fork of R as in *C. tonnoiri*. Halteres large; stem creamy yellow, knob dark brown.

Abdomen.—Dark greyish brown dorsally, with a fringe of golden hairs near distal edge of each segment. Spermatheca nearly smooth; external genitalia and genital fork as in *C. tonnoiri*.

Male.

The upper facets of the eyes are about 0·06 mm. in diameter and in about 15 rows vertically, 12 across. The antennae are pale on only the first and basal half of the second segment; the scutal vittae are less well defined, and there is a rather indefinite patch of golden scales above and in front of wing roots; the legs are less darkened; otherwise as in female.

Hypopygium (Text-fig. 3) with the distal end of the anterior part of the phallosome lightly chitinized and produced into a prominent process, which at first sight resembles a median piece, but is seen on close examination to be continuous with the distal edge. There is a rather prominent, hairy lobe on the inner side of the coxite.

Cocoon.

Length 3–4 mm. Like *C. tonnoiri*, soft, simple bag-like; dark, and usually including foreign material. Attached to substrate all along ventral wall, generally in groups of two or more. The head and thorax of the pupa often project from the cocoon.

Pupa.

Length 3–4·5 mm. The respiratory organ has a short, wide stem, which divides at once into several wide branches, the posterior being the longest. Each main trunk

gives off a number of spreading, antler-like branches. Many of these divide again, forming a complex group of about 50 to 55 filaments arranged as in Text-fig. 4, in which, however, only about two-thirds of the filaments are shown.

The pupa cannot be distinguished on these and other easily seen characters from *C. aurantiacum*, but does appear to differ, in that the third abdominal sternite is more strongly chitinized and bears three instead of two hooks laterally on each side, the tergites are finely tuberculate on their posterior portions only (tuberculate all over in *aurantiacum*), and there are usually more than five stiff hairs on each side of the ninth tergite (five in *aurantiacum*). The first of these characters may be variable, and the others can only be seen in cleared and mounted material.

Larva.

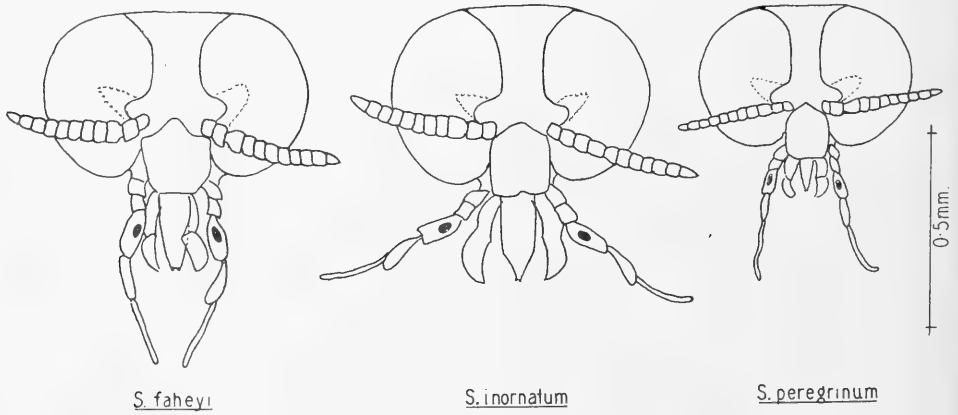
Length of gill-spot larvae 8–11 mm. Very large, thick, brownish larvae, with paler integument ventrally at posterior end. Head capsule and its appendages very heavily pigmented, dark brown to black, obscuring pattern on dorsum of head. Submental plate characteristic, with prominent central tooth and eight or nine pairs of stout, blade-like spines on each side (Text-fig. 5). Gill-spot as in Text-fig. 14. Thoracic proleg robust and armed with a conspicuous circlet of dark hooks, which are more closely set than in other known species; the lateral cuticular plates are well developed and bear numerous long spines, and there are two pointed palp-like processes at the base of the apical narrower portion of the proleg (Text-fig. 14).

Anal sclerite with median notch closed posteriorly to form a "hole"; its posterior limbs double. There is a conspicuous row of 10–12 tubercles bearing short spines on each side between the posterior limb of the sclerite and the upper edge of the circlet, and also a patch of fine, short hairs on each side above the posterior limbs. Posterior circlet exceptionally well developed, the rows of hooks being so closely packed and the hooks so numerous as to render counting them impracticable. Individual hooks are rather smaller than in *C. tonnoiri*, and are also smaller than the hooks on the proleg.

Distribution.—Known only from the type locality in North Queensland.

Biology.

This is a remarkable species. The larvae are enormous, so large that we scarcely believed that they were Simuliid larvae when we first saw them; yet their bulk must be made up of muscle and essentially larval organs, for the pupae and adults are little if



Text-fig. 6.—Heads of females of *Simulium* spp.

any bigger than their near relatives. Most larvae were found in cascades, at points where a powerful jet of water was concentrated between narrow walls of rock and shooting over a sharp ledge. The depth of water was about twelve inches, and the flow so strong that an arm or leg could only be held against it with the greatest difficulty. The larvae were clustered thickly on the bottom ledge, where the flow was strongest. It is from their adaptation to withstand such a battering that the specific

name is derived. By contrast, a few were also found in company with *Simulium aureonigrum* in a small tributary, on a nearly vertical face of rock over which the water poured in a thin, fast layer. When removed from the water, the larvae adhered to our hands in a way we had not seen previously, and were quite difficult to remove.

Pupae were in groups on the rocks, in rougher, more sheltered places below the brink, where the water spread out fanwise into a fast, but much thinner and less forceful layer. They were quite difficult to collect intact. No adults were seen in the field.

The Genus Simulium Latr.

So many species have been added that our previous keys have become obsolete.

Keys to Species.

Females.

1. Pre-alar area bare 2
   Pre-alar area with conspicuous pale scales 3
2. Medium-sized species; legs conspicuously marked with black and yellow ..... *ornatipes* Sk.
   Small species; legs predominantly dark (basal two-thirds of hind metatarsus pale) ..... *peregrinum* n. sp.
3. Minute, pale species; antennae entirely yellowish fawn; femora and tibiae predominantly creamy yellow. (Northern Territory.) ..... *Simulium* sp. *B.*
   Larger, darker species; antennae with at most the basal segments pale; femora and tibiae predominantly dark 4
4. Scutum with golden median and dorsocentral lines; abdomen with tergites 2-4 black scaled; 5-8 bare, greyish black, rather shining ..... *clathrinum* M. & M.
   Scutum without discrete golden lines; abdomen with pale scales on some tergites, including 5-8 5
5. Basal segments of antennae brown; very dark species 6
   Basal segments of antennae orange to brownish yellow; not such dark species 7
6. Scutum and second abdominal tergite adorned with rich golden scales; tergites 3-5 entirely dark, 6-8 sprinkled with creamy gold scales ..... *aureonigrum* n. sp.
   Scutum and second abdominal tergite with dull golden to silvery scales; tergites 3-7 with incomplete golden or creamy fringe and few or no pale scales on the disc, 8 with a few scattered pale scales ..... *inornatum* n. sp.
   Scutum and second abdominal tergite with creamy gold to silvery scales; tergites 3-5 with a few apical silvery scales, 6-8 speckled with silvery scales ..... *melatum* Wh.
7. Brownish black and golden species; second abdominal tergite with at most an incomplete golden fringe; claws with small, sub-basal tooth ..... *faheyi* Tayl.
   Brownish black and creamy gold species; second abdominal tergite quite densely covered with creamy gold scales; claws with small, sub-basal tooth ..... *papuense* Wh.
   Black and silvery species; second abdominal tergite with median patch or apical band of silvery scales; claws without teeth ..... *nicholsoni* M. & M.

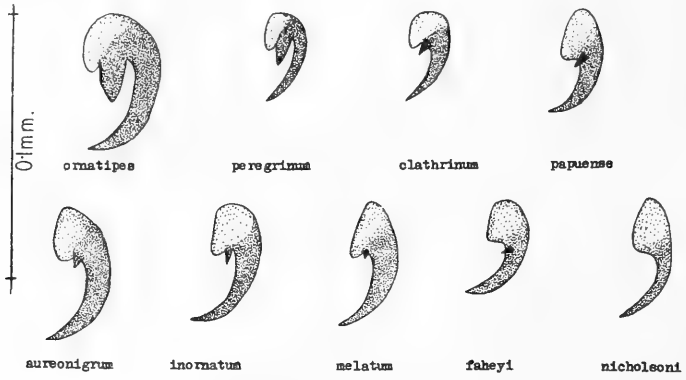
Notes.—(1) We have omitted two New Guinea species, *S. oculatum* End. and *S. wilhelmlandae* Smart, because it is uncertain to which groups they belong; the former may be close to *S. clathrinum*, the latter to *S. peregrinum*. (2) The claw characters are useful, but can only be seen properly in cleared and mounted preparations. Females of *S. ornatipes* and *S. peregrinum* have very large basal teeth on the claws, *S. clathrinum* has medium-sized teeth, often detectable in pinned specimens, *S. aureonigrum*, *S. inornatum*, *S. faheyi* and *S. papuense* have small sub-basal teeth, *S. melatum* has minute teeth deeply set in the concavity of the claw, and *S. nicholsoni* has none (Text-fig. 7).

Males.

1. Pre-alar area bare 2
   Pre-alar area with conspicuous pale scales 3
2. Medium-sized species; upper facets of eye less than 0·04 mm. in diameter, in about 16 rows; legs conspicuously marked with black and yellow ..... *ornatipes* Sk.
   Small species; upper facets of eye more than 0·05 mm. in diameter, in 10 rows; legs predominantly dark ..... *peregrinum* n. sp.
3. Upper facets of eye more than 0·04 mm. in diameter, in about 12 rows 4
   Upper facets of eye less than 0·04 mm. in diameter, in about 16 rows 6
4. Abdominal tergites 2 and 5-8 with golden scales arranged much as in ♀ ..... *papuense* Wh.
   Abdominal tergites 2-8 velvety black, tomentose 5
5. Scutum with three golden lines usually discernible ..... *clathrinum* M. & M.
   Scutum without indication of median and dorsocentral golden lines .... *aureonigrum* n. sp.

6. Anterior part of phallosome with straight or gently sinuous distal edge ..... *nicholsoni* M. & M. *faheyi* Tayl.
   Anterior part of phallosome with markedly concave distal edge 7
7. Anterior part of phallosome forming an open bay distally ..... *melatum* Wh.
   Anterior part of phallosome with bay almost enclosed distally ..... *inornatum* n. sp.

Note.—The abdominal adornment of *S. papuense* is most unusual. Our specimen may be an intersex, but the eyes, legs and genitalia are normal, and Wharton's description of the allotype agrees.



Text-fig. 7.—Claws of females of *Simulium* spp.

Pupae.

1. Gill filaments numerous, arborescent ..... *papuense* Wh.
   Gill filaments 8 on each side ..... *peregrinum* n. sp.
   Gill filaments 6 on each side 2
   Gill filaments 4 on each side 3
2. Respiratory organ with well-marked stem; filaments directed forward close together ..... *nicholsoni* M. & M.
   Respiratory organ with short stem; filaments spreading more widely ..... *faheyi* Tayl.
3. Cocoon with deep collar; filaments narrow, subequal, directed forward close together ..... *clathrinum* M. & M.
   Cocoon with narrow collar or none 4
4. Filaments very thick, pale, relatively smooth, subequal, spreading widely .... *ornatipes* Sk.
   Filaments of moderate thickness, darker, irregularly mammillated, subequal, directed forward close together ..... *melatum* Wh.
   Filaments of intermediate thickness, dark, finely patterned, of unequal length, spreading widely ..... *inornatum* n. sp.
   Filaments narrow, dark, finely patterned, subequal, spreading ..... *aureonigrum* n. sp.

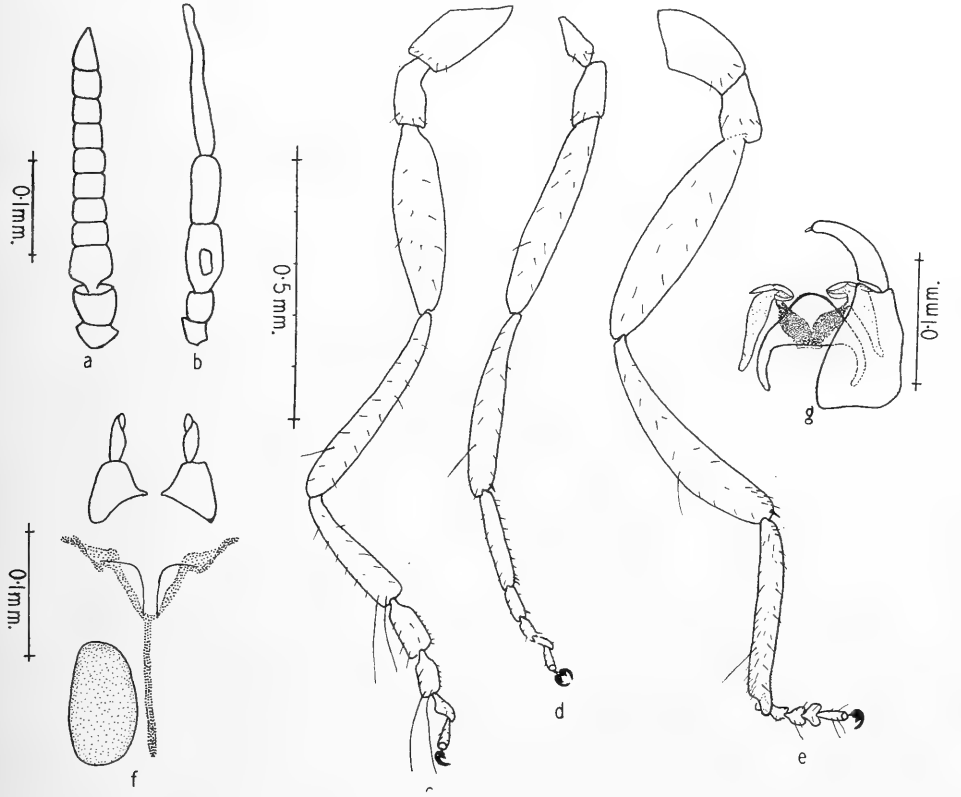
Larvae.

1. Rectal gills simple 2
   Rectal gills compound 5
2. A broken line of brown scales ventro-laterally on each side anterior to the circlet 3
   No brown scales in this position 4
3. Posterior part of head rather uniformly pigmented; ventral papillae usually indefinite or absent ..... *melatum* Wh.
   Posterior part of head with a W-shaped pattern; ventral papillae well defined ..... *inornatum* n. sp.
4. Smaller, more yellowish larvae; head pattern negative type ..... *nicholsoni* M. & M.
   Larger, darker larvae; head pattern positive type ..... *ornatipes* Sk.
5. Small larvae; antennae conspicuous, nearly as long as head ..... *peregrinum* n. sp.
   Larger larvae; antennae inconspicuous, about half as long as head 6
6. A broken line of brown scales ventro-laterally on each side anterior to the circlet ..... *aureonigrum* n. sp.
   No brown scales in this position 7
7. Head pattern indefinite or partly negative in type; ventral incisure extending about half-way to base of submentum ..... *faheyi* Tayl.
   Head pattern positive type; ventral incisure extending more than half-way to base of submentum 8
8. Ventral incisure reaching base of submentum; head pattern conspicuous, bullet-shaped ..... *papuense* Wh.
   Ventral incisure not reaching base of submentum; head pattern cruciate .. *clathrinum* M. & M.

Notes.—(1) The ventro-lateral scales at the posterior end of the abdomen are sometimes inconspicuous or absent in young larvae of *S. melatum*. (2) The ventral papillae do not provide such clear-cut distinctions as in *Austrosimulium*. They are large in *peregrinum*, medium to small in *ornatipes*, *nicholsoni*, *faheyi*, *aureonigrum* and *inornatum*, indefinite or ventro-lateral in *melatum* and *papuense*, and absent (though ventro-lateral swellings are present) in *clathrinum*.

Simulium ornatipes Sk.

New distribution.—Queensland: Springbrook, 2,000 ft. (south coast district), December; various coastal streams between Nambour and Gympie, February, April, May; Babinda, N.Q., September (the most northerly record so far on the mainland).



Text-fig. 8.—*S. peregrinum*. ♀: a, antenna; b, palp; c, fore leg; d, mid leg; e, hind leg; f, genitalia. ♂: g, hypopygium.

Simulium peregrinum n. sp.

Types: Holotype ♀, allotype ♂, morphotype pupa and larva, from Black Camp Creek, Cape York, August, in the Division of Entomology, C.S.I.R.O., Canberra, A.C.T.

Distinctive features.

A very small species, lacking pre-alar scales, but possessing lower sternopleural ("mesosternal") hairs and largely pale hind metatarsus like the *clathrinum* group. Adults with base of abdomen creamy to yellowish in both sexes; head of male wider than thorax, upper facets of eye greatly enlarged. Pupae with respiratory apparatus longer than the body, dichotomously branched, forming eight slender filaments. Larvae with very prominent ventral papillae, compound anal gills, and antennae which are nearly as long as the head.

This is the first species of Edwards' sub-group C to be found on the mainland of Australia. It is typical of the sub-group in all respects, and quite distinct from the

clathrinum group, though it shows features which suggest that the latter may have evolved from the former in the isolation of their Australasian extension. Similarly, *S. ornatipes*, which lacks lower sternopleural hairs, would appear to represent the sub-group D in a somewhat modified form in this region.

S. peregrinum is separable from the species of sub-group C described by Edwards from Java most conspicuously on pupal characters, and from *S. wilhelmlandae* Smart. (= *Morops pygmaea* End.) by the colouration of the antennae and abdomen. Its name is derived from the fact that it is, in a sense, a wanderer from the home of its relatives.

Female.

Length: Body 1·5 mm.; wing 1·4 mm.

Head wide and rounded. Frons about one-sixth of head width (Text-fig. 6) brownish black, shining. Antennae eleven-segmented; basal three or four segments brownish yellow, remainder dark brown. Proboscis very short, so that the palpi appear to be long; both dark brown, with the terminal segment of the palp paler.

Thorax.—Scutum and scutellum uniformly greyish black, rather shining; disc thinly and evenly covered with short, fine, golden hairs, which are rather denser and stronger at the sides and on the scutellum; apical edge of scutellum with a row of long black hairs. Pleurae uniformly dark greyish brown. Pre-alar area bare; a tuft of white propleural hairs present; lower sternopleural hairs fine, pale, and rather scattered.

Wings clear, veins brownish yellow, hairs black. There is the usual row of spinules on the costa and a few on the distal part of R_1; upper surface of R with a single row of black hairs. Halteres with stem light brown, knob pale lemon yellow.

Legs (Text-fig. 8).—Fore tarsi larger and more powerful than mid, distinctly thickened but not flattened; the enlargement is more obvious than in the *clathrinum* group. Hind femora and tibiae swollen, about twice as thick as fore or mid, the tibiae angulated beyond the middle posteriorly. Calcipala and pedisulcus well developed. All claws with a powerful basal tooth.

Fore and mid coxae cream; femora brown, covered with golden scales on most of anterior surface; tibiae brown, with creamy knees and golden scales on about proximal third; tarsi deep brown, almost black. Hind coxae brown, cream apically; femora brown, with rather scattered pale golden scales; tibiae brown, with creamy knees and a covering of pale golden scales on basal half anteriorly; metatarsi cream and covered with creamy golden scales on basal four-fifths, brown on distal fifth; remaining tarsal segments dark brown, but with a ring of pale scales at bases of second and third segments.

Abdomen.—First tergite brown, creamy in centre and with a creamy fringe; second largely cream, but more or less narrowly brown along apical edge; 3 to 5 brownish black, tomentose, with dark hairs; 6 to 8 black and shining. Venter cream basally, light brown apically. Terminalia, genital fork and spermatheca as in Text-fig. 8.

The female from Smoko Creek is a little larger; tergites 1 and 2 of its abdomen are more completely yellow; only the basal three-fourths of the hind metatarsi are pale; but otherwise it is similar to the Cape York specimens. It came from an exactly similar pupa.

Male.

Head large, rounded, wider than thorax. Upper facets of eye about 0·05 mm. in diameter,* in ten rows vertically and transversely. Antennae creamy yellow, except for the distal 2 to 4 segments, which are darkened. Scutum fairly densely covered with creamy gold scales, which are larger and more conspicuous than in the female. Pleurae, wings and legs as in female; claws simple, of typical male form. Abdomen with first tergite dark brown, with long brown fringe; second mostly yellow, with apical brown zone widening at sides, the pale part covered with rather shining tomentum; remaining segments velvety black, except for conspicuous, silvery, tomentose sublateral patches on 5 to 7; venter as in female.

* Eye facet measurements given in this paper were made on cleared preparations mounted in Canada balsam.

Hypopygium (Text-fig. 8) with style shorter than coxite, ending in a single spine. Anterior part of phallosome strongly convex and longitudinally striate ventrally; posterior part with a pair of heavily chitinized, deeply pigmented, somewhat irregular plates, possibly corresponding to the chitinized bars in *Cnephia*. Apodemes large and powerful, terminating in a beak-like structure with three divisions. No median piece detected.

Cocoon.

Wall-pocket type; smooth, very thin and delicate; no collar and no central dorsal projection.

Pupa.

Length: Body 2·1 mm., filaments 2·2 mm.

There is a group of four long hairs on each side of the scutum just anterior to its highest point; abdominal armature weak, inconspicuous, of normal distribution, except for the presence of patches of minute spines on the ventral surface of segments 4 to 6. Respiratory apparatus (Text-fig. 10) with a distinct stem, branching dichotomously to form eight very long, slender filaments; the lower and outer pairs are longer than the upper and inner. The tips of these delicate filaments break off easily, so that discrepancies in their relative lengths are seen in different specimens.

Larva.

Small; length of gill-spot larva about 3·7 mm.

Head pattern (Text-fig. 11) of positive type, rather indefinite; generally of broadly rectangular form, with a somewhat darker median zone merging into a darkened, transverse, posterior triangle. Antennae very large, more than three-fourths the length of the head; basal segment longer than apical, and without apparent subdivision into two parts. Submentum as in Text-fig. 12.

Gill-spot brown, characteristic, the long stem and posteriorly coiled filaments being easily seen (Text-fig. 14).

Abdomen.—Ventral papillae triangular, very large and conspicuous. Rectal gills compound, each with about four lobes. Posterior circlet narrow, rows well spaced, each composed of 12 rather large teeth. Anal sclerite as in Text-fig. 12. There are no ventro-lateral dark scales in front of the circlet.

Distribution.—North Queensland: Cape York (Black Camp Creek, Black Gin Creek), August, September; Russell River, near Babinda, and Smoko Creek, near Bramston Beach, September.

Biology.

In the type locality larvae were abundant on reeds, sticks, and dead leaves in the swifter parts of small, clear, shaded creeks running through fairly dense bush. Pupae were present in the same situations, but only a few could be found, although gill-spot larvae were not uncommon. Conditions in Smoko Creek were similar to those on Cape York, but the Russell River was a swifter, more open stream. Adults were not seen in the field.

Simulium clathrinum M. & M.

Adults, pupae and larvae from North Queensland resemble those from the south, but the cocoons (Text-fig. 15) differ in that, although they have the same lattice-like construction and well-developed collar, the mouth has a clearly defined, often thickened edge, which gives it something the appearance of an *A. bancrofti* cocoon under a hand lens. Many pupae in the Babinda district had the filaments broken off short, and none of these gave rise to adults.

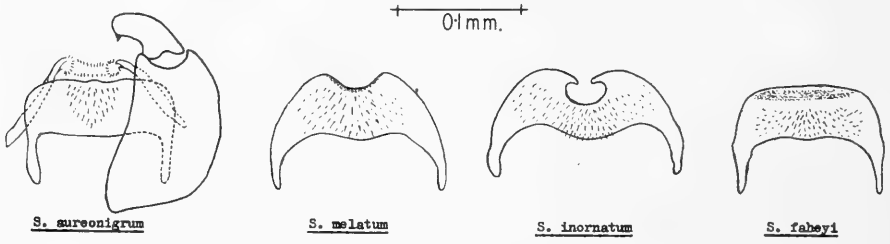
New distribution.—Queensland: Currumbin Creek, near N.S.W. border, January; swifter coastal streams between Nambour and Gympie, February, April, May (southern race). Cape York, Black Gin Creek, August; Babinda district, abundant in larger, swifter streams and also in a small jungle creek near Bramston Beach, September; Innisfail district, Berner Creek, September (all these being northern race).

Simulium aureonigrum n. sp.

Types: Holotype ♀, allotype ♂, morphotype pupa and larva, from a small tributary near the Cascades, Freshwater Creek, Cairns district, October, in the Division of Entomology, C.S.I.R.O., Canberra, A.C.T.

Distinctive features.

Five Australian species of *Simulium* are now known to have pupal respiratory organs with four filaments. *S. ornatipes* stands apart on group and general characters, and *S. clathrinum* is well differentiated in all stages, but the other three (*aureonigrum, melatum, inornatum*) are closely related. They are dark species, with more rounded heads than *S. nicholsoni* and *S. faheyi* (Text-fig. 6), and with larvae having ventro-lateral patches of brown scales just in front of the posterior circlet, suggesting at first sight a rudimentary form of the ventral chitinous ring found in some species of *Austrosimulium.*



Text-fig. 9.—♂ hypopygium of *S. aureonigrum*; anterior part of phallosome of other species.

S. aureonigrum is distinguished from its near relatives: in the female by the rich golden scales on the jet black scutum and by the arrangement of the golden scales on the abdomen; in the male by the large upper facets of the eyes and gently sinuous distal margin of the anterior part of the phallosome, in both of which respects it is nearer to *S. clathrinum*; in the cocoon by its close weave and lack of a central dorsal projection; in the pupa by the form and arrangement of the gill-filaments (Text-fig. 15); and in the larva by the compound rectal gills.

Female.

Length: Body 2·3 mm., wing 2·3 mm.

Head.—Frons about one-fifth of head width, tapering towards antennae; grey, with scattered golden scales. Face grey, with some pale scales. There is a rim of golden scales on the occiput behind the eyes. Antennae with first two segments light brown, remainder brownish black. Proboscis dark brown, palpi brownish black.

Thorax.—Scutum black, fairly densely covered with rich golden scales, mixed on the disc with some patchily distributed black ones. The black scales also form fairly definite, narrow dorsocentral lines, which converge slightly from behind forward and then diverge for the anterior fourth of their length as in *S. nicholsoni.* Pleurae uniformly dark grey, with paler grey reflections in certain lights. Pre-alar scales rich to creamy gold; pronotal, propleural and lower sternopleural scales pale gold.

Legs.—Fore and mid coxae covered with golden scales, hind coxae black. Femora black, with rather irregularly distributed golden scales. Tibiae black, with paler gold (silvery in certain lights) scales on basal half, extending more distally on outer surface. Fore and mid tarsi black. Hind metatarsus with creamy zone on basal two-thirds; remaining segments completely dark. Calcipala and pedisulcus as in *S. clathrinum.* Claws with a small sub-basal tooth.

Wings hyaline, veins dark. Halteres with brown stem and creamy yellow knob.

Abdomen.—First segment dark brown laterally, paler medially, with a deep golden fringe. Second deep brown, blackish at apex, and bearing a conspicuous median patch of rich golden scales, which extend laterally as an apical golden line to join the golden patches on the side of the abdomen. Third to fifth entirely black dorsally. Sixth with

a smooth, somewhat tomentose area in centre, seventh and eighth entirely smooth, not as shining as *S. clathrinum*; all three bearing scattered golden scales, especially near their apical edges. Side of abdomen with broad patches of rich golden scales on segments 2 to 5, a few on 6, and only two or three on 7. Venter dull yellowish brown. External genitalia and genital fork as in other species of the group.

Male.

Upper facets of eyes as in *S. clathrinum*, averaging 0·042 mm. in diameter and in about 12 rows vertically and across. The antennae are entirely dark. The scutum is jet black, fairly densely covered with rich golden scales, which are somewhat irregularly mixed with black ones in the central area, though with no indication of definite lines. The legs are similar to the female, except that the zone of pale scales on the hind tibia is more sharply limited distally, and the pale area on the hind metatarsus is rather vague and indefinite.

Abdomen dark brown to black, with apex of first segment lighter brown; second to fourth covered with velvety black tomentum, remainder with velvety black tomentum in centre, somewhat shining at sides, where there are silvery reflections in certain lights. Venter with basal two or three segments dull yellowish brown, remainder dark. Hypopygium similar to that of *S. clathrinum*.

Cocoon.

Length about 2·7 mm. along base. An open wall-pocket type, smooth and fairly closely woven; with a well-defined, darkened, rolled edge, but without collar or central dorsal projection.

Pupa.

Length 2·5 mm. Cephalic and thoracic hairs slender. Head and thorax densely covered with minute, irregularly arranged tubercles, which become more triangular and spiny on the under side of the head, legs and wing covers. Respiratory organ (Text-fig. 15) with a very short stem, which is covered with minute triangular spines. The four filaments come off almost simultaneously; the first sweeps upwards and directly forwards; the second upwards, inwards and forwards; the third downwards, inwards, forwards and upwards; and the fourth downwards, outwards, forwards and finally curves upwards and inwards. Abdominal chaetotaxy normal.

Larva.

Length 5 to 6 mm. when full grown. Body white to slaty grey in colour, with the usual darker mottling. Antennae about half the length of the head, projecting a little beyond basal segment of mouth brushes. Head pattern positive type, usually forming a well-defined "W" as in Text-fig. 11, but sometimes rather diffuse. Ventral incisure deeper than wide, extending about half-way to base of submentum, and forming a rounded triangle anteriorly. Submentum as in Text-fig. 12; five pairs of hairs on submental plate.

Gill-spot (Text-fig. 14) L-shaped, with three main trunks showing in the anterior, upright limb, which is shorter than the horizontal limb.

Ventral papillae present, small and triangular, rather better defined than in related species. Rectal gills compound; accessory lobes variable, usually three on each main lobe, but sometimes fewer and occasionally none on one or two lobes. Anal sclerite as in Text-fig. 12. Posterior circlet composed of about 90 rows of hooklets, with about 12 hooklets per row. There are two patches of flat, brownish scales ventro-laterally on each side just anterior to the circlet.

Distribution.—Known only from the type locality in north Queensland.

Biology.

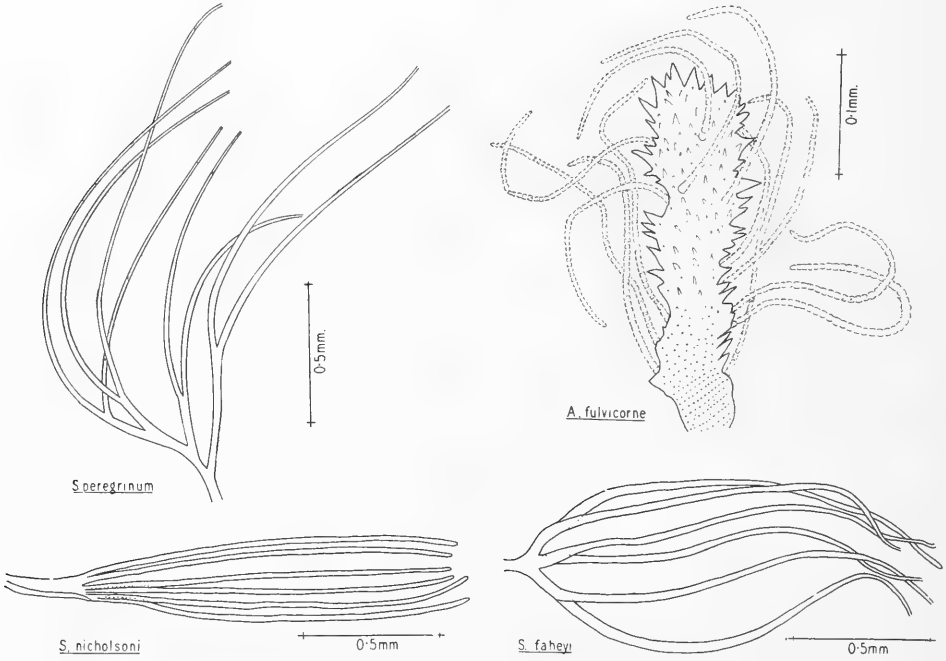
Larvae and pupae were found adhering to rock and dead leaves, in company with a few *Cnephia strenua*, in a thin layer of clear water coursing rapidly over an almost vertical face of rock. The stream was quite small and well shaded, in contrast to the powerful, turbulent flow in the adjacent, larger, open Freshwater Creek, where *C. strenua* was abundant but no *Simulium* larvae were found. Adults were not seen in the field.

Many of the larvae were parasitized by Mermethid worms, which sometimes almost filled the posterior swollen part of the abdomen of the host. In one larva a Microsporidian occupied much of the hinder part of the abdomen.

SIMULIUM MELATUM Wh.

Wharton, 1949, p. 406; from Blue Mountains and Sydney district, N.S.W. (type locality Lett River, Hartley).

Our material agrees substantially with Wharton's description and with specimens he kindly presented to the Institute, though with minor differences.



Text-fig. 10.—Respiratory organs of pupae.

Female.

Black and creamy gold to silvery, as compared with the black and rich gold of *S. aureonigrum*. The dorsum of the abdomen shows a silvery fringe to first segment, 2 with a conspicuous patch of silvery scales, 3 to 5 black, with an occasional apical silvery scale, 6 to 8 smooth, slightly shining, and speckled with silvery scales. The pale area on the hind metatarsus is better defined than in Wharton's description. Claws with a very small tooth, which is deeply set in the concavity (Text-fig. 7).

Male.

The upper facets of the eyes, as Wharton points out, are relatively small, resembling those of *S. nicholsoni* rather than *S. clathrinum*; they average 0·033 mm. in diameter and are in about 16 rows. The abdomen is covered with velvety black tomentum, with the usual ashy reflections from the shiny lateral patches on segments 5 to 7. The hypopygium is distinguished by the deep but widely open bay in the distal end of the anterior part of the phallosome (Text-fig. 9). This is a true indentation, and its appearance is different from that of the arched anterior part when seen more or less end on.

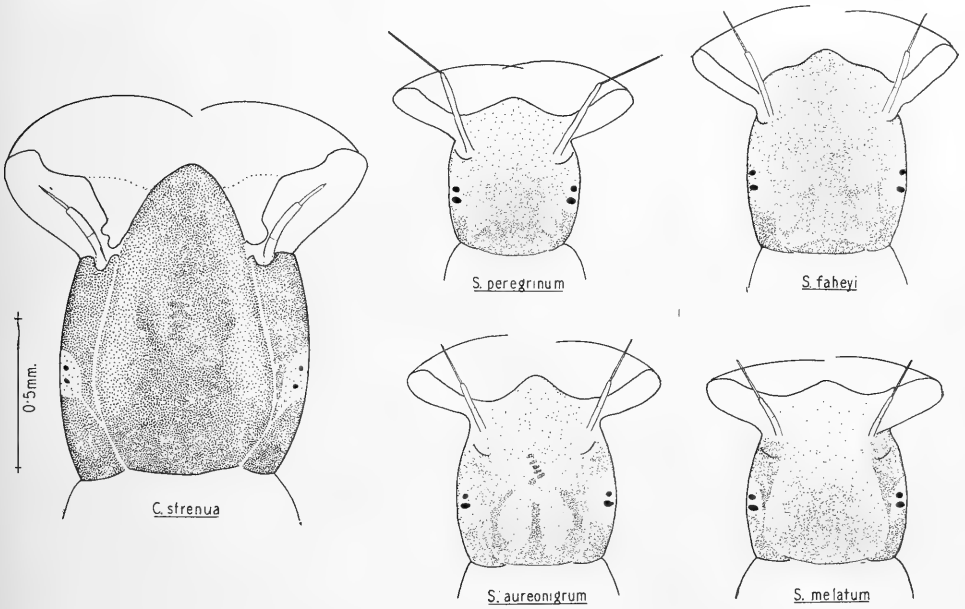
Cocoon and Pupa.

As described by Wharton; the cocoon has a well-marked central dorsal projection and is coarsely woven.

Larva.

Difficult to separate from *S. aureonigrum* and *S. inornatum*. The head pattern is generally more diffuse, and the antennae and bases of the mouth-brushes are shorter relative to the length of the head. The gill-spot is bigger in all dimensions (Text-fig. 14). Ventral papillae variable, usually none, but sometimes as well developed as in *S. inornatum*. The rectal gills are simple. A single group of flat brown scales is present ventro-laterally on each side in front of the circlet in grown larvae, but sometimes cannot be seen in young specimens.

New distribution.—New South Wales: Gara River, Armidale district (about 3,000 ft.), May, A. F. O'Farrell. Queensland: Purling Brook (2,000 ft.), Springbrook area, December, larvae and pupae at the edge of a fast, clear stream in company with *Cnephia tonnoiri orientalis, S. ornatipes, Austrosimulium mirabile, A. furiosum* and *A. victoriae*; tributary of Freshwater Creek, Cairns district, October, in company with *S. aureonigrum* (two pupae, from one of which a ♀ emerged).



Text-fig. 11.—Heads of larvae.

SIMULIUM INORNATUM n. sp.

Types: Holotype ♀, allotype ♂, morphotype pupa and larva, from a small unnamed creek at 1,300 ft. on the Springbrook Road, south Queensland, December, in the Division of Entomology, C.S.I.R.O., Canberra, A.C.T.

An undistinguished species, intermediate in many respects between *S. aureonigrum* and *S. melatum*, but sufficiently distinct on characters of the male and pupa to be regarded as a separate species.

Female.

Head, thorax, legs, wings and halteres generally as in *S. melatum*, but with more of a tendency to a dull gold coloration. Scutum with broad though rather indefinite black dorsocentral stripes, which widen posteriorly almost to reach the median line and lateral margins. Claws with a small sub-basal tooth. First segment of abdomen with a conspicuous creamy fringe; second with a patch of golden scales on tergite; third to fifth with brownish black scales on disc and irregularly scattered creamy golden ones along the apical edge; sixth rather shiny in centre, seventh and eighth rather shiny over whole dorsum, all three bearing some dull golden scales which are mainly apical in position; the lateral patches on segments 2 to 5 are silvery.

Male.

Velvety black and golden, as in the other species, and with the usual lateral ashy patches on the abdomen. Resembles *S. melatum* in the upper facets of the eyes, being only moderately enlarged and in about 16 rows. Distinguished from all Australian species of the genus by the hypopygium, the anterior part of the phallosome being deeply excavated distally to form a nearly circular bay, which is almost closed by inwardly projecting arms (Text-fig. 9).

Cocoon.

Wall-pocket type, rather finely woven, with no collar, a rolled anterior edge, and a well-marked central dorsal projection.

Pupa.

Length about 3 mm. Head and thorax covered with minute tubercles. Chaetotaxy normal. Respiratory organ (Text-fig. 15) with short dark stem which gives rise simultaneously to four diverging filaments of unequal length, the ventral and ventro-lateral branches being the longest. The filaments are stiff, and curve forward and upward; the tips may be produced into a very delicate flexible extension which is readily broken off.

Larva.

Resembles *S. aureonigrum* in all respects, except that the rectal gills are simple, and the gill-spot is larger, though not as broad as that of *S. melatum*. From the latter it is distinguished by the more definitely W-shaped head pattern, somewhat longer antennae, and well-defined ventral papillae.

Distribution.—Only known from the type locality in south Queensland.

Biology.

Larvae and pupae were present in moderate numbers on rock, dead leaves, and the fine roots of a semi-aquatic plant in a small, steep, shady creek. The water was clear and moderately fast, but only an inch or so deep and a foot or so wide. The stream appeared to be drying out fairly rapidly. In the previous year we had taken a few larvae and pupae (which failed to emerge) about two miles lower down the same road at an elevation of 900 ft. They were in a similar creek, but on an edge of rock where the water poured over in a miniature fall.

SIMULIUM NICHOLSONI M. & M.

New distribution.—Queensland: Several coastal streams between Nambour and Gympie. The most northerly limits known for *S. nicholsoni* are the Mackenzie River inland and just south of Fraser Island on the coast.

SIMULIUM FAHEYI Tayl.

Types: Taylor's type ♀ is in the School of Public Health and Tropical Medicine, Sydney. We have designated an allotype ♂ from Lennon's Creek, near Babinda, N.Q., and morphotype pupa and larva from Berner Creek, and lodged them in the same collection. Additional specimens (♀, ♂, pupa, larva) are in the Division of Entomology, C.S.I.R.O., Canberra, A.C.T.

The status of this species is established on the basis of fresh material from Taylor's type locality, and a re-examination of the holotype, which was kindly lent us by Mr. D. J. Lee of the School of Public Health and Tropical Medicine, Sydney, who also permitted us to mount a leg. It is close to *S. nicholsoni*, but the differences in the female, pupa and larva are sufficient to warrant specific separation.

Female.

S. faheyi differs from *S. nicholsoni* in possessing a small sub-basal tooth on the claw (Text-fig. 7), so doubtful specimens can be identified by clearing and mounting a leg. Its general coloration is brownish black (darker in fresh material than in the type, which is thirty years old) and golden, as compared with the black and silvery coloration of *S. nicholsoni*. On the abdomen, the fringe of the first segment is golden; the dorsum of the second is usually entirely brown, but sometimes with a more or less incomplete golden fringe; third and fourth entirely brown; sixth to eighth tomentose and sprinkled with brown and pale golden scales, which are not so numerous and conspicuous (nor as pale) as in *S. nicholsoni* or *S. papuense*. Other differences are listed in the key. It is

interesting that *S. papuense*, which has very different pupae and larvae, resembles *S. faheyi* so closely in the female.

Male.

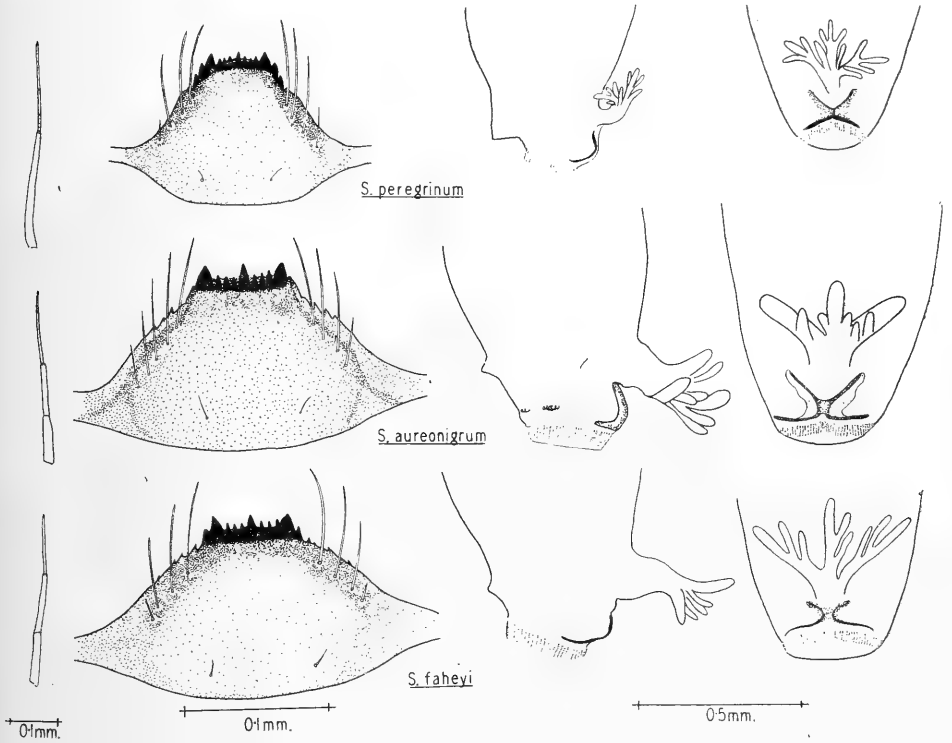
Similar to *S. nicholsoni* in all respects, including the hypopygium.

Cocoon.

Wall-pocket type, similar to *S. nicholsoni*, though usually more coarsely woven.

Pupa.

Northern specimens have the head and thorax fairly densely but not quite uniformly covered with small, blunt tubercles, like *S. nicholsoni*, and the thoracic and abdominal chaetotaxy also resembles *S. nicholsoni*. The chief differences are in the respiratory



Text-fig. 12.—From left to right: antenna, submentum, lateral and dorsal views of tip of abdomen of larvae of *Simulium* spp.

apparatus (Text-fig. 10). Stem very short, 0·07 mm. (0·35 in *nicholsoni*), dark and spiny (paler and with smaller, paler spines in *nicholsoni*). The stem divides into four branches (immediately into six in *nicholsoni*), the medial and lateral of which again divide into two. The six filaments diverge more than in *S. nicholsoni*, but continue forward, and the tips curve inward towards each other. The filaments are about 2 mm. long, but their distal portions are delicate and readily broken off, perfect specimens being difficult to find.

In southern specimens the tubercles on the head and thorax are sparse and sometimes restricted to a narrow row on each side of the mid line. The gill filaments also tend to be shorter. In pupae from Tin Can Bay they are about 1·5 mm. long, and in those from Fraser Island they are remarkably short (about 1 mm.), and usually more robust, or at any rate the tips are usually intact. This is reflected also in the gill spot of the larva, the filaments being only long enough to complete one circle and the tips sometimes projecting out from the top of the spot.

Larva.

S. faheyi can be distinguished immediately from *S. nicholsoni* by its possessing compound rectal gills, each main digitation usually having three accessory lobes, though sometimes fewer. Other differences are minor. The head pattern of *nicholsoni* is negative, the median longitudinal stripe and two oval areas at the base on each side being pale; in *faheyi* the pattern (Text-fig. 11) is of similar form but indeterminate type, the median stripe often being pigmented, but the oval areas at base usually pale. The antennae are longer (about 0·35 mm.) in *nicholsoni* than *faheyi* (0·30 mm.). The ventral incisure extends about half-way to the base of the submentum and is rather square ended in *nicholsoni*; it is deeper and usually U- or V-shaped in *faheyi* (Text-fig. 2). In the gill-spit, the long, pale stem shows conspicuously on the anterior edge in *nicholsoni*, whereas in *faheyi* this edge is also pale, but the dividing filaments can be made out (Text-fig. 14). The ventral papillae are more conspicuous in *faheyi* than in *nicholsoni*.

Distribution.—Queensland: Innisfail district, Berner Creek (type locality), September, numerous larvae, pupae and bred adults; Babinda district: Lennon's Creek, small creeks on Babinda-Boulders Rd., jungle creek near Bramston's Beach, Russell River, Fishery Creek, all September. Southern Queensland: Figtree Creek, Fraser Island, April; creek near Tin Can Bay, April; Kin Kin Creek, near Lake Cootharabah, April; Six-Mile Creek, Cooroy, May—last three in company with *S. nicholsoni*; Blunder Creek, Oxley, April, May. The Lawn Hill specimen previously recorded appears to be correctly placed, but more material is needed to check the record from the Northern Territory.

Biology.

S. faheyi seems to be as abundant and widespread in the north as *S. nicholsoni* is in the south, and the early stages were found in broadly similar situations, having a preference for moderately fast, smoothly flowing, open streams, and especially for attachment to reeds and grass beneath the water. It was found alone or in company with *S. clathrinum* (which was equally abundant) in the north, and in company with one or more of *S. ornatipes*, *S. nicholsoni*, *A. furiosum* and *A. bancrofti* in the various southern streams. A female was taken at Bramston Beach attempting to bite man; in view of the abundance of the early stages, this habit would appear to be as occasional as in *S. nicholsoni*.

The Genus Austrosimulium Tonn.

Austrosimulium mirabile M. & M.

New distribution.—Queensland: Babinda district, Smoko Creek, small jungle creek near Bramston's Beach, and small creek on Babinda-Boulders Road, all September. Springbrook area: unnamed creek at 1,300 ft., in company with *S. inornatum*, November; Purling Brook (2,000 ft.), December. (Previously only known from the type locality at Dawson's Creek on the slopes of Mt. Glorious, S.Q.)

Austrosimulium fulvicorne n. sp.

Types: Holotype ♂, mounted on a slide, morphotype pupal skin and larva, in spirit, from Yanky Jack Creek, Fraser Island, February, D. Mackerras, in the Division of Entomology, C.S.I.R.O., Canberra, A.C.T.

Distinctive features.

Belongs to the *mirabile* group and most nearly related to that species, but so well differentiated that we feel justified in describing it on less material than we would ordinarily require. The male is distinguished from *A. cornutum* by the shape of the antennae and entirely yellow segments 4 to 10, and from *A. mirabile* by antennal coloration and absence of dark spots on the wing; the pupa from *A. mirabile* by the rounded, club-shaped end of the spiny respiratory horn; and the larva from *A. mirabile* by the longer, darkened basal segment of the antenna and by the head pattern.

Male (in spirit).

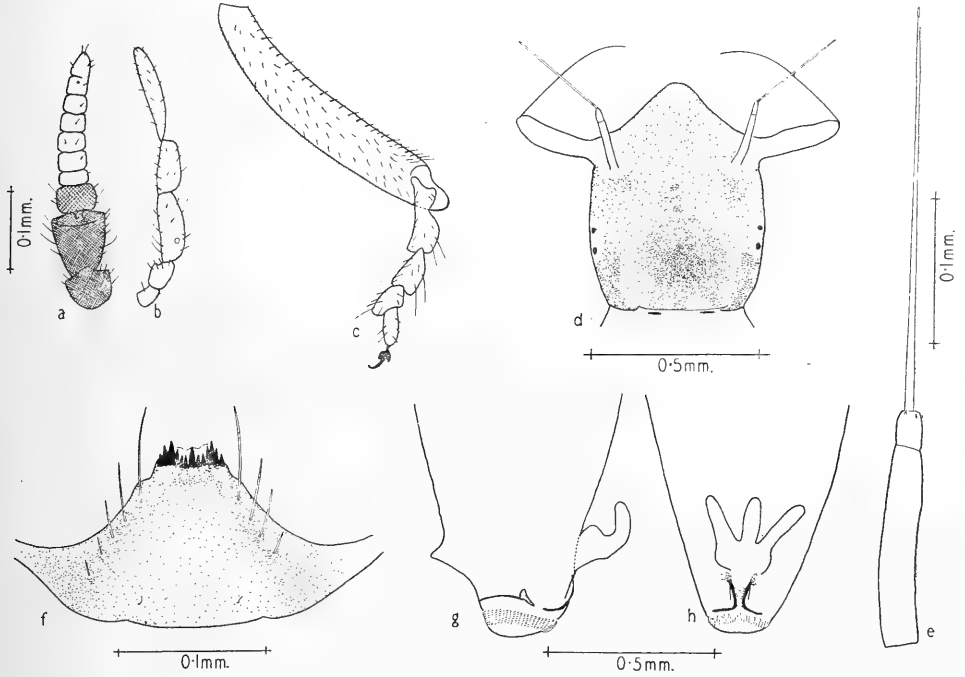
Length: Body (abdomen over-extended) 4 mm.; wing 1·8 mm. (the true size is about the same as *A. mirabile*).

Head.—Wider than thorax; upper facets of eyes moderately enlarged, in 15–16 rows. Antennae (Text-fig. 13) distinctly shorter than in *A. mirabile* but of same general form,

with second segment much longer than others; the ninth and tenth are incompletely separated (cf. *A. bancrofti*); first and second segments dark brown, third a brighter brown, fourth to tenth yellow. Face dark brown, with silvery hairs; mouth parts and palpi brown. There appear to be some golden hairs around the occiput.

Thorax.—Scutum dark brown, and appears to be covered with silvery scales. Pleurae brown.

Legs.—Not so uniformly dark brown as in a spirit specimen of *A. mirabile*. The fore and mid femora and tibiae are pale brown, almost yellowish, and the hind femur and tibia are also pale, but margined with dark pigmentation, especially on the tibia. All knees are yellowish. All tarsi (including hind metatarsus) are dark brown. Calcipala and pedisulcus as in Text-fig. 13. The claws are of normal male form.



Text-fig. 13.—*A. fulvicorne.* ♂: a, antenna; b, palp; c, hind tarsus. Larva: d, head pattern; e, antenna; f, submentum; g and h, lateral and dorsal views of tip of abdomen.

Wings.—Veins dark, and hairs on veins strong, with a few outstanding black hairs on basal section of R as in *A. mirabile*; but without the dark pigmented spots, except a trace at the fork of R, and completely without the groups of long dark bristles which are associated with these spots in *A. mirabile*. Halteres with stem brownish and knob creamy.

Abdomen.—Chitinous parts of tergites dark brown, membrane pale; hairs appear to be black. No trace could be seen of the silvery, tomentose patches, which are characteristic of *A. mirabile* and clearly visible on a spirit specimen as well as on pinned material. The hypopygium resembles that of *A. mirabile*, and has a similar acute setulose ventral swelling on the anterior part of the phallosome. The style, however, has only two spines, as in *A. cornutum* and *A. crassipes*. The spines are variable in *A. mirabile*; one specimen now before us has three on each side, and another four on one side and two and a small one on the other.

Note.—The colour description is to be taken as general rather than precise, and may require amendment when fresh material is available. The comparison with the male of *A. mirabile* was, however, made entirely on spirit specimens.

Cocoon.

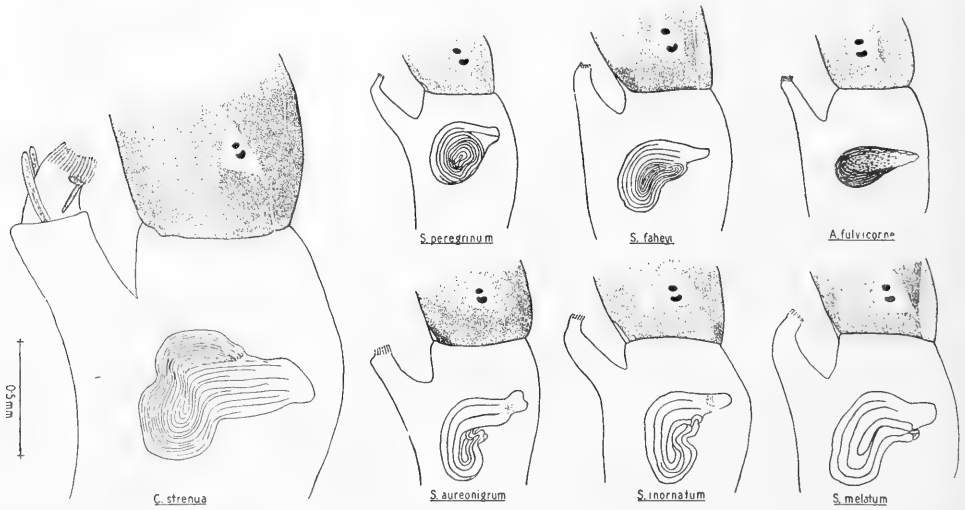
Length: 1·8 mm. Simple, finely woven wall-pocket type, with central dorsal projection, like that of *A. mirabile.*

Pupa.

Thoracic integument with exceedingly minute, irregularly distributed tubercles, and a large, stout, curved pair of posterior thoracic hairs as in *A. mirabile.* The abdominal chaetotaxy is apparently normal, but the specimen is somewhat damaged. The respiratory horn (Text-fig. 10) is flattened, blade-like, with rounded end, and covered with numerous strong, sharp, black spines. The filaments are not very numerous, arise mainly from the sides and internal surface and but few from the lateral surface, are rather longer than the horn, and of the usual beaded appearance.

Larva.

Length: 5 mm. Creamy white, with greyish brown mottling. Head with broad, dark pattern (Text-fig. 13), which is distinctly wider than in *A. mirabile.* Antennae similar to *A. cornutum,* with brownish basal segment, shorter than the paler, slender



Text-fig. 14.—Lateral view of full-grown larvae, showing gill-spots; note size of *C. strenua.* (This figure is intended for use in conjunction with our earlier figure—1949, Text-fig. 19, p. 403.)

distal segment (Text-fig. 13). Ventral incisure shallow, wider than deep (Text-fig. 2). Submentum as in Text-fig. 13, with five strong hairs on each side.

Gill-spot (Text-fig. 14) pear-shaped, dark, with the spiny horn distinctly visible.

Rectal gills simple. Large ventral papillae present. Anal sclerite with the usual backwardly directed struts and the incomplete ventral chitinous ring characteristic of the group, the upper end being swollen as in *A. mirabile* (Text-fig. 13). Circlet similar to other species.

Distribution.—Queensland: Only known from the type series from Fraser Island, comprising about 20 larvae and one adult male with its pupal shell and cocoon.

Biology.

The larvae and pupa were on grass in fairly swift, evenly flowing clear water in a small creek, deeply cut in the sand and shaded by vegetation growing over from the banks.

AUSTROSIMULIUM CRASSIPES (Tonn.).

New distribution.—Queensland: Small tributary of Cave Creek (upper Numinbah Valley), March. (Previously known only from Victoria and the Blue Mountains, N.S.W.)

AUSTROSIMULIUM BANCROFTI (Tayl.).

New distribution.—Queensland: Coastal creeks between Nambour and Gympie, February, April.

AUSTROSIMULIUM PESTILENS, M. & M.

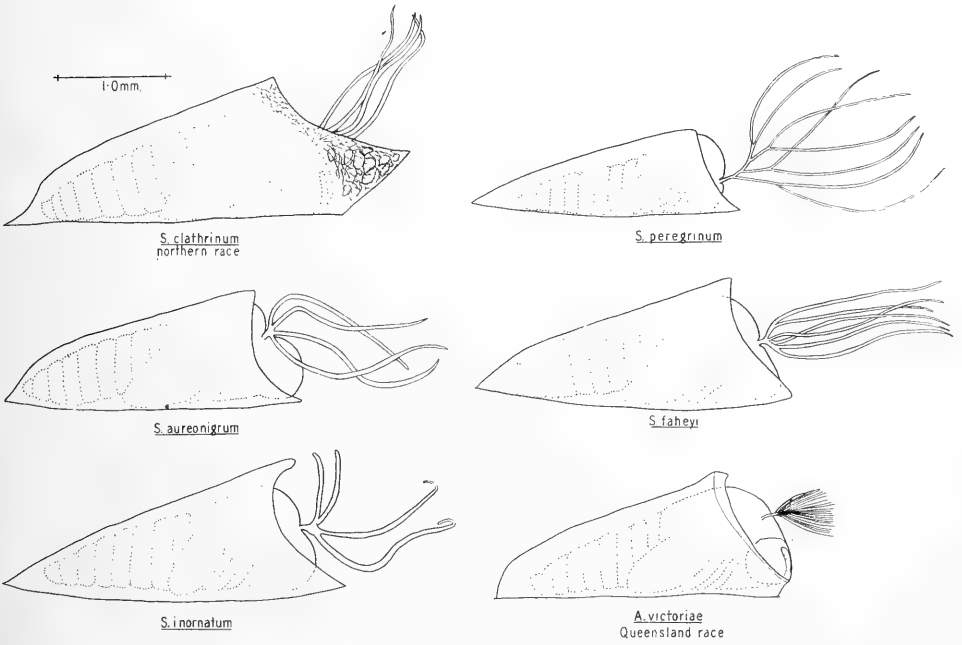
New distribution.—New South Wales: Nyngan, March, August, J. Armstrong. Not previously recorded outside Queensland; specimens kindly submitted by Mr. D. J. Lee, School of Public Health and Tropical Medicine, Sydney.

AUSTROSIMULIUM FURIOSUM (Sk.).

New distribution.—Queensland: Fig-tree Creek, Fraser Island, April; several small coastal streams between Nambour and Gympie, April, May; Purling Brook, Springbrook area (2,000 ft.), December.

AUSTROSIMULIUM VICTORIAE (Tonn.).

Queensland specimens differ from southern ones in that the long dorsal projections on the cocoon are completely missing (Text-fig. 15). Adults, pupae and larvae are, however, not to be separated from Canberra specimens, and we hesitate at present to use



Text-fig. 15.—Cocoons and pupae (for use in conjunction with our earlier figure—1949, Text-fig. 20, p. 404, but it is to be noted that the respiratory organ of one side only is shown here).

the cocoons alone to differentiate species or subspecies. Nevertheless, differences in the shape of the cocoon in different parts of the geographical range have been observed in other species also (*S. ornatipes*, *S. clathrinum*), and may indicate that genetic differences are developing. Much more material would be needed to attack this problem.

New distribution.—Queensland: Little Nerang Creek (300 ft.), September; Purling Brook and a small creek in the rain forest, Springbrook area (2,000 ft.), December. (Not recorded previously north of Wentworth Falls, N.S.W.)

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REVISION OF THE GENUS SOLENOGYNE CASS.

By Gwenda L. Davis, Department of Biology, New England University College, Armidale.

(Fourteen Text-figures.)

[Read 26th July, 1950.]

Introduction.

Cassini (1828) described the genus *Solenogyne* from a dry specimen in Mérat's herbarium, which had been collected in the Port Jackson district. He wrote: "this genus is distinguished from *Lagenophora* by its non-radiate and discoidal inflorescence, the crown composed of tubular flowers in many rows, by the ovaries lacking a collar and by the involucral bracts being applied and foliaceous all over."

The genus *Emphysopus* was erected by J. D. Hooker in 1847 to accommodate certain specimens collected in Tasmania by Gunn, but no comparison was published between this new genus and either *Lagenophora* or *Solenogyne*.

In 1860 Hooker incorporated *Emphysopus* in *Lagenophora* with the comment "at one time, as I thought, worthy of being kept generically separate, but upon reconsideration I am induced to unite it with *Lagenophora*".

Mueller (1866) listed Hooker's genus in the synonymy of a new species of *Solenogyne*, but Bentham (1867) sank both *Solenogyne* and *Emphysopus* in *Lagenophora*.

Although Moore and Betche (1893) once more separated *Solenogyne* (in which they included *Emphysopus*) from *Lagenophora*, most later workers have followed Bentham, whose treatment was retained by Maiden (1916).

The investigations of the present writer have supported Moore and Betche, and the genus *Solenogyne* is now reinstated in generic rank. *Lagenophora* and *Solenogyne* agree in that the capitulum is made up of two types of florets, the outermost of which are arranged in three or four rows and are pistillate. The central florets in both genera are staminate and although the stylar branches are densely papillose and relatively large, they function only in brushing the pollen out of the anther tube, and the ovaries are abortive. These two genera are undoubtedly closely allied but the tubular or lipped outer florets of *Solenogyne* and the marked difference in fruit are characters which merit generic recognition.

Affinities.

In the absence of cytological evidence the affinities of *Solenogyne* and *Lagenophora* can only be discussed in a general way. Nevertheless Babcock (1947) states: "the phylogenetic significance of chromosome number, size and shape can be interpreted only in relation to or with the aid of other criteria", and although "the evidence from comparative morphology, cytology and genetics has been combined in the determination of interspecific relationships in *Crepis*", he asserts that differences in chromosomes are always reflected in morphology. In view of this a discussion of some evolutionary trends shown by these genera, though based on comparative morphology alone, may not be entirely worthless.

In *Brachycome* it has already been noted (Davis, 1948) that the innermost disc florets seldom develop fruit, and it was suggested that this might be due to the earlier maturing outer florets commandeering the vascular supply of the receptacle. When the ray and outer disc florets of *B. marginata* Benth. were removed those at the centre still failed to produce normal fruits. Although these experiments were not sufficiently extensive to be of great value in themselves, they did suggest another explanation, that is, that the inner disc florets might be partially or wholly sterile, and further work is being carried out along these lines. If in *Brachycome* the species can be arranged in

a series from normal fertility of the inner disc florets to their partial and finally complete sterility, then the evolution is indicated of the condition in *Lagenophora* and *Solenogyne* where all the disc florets are sterile. The strap-like ray florets of *Lagenophora* are identical with those of *Brachycome*, and if the relationship with *Solenogyne* is as close as comparative morphology indicates, then the question must be decided whether tubular ray florets are primitive or derived. It is suggested that had this tubular condition been the retention of a primitive character, little or no variation would be shown. The fact that all conditions, from completely tubular to 2-lipped and finally 1-lipped with minute ray are found, indicates that *Solenogyne* originated from a *Lagenophora*-like ancestor, and that genetic stability has not yet been attained. The opinion that *Solenogyne* is of more recent origin than *Lagenophora* is supported by its more restricted distribution.

Distribution of the Genus.

Solenogyne is confined to eastern and southern Australia from south-eastern Queensland through New South Wales and Victoria to south-eastern South Australia and is widely distributed in Tasmania. A single record exists for "North Queensland", but unfortunately no exact locality accompanies the specimen.

According to Cheeseman (1925) one variety has become naturalized in New Zealand on Banks' Peninsula and near Wellington. Specimens have been examined from the latter locality and were identical with those from New South Wales.

Nomenclature.

Some changes in nomenclature have been necessary on priority grounds and are discussed in the groups concerned.

Type terminology is that of Davis and Lee (1944).

Categories.

This genus consists of two populations which, although vegetatively distinct, bear identical fruits. As in *Brachycome* (Davis, 1948) and *Lagenophora* (Davis, 1950), the principle has been followed that only morphological differences in the fruits justify specific status, and that constant and discontinuous differences in vegetative characters are varietal in nature. It therefore follows that *Solenogyne* is a monotypic genus of two varieties.

Evaluation of Taxonomic Characters.

All morphological characters were examined and limits of variation noted. Although few of these characters were found to be continuously variable, and therefore of no value in distinguishing between populations, the results of these examinations are discussed below. *Habit*: scapigerous, but considerable variation in size was noted, which could probably be largely correlated with habitat. Unfortunately collector's notes seldom supply details of habitat. *Indumentum* of a septate-hairy nature was always present, but the degree of development was variable. *Leaves* showed a slight amount of variation in the dissection of their margins and in one variety the specimens fell into three geographic groups on this and other vegetative characters. Leaves alone, however, have little diagnostic value. *Scapes* supply the primary taxonomic character in distinguishing between the two varieties, and although some variation was observed both in breadth and length, intermediate forms were not found. *Capitula*, *florets* and *fruits* were identical in both varieties.

Specimens Examined.

All specimens examined are listed under the appropriate category, together with collector's notes. The source of each specimen is indicated in brackets as in a previous paper. (Davis, 1950.)

Descriptions.

It is only when a genus is made up of two or more species that generic characters can be recognized apart from specific. Since *Solenogyne* is a monotypic genus no generic diagnosis is given.

Specific and varietal descriptions are based on available material, consequently as further specimens are collected these descriptions may require modification. Limits of variation are indicated for all characters, but size measurements are to be regarded merely as a guide.

Type Species: SOLENOGYNE BELLIOIDES Cass.

SOLENOGYNE BELLIOIDES Cass.

Dict. Sci. Nat., lvi (1828), 174.

Perennial herbs, 1·5–18 cm. high, more or less septate-hairy on leaves and scapes. Leaves up to 11 cm. long, 2·1 cm. broad, radical, oblanceolate, dentate or coarsely toothed, rarely entire. *Scapes* filiform or robust, provided with 1–4 broad-linear bracts, the lowest of which are up to 1·3 cm. long, and the uppermost scale-like. *Capitula* 2–26 on each plant, 5–7 mm. diameter. *Involucral bracts* 22–30 in 3–4 rows, 1·5–2·2 mm. long, 0·5–1·3 mm. broad, glabrous or microscopically glandular, linear to narrow-obovate, obtuse, and the margins torn ciliate. *Outer florets* about 55, in 3–4 rows, tubular at the base with distal half to third split to form a short entire or toothed ray, 0·7 mm. long, female. *Central florets* about 10, 5-toothed, tubular, regular, 2·2 mm. long, staminate, ovary abortive. *Stylar branches* of outer florets linear, smooth and diverging at maturity, those of the inner florets unequal in length, densely glandular hairy and appressed. *Stamens* 4–5, present only in the central florets. *Anthers* united into a tube, and the connective is prolonged distally to form a terminal appendage. *Receptacle* 1·1–3 mm. broad, 1 mm. high, convex, shallowly pitted. *Fruit* an inferior achene, 2·2–2·6 mm. long, 0·7–1·2 mm. broad, light to dark brown, narrow-elliptical to narrow-obovate, flattened, smooth. Pappus absent.

Key to the Varieties.

Scapes filiform, usually exceeding the leaves, rarely less ..... var. *α bellioides*
Scapes robust, usually not exceeding the leaves ..... var. *β Gunnii*

Solenogyne bellioides Cass. var. *α bellioides* comb. et stat. nov. (Text-figures 1–11.)

Synonymy: *Solenogyne bellioides* Cass., Dict. Sci. Nat., lvi (1828), 174; *Solenogyne brachycomoides* F. Muell., Fragm., v (1866), 62; *Lagenophora Solenogyne* F. Muell., ex Benth, Fl. Aust., iii (1866), 506.

Herbs 5·5–18 cm. high in which the indumentum of septate hairs is not dense but can be seen macroscopically. Leaves up to 6·5 cm. long, 2·1 cm. broad. Scapes filiform, in thickness one-seventh to one-tenth the breadth of the inflorescence, and usually at least twice as long as the leaves, rarely less. *Involucral bracts* turn downwards when fruit are mature.

Habitat: Grassland and open forest.

Range: South-eastern Queensland, northern tablelands and middle western districts of New South Wales, and central coast south to Hill Top.

Specimens examined.—Queensland: Ridges about Brisbane, F. M. Bailey (BRI); Brisbane River (MEL); Botanic Gardens, Brisbane, 2.1915, C. T. White (BRI); Goodna, forest country, edge of scrub, 17.3.1917, C. T. White (BRI); Laidley, 3.1920, C. T. White (BRI); Gatton, 10.1906, J. F. Bailey (BRI); Gatton Agriculture College, moderately common in mixed native pasture, poor soil, badly drained subsoil, 19.2.1930, C. T. White, No. 6637 (BRI); Toowoomba, 4.1916, C. T. White (BRI).

New South Wales: Tenterfield, 10.1886, E. Betche (NSW); Tenterfield, C. Stuart, No. 939 (MEL); Timbarra, C. Stuart, No. 99 (MEL); Clarence River, Beckler (MEL, NSW); Warialda, 3.1906, H. M. R. Rupp (NSW); Glen Innes, 29.10.1886, E. Betche (NSW); Clifton, C. Stuart, No. 41 (MEL); Tingha, 6.1917, J. L. Boorman (NSW); Armidale, 31.1.1941, G. L. Davis (FAR); University College, pasture land, under trees, 28.11.1949, G. L. Davis (FAR); Bundarra-Uralla Road "pasture land", 13.11.1949, G. L. Davis (NSW); Namoi River, 1890, Musson (MEL); Coonamble, 4.1913, W. H. Potts (NSW); Murrurundi, 3.1895, F. Fraser (NSW); Moonan Brook, near Scone, 1883, H. Carter (MEL); Gulgong, 4.1901, J. H. Maiden and J. L. Boorman (NSW); Barrangan, beyond Mudgee (MEL); Hill End, 4.1885, J. Lauterer (MEL); Hornsby, 4.1914, W. F. Blakely (NSW); Botanic Gardens, Sydney, 4.1914, W. F. Blakely (NSW); Hurstville, 3.5.1899, E. Cheel (NSW); Penshurst, 10.1909, E. Cheel (NSW); Liverpool, "amongst grasses on hill in Eucalypt forest", 15.4.1931, C. E. Hubbard and E. Cheel, No. 8486 (BRI); Hill Top, J. Shirley (BRI).

The first record of this population was in 1828 when Cassini erected the monotypic genus *Solenogyne* (*S. bellioides*) to accommodate "a dry specimen which appears to have been collected in the neighbourhood of Port Jackson, and which is to be found among the unnamed Synantherée in the herbarium of M. Mérat" (original description). Although this specimen has not yet been traced, Cassini's thorough description leaves no doubt as to its identity.

This same population was later described by Mueller (1866) as *S. brachycomoides*, and Cassini's name was listed in synonymy. In the original description and immediately following the name of the new species, Mueller inserted the words "*Lagenophora Solenogyne* F.M. coll.", apparently referring to specimens distributed by him with that identification. When Bentham (1866) incorporated *Solenogyne* in *Lagenophora* he used Mueller's manuscript name which he attributed to him. Since Bentham's description is the first to which the name *Lagenophora Solenogyne* is formally applied, the epithet itself should date from that time and not, as is generally quoted, from Mueller's earlier mention in synonymy.

The type locality of *S. brachycomoides* is "near the town of Clifton, New England, C. Stuart", while Bentham, in connection with *L. Solenogyne*, quoted specimens from three localities, one of which was "New England, C. Stuart". In the National Herbarium, Melbourne, is a folder of specimens labelled by Mueller "*Solenogyne brachycomoides*, Clifton, New England, C. Stuart", and marked as having been examined by Bentham. One of these specimens (Text-figure 1) has therefore been selected as lectotype of both *Solenogyne brachycomoides* and *Lagenophora Solenogyne*.

Since the genus *Solenogyne* is reinstated in the present paper, Cassini's original epithet is retained and the source of the slightly different spelling in Index Kewensis (*S. bellidioides*) has not been traced.

Variation within this variety is confined to the size of the plants, other characters remaining constant. One specimen was examined in which the indumentum was so dense that the plant had a woolly appearance, but owing to the uncertainty of the locality from which it was collected, it has not been listed above. A field label is attached to the specimen, and bears, in Leichhardt's writing, the data "the wool leaved daisy, near water, 9th March, 1843".

Solenogyne bellioides Cass. var. *β Gunni* (Hook. f.), n. comb.

(Text-figures 12–14.)

Synonymy: *Emphysopus Gunnii* Hook. f., *Lond. Journ. Bot.*, vi (1847), 114; *Solenogyne bellioides* Sond. in Linnaea, xxv (1852), 480; *Lagenophora Emphysopus* Hook. f., Fl. Tasm., 1 (1860), 187; *L. Gunnii* (Hook. f.) J. M. Black, Fl. S.A., Pt. 4 (1929), 580.

Haptotype: Tasmania, 1835, Gunn, No. 512 (NSW).

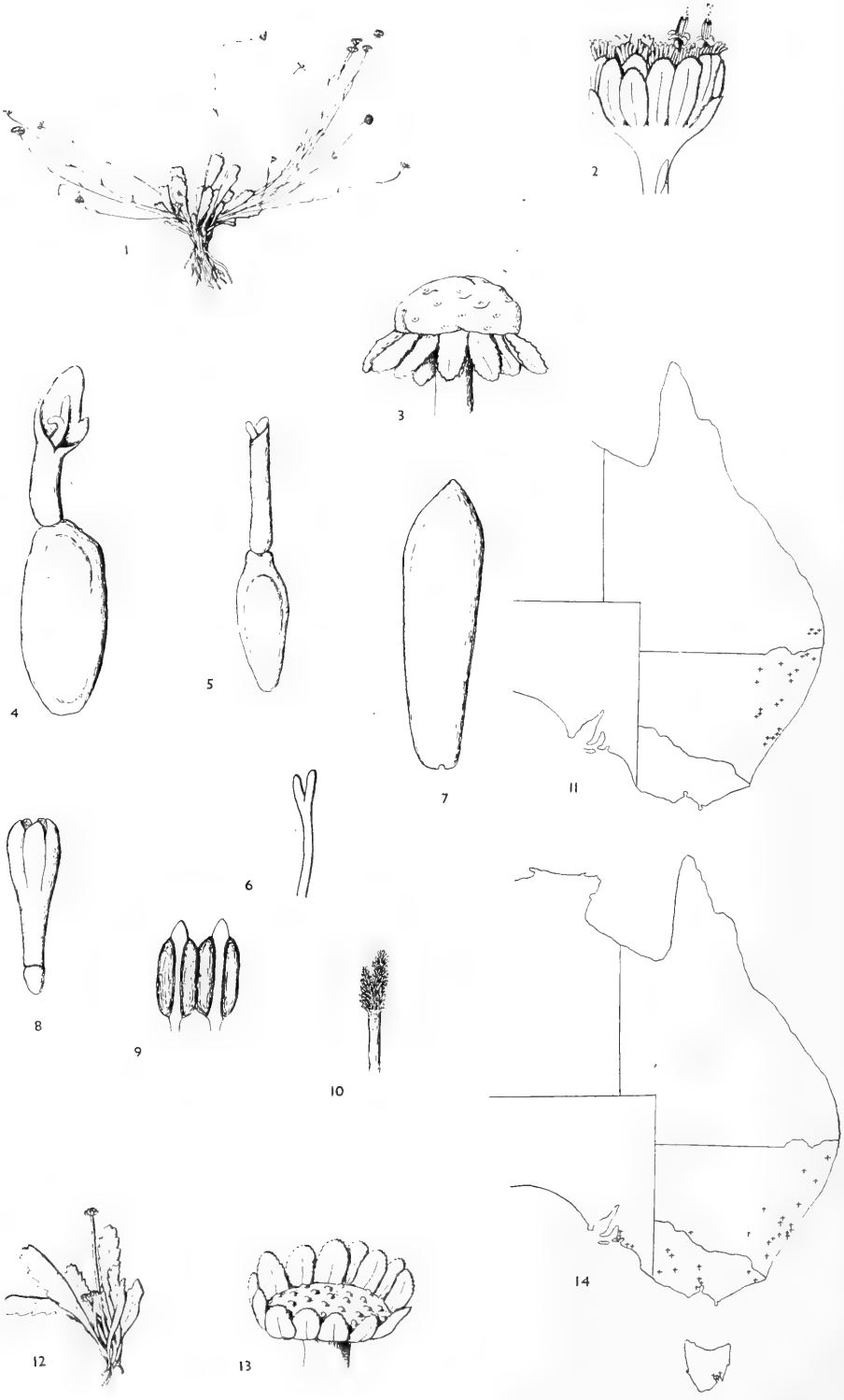
Septate-hairy herbs, 1·5–11·3 cm. high. Leaves up to 11 cm. long, 1·4 cm. broad. Scapes robust, in thickness one-fifth to one-sixth the breadth of the inflorescence. *Involucral bracts* do not turn downwards at maturity of fruits.

Habitat: Grassland and open forest.

Range: Tablelands, middle west and central coast of New South Wales, throughout Victoria and south-eastern South Australia; east coast of Tasmania, and a single record from Queensland. Naturalized on Banks' Peninsula and near Wellington in New Zealand (Cheeseman, 1925).

Specimens examined.—Queensland: North Queensland, J. E. Tenison-Woods (MEL).

New South Wales: Clarence River, Beckler (MEL); Glen Innes, 6.1917, J. L. Boorman (NSW); Ebor Falls, 31.1.1941, G. L. Davis (FAR); University College, Armidale, 28.11.1949, G. L. Davis (FAR); Moona Plains, Walcha, 1884, A. R. Crawford (MEL); Warrambungle Ranges, 10.1899, W. Forsyth (NSW); Barrington Tops, on granite, 7.1.1934, L. Fraser and J. Vickery (NSW); Swashfield, via Oberon, 31.1.1941, F. Johnson (NSW); west of Blue Mountains, 7.1893, G. King (MEL); Putting Green, Blackheath, 19.1.1937, N. Popel (NSW); Jenolan Caves, 3.1900, W. F. Blakely (NSW); Ryde Bowling Green, 10.1944, Cruth (NSW); near Tempe, 10.1897, W. Forsyth (NSW); Hurstville, 18.5.1899, E. Cheel (NSW); Moss Vale, 28.10.1910, E. Cheel (NSW); Lake George, 4.1898, E. Betche (NSW); Queanbeyan, 1.1888, E. Betche (NSW); Nelligen-Braidwood Road, Top of Clyde Mt., in grass at roadside, 25.4.1932, F. A. Rodway (FAR); Wagga Wagga, 3.1885, R. Thom (MEL); Upper Murray River, 1887, C. French, Jr. (MEL); Edward's River, 10.1875, F. Mueller (MEL).



Text-figures 1-14.

Victoria: Snowy plains on the Limestone River (MEL); Genoa district, 3.1885, W. Bauerlen (MEL); in meadows above the Snowy River, F. Mueller (MEL); Kilmore, 1883, R. Thom (MEL); Laverton, 4.1911, J. Starr (MEL); near Station Peak (MEL); sources of the Campaspe River, 6.1887, J. Dickinson (MEL); Moyston, 4.1872, D. Sullivan (MEL); Wando Vale, 21.6.1842, J. G. Robertson (NSW); Wimmera, 10.1899, D'Alton (MEL); Wimmera, 1890, H. Davis (MEL); Wimmera, swampy places, 16.4.1898, F. M. Reader (MEL).

Tasmania: East coast, south of Elephant Pass, on a grazed short-grass roadside bank near sea-level, 18.11.1942, H. D. Gordon (HO); Mt. Rumney, Mountain Top, 1,236 ft., 25.4.1931, F. H. Long (HO); New Town, Spicer (MEL); Domain, Hobart, 5.1892, L. Rodway (HO); Mt. Nelson Range, 1.1893, L. Rodway (NSW); Tasmania, 1835, Gunn, No. 512 (NSW).

South Australia: Open pastures on State hills, Belair, 26.5.1882, R. Tate (AD); entrance to Long Gully, North Park, 22.4.1910, H.H.D.G. (JMB); Port Elliot, in paddock near Cliff House, 9.5.1918, H. W. Andrews (JMB); Clarendon, 5.1882 (MEL); the Bluff, Encounter Bay, on cropped grassy hills, 1.1924, J. B. Cleland (JBC, JMB); Encounter Bay, 12.5.1928, J. B. Cleland (JBC); Hall's Creek, Encounter Bay, 23.5.1932, J. B. Cleland (JBC).

J. D. Hooker (1847) described *Emphysopus Gunnii* from a specimen collected in Tasmania by Gunn, but later (1860) he included this species in *Lagenophora* as *L. Emphysopus*. In Gunn's collection in the National Herbarium, Sydney, is a specimen with the label "512.1835", which has been nominated *haptotype* (Text-figure 12).

Solenogyne bellioides Sond. is listed in the above synonymy on Bentham's authority and the fact that the original description agrees with this variety, but no syntype specimens are available in Australia.

The specimens examined fall into three groups on vegetative characters:

1. Small plants from 1·7–6·3 cm. high with 2–8 inflorescences. The indumentum of septate hairs is very sparse, so that the plants are almost glabrous. Leaves are up to 3·4 cm. long, 1·3 cm. broad, closely and regularly serrate. This form has only been seen from New South Wales localities.

2. Larger plants from 4·2–11 cm. high bearing up to 17 inflorescences. Septate hairs are more abundant and visible macroscopically on stems and leaves, but are not sufficiently numerous to appear woolly. Leaves up to 9·8 cm. long, 1·6 cm. broad, the teeth further apart and the margin not closely serrate. Only seen from southern New South Wales, western Victoria, Belair and Mt. Graham in South Australia.

3. Densely septate-hairy plants of a rather woolly appearance, 2·5–8·8 cm. high with 3–9 inflorescences. Leaves up to 7·6 cm. broad, the teeth of which are similar to the preceding form. This is the most widely distributed form, occurring from the Clarence River, through the highlands of New South Wales, Victoria and Tasmania.

It is considered that the series of specimens available is not sufficiently large to justify these three apparently distinct forms being recognized under separate status, since more intensive collecting may yield specimens which bridge the apparent discontinuities.

No other variation was seen in this variety except an abnormal condition in a specimen from the Snowy River, in which two of the scapes bifurcated distally and each branch was terminated by an inflorescence.

Acknowledgements.

The specimens on which this revision was based were lent for that purpose by the Directors of the various public herbaria, and I would like to acknowledge the co-operation in this respect of Mr. R. H. Anderson, National Herbarium, Sydney; Mr. A. W. Jessep and Mr. J. H. Willis, National Herbarium, Melbourne; Mr. C. T. White, Brisbane Herbarium; Miss W. Curtis, University of Tasmania; as well as the following private collectors: Professor J. B. Cleland and Mr. J. M. Black of University of Adelaide, and Dr. F. A. Rodway of Nowra.

Text-figures 1-11, *S. bellioides* var. *bellioides*.—1, habit. lectotype of *S. brachycomoides* and *Lagenophora Solenogyne*, × ½. 2, *capitulum*, × 5. 3. receptacle after shedding of fruits, × 5. 4 and 5, ray florets and young fruits showing extremes of variation, × 20. 6, stylar branches of ray florets, × 20. 7, fruit, × 10. 8, disc florets, × 10. 9, anthers, × 20. 10, stylar branches of disc floret, × 20. 11, distribution.

Text-figures 12-14.—*S. bellioides* var. *Gunnii*.—12, habit. Haptotype of *Emphysopus Gunnii*, × ½. 13, receptacle after shedding of fruits, × 5. 14, distribution.

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THE CLASSIFICATION OF BACTERIA.

WITH SPECIAL REFERENCE TO NON-PATHOGENIC EUBACTERIA.*

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Introduction.

An urgent need in bacteriology to-day is a revision of the classification of bacteria. At the present time, two determinative systems are in common use, that of Lehmann and Neumann (1930), which is used on the Continent of Europe and to a large extent in Britain, and that given in Bergey's Manual of Determinative Bacteriology (5th Ed., 1943), which is used in America and by workers in general bacteriology in Australia. In Britain, Topley and Wilson's textbook on the Principles of Bacteriology and Immunity deals with classification using a slight modification of the classification of Buchanan (1917) and its later development by Bergey *et al.* But the section on non-pathogenic forms is weak.

In my work on bacteria of marine origin I have found that a revision of the systems now in use is essential before any studies on the relationships of any flora can be profitably undertaken. In a previous study (Wood, 1940) I found that a number of types isolated did not fit any species described by Bergey, whereas Lehmann and Neumann's classification proved too general. It was felt at the time that many of the strains did not deserve specific rank and I begged the question by using a decimal system to describe them. It may well be that, with some genera, such a system may be the best method of describing the strains until further work has shown what can be justly regarded as differentiating characters.

A growing amount of literature deals with bacterial classification, while earlier work has been ably summarized by a number of authors. None of the classifications is free from serious faults, the conclusion being that bacterial classification is a subject of considerable difficulty, owing to the apparently simple nature of the organisms and more especially to their extreme variability.

The best starting point appears to be a critical study of some of the more prominent suggestions which have been made. There seems, however, to be a limit to this sort of work; at a certain stage one can only go back to the bacterial groups with which one is familiar to see how they fit into the suggested scheme. This suggestion has already been made by Bruce White (1937). Once one knows the range of variation of his organisms and can show how to arrange them into groups and sub-groups, so that other workers can compare different floras, one has a working classification of genera and species. If this working system fits one of the determinative classifications, the latter may be claimed to be satisfactory for the purpose. I feel sure that such a combination of the practical with the speculative will give a much clearer picture than can be seen at present. Lately van Niel with the non-sulphur purple bacteria and Stanier with the Cytophagas have successfully used the method.

In any classification there are bound to be difficulties, for, to the taxonomist, "natura non facit saltem". Frequently species and often genera can only be determined by making arbitrary divisions at convenient points—e.g., in the case of *Pseudomonas* and *Vibrio*. The student should realize that these divisions are arbitrary and that they

* The work described in this paper was carried out as part of the programme of the Division of Fisheries, Commonwealth Scientific and Industrial Research Organization, Australia.

will only rarely mark real breaks in continuity. Observed breaks are often due to lack of knowledge of intermediate forms. Therefore, in recording his results, a writer should state where in each group his divisions occur and why he has drawn them. Then if he or another writer wishes to alter the divisions it can be done without invalidating the records of other workers or throwing the whole system out of gear. The debated question (Zobell and Upham, 1944, Zobell and Feltham, 1934, Stanier, 1941) of whether a distinctive marine flora should be recognized cannot be resolved until it is agreed by bacteriologists whether failure to grow on fresh water media is sufficient grounds for creating new species. On this distinction depends whether or not a marine flora can be regarded as homologous with a land flora. This question of the possibility of continued interchange of sea and soil bacteria is highly important in marine biology. Hence my own enforced excursion from marine bacteriology into the taxonomic field and my insistence that taxonomy must be pragmatic.

Difficulties in the Way of Adequate Classification.

A. bacterial variation.

Recent literature abounds with reports of variation in bacteria which some authors claim is mutational in character and therefore bound up with the genetics of the organisms—e.g., Severens and Tanner (1945). If mutants occur, as now seems certain, we should have to regard each culture as containing potentially several mutant strains. By selective culture we can increase the numbers of the strain most suited to our chosen conditions till we get a pure culture which may or may not retain the tendency to mutate. On the other hand, we may, for example, by growing a culture in 3% sodium chloride, induce a salt-tolerant strain, or the whole culture may become salt tolerant and have to be trained again to grow on fresh water media. Such strains would be more labile than the mutant strains.

We have, in the literature, records of variation in morphology, serology, biochemical reactions, nutrition requirements, pigmentation, enzymic activity, phage resistance, resistance to bactericides and loss of function. A study of the papers given or referred to in the Cold Spring Harbour Symposium of Quantitative Biology II, 1946, will show this.

This brings us to the question of type cultures. Lehmann and Neumann (1930) give numerous examples of type cultures which in their laboratory behaved very differently from the reactions given for them by the original recorder. I have found that my own cultures, when kept for a year or more, showed considerable variation from the original, and that the confirmation of a reaction of such cultures for comparison with a given strain is often impossible. Type cultures of these organisms may therefore be quite misleading and, although they are a valuable aid to diagnosis, they must be used with caution. In other words, a worker must know the probable expected variation before he decides whether his organism can be classed with the type culture of the species.

Another point is that reactions which will serve to differentiate one species may be useless in differentiating another. I took four pure cultures, two each of *Staphylococcus** and *Corynebacterium*, plated them and selected ten colonies of each. Each of the forty colonies was then tested as a separate organism. Table 1 shows the reactions of these forty cultures and the originals on the usual diagnostic media.

It will be seen that *Staphylococcus* was reasonably constant in its fermentation of certain sugars—e.g., glucose, maltose, mannitol, salicin, etc.—and inconstant in xylose, raffinose, etc. *Corynebacterium*, on the other hand, was inconstant throughout so that it is doubtful whether sugars can be used to determine species of this genus.

Lehmann and Neumann, while admitting that there appears to be a reason for separating *Pseudomonas* and *Vibrio*, have pointed out that the separation is difficult at times, as one frequently finds cultures which show only a proportion of curved or straight rods. In such cases, one must rely on the "scientific tact" of the observer until

* I have adopted Cowan's suggestion (private communication) that Cohn's description of *Micrococcus* is inadequate and therefore *Staphylococcus* must be used for these forms.

TABLE 1.

Table of Reactions of Strains Selected at Random from Poured Plates of Pure Cultures.

Culture.	Strain.	Milk.	Gelatin.	Nitrite.	Glucose.	Lactose.	Saccharose.	Maltose.	Mannitol.	Laevulose.	Glycerol.	Galactose.	Inulin.	Salicin.	Xylose.	Raffin.
15/43 *Staphylococcus.*	1	P.D. Ac.	+	—	A.	—	A.	A.	A.	A.	A.	A.	—	A.	A.	—
	2	,,	+	—	A.	—	A.	A.	A.	A.	A.	A.	—	A.	A.	—
	3	P.D.	+	+	A.	—	A.	A.	A.	A.	A.	A.	—	A.	—	—
	4	,,	+	+	A.	—	A.	A.	A.	A.	A.	A.	—	A.	—	—
	5	,,	+	+	A.	—	A.	A.	A.	A.	A.	A.	—	A.	A.	—
	6	P.D. Ac.	+	—	A.	—	A.	A.	A.	A.	A.	—	—	A.	—	—
	7	,,	+	+	A.	—	A.	A.	A.	A.	A.	—	—	A.	—	—
	8	,,	+	—	A.	—	A.	A.	A.	A.	A.	A.	—	A.	A.	—
	9	,,	+	+	A.	—	A.	A.	A.	A.	A.	A.	—	A.	—	—
	10	P.D.	+	—	A.	—	A.	A.	A.	A.	A.	—	—	A.	—	—
	Orig.	P.	+	+	A.	A.	A.	A.	A.	A.	—	—	—	A.	—	—
CW 3/1 *Staphylococcus.*	1	P.D. Ac.	+	—	A.	F.	A.	A.	A.	A.	A.	A.	—	A.	A.	A.
	2	,,	+	+	A.	A.F.	A.	A.	A.	A.	A.	A.	—	A.	A.	—
	3	,,	+	+	A.	A.	A.	A.	A.	A.	A.	A.	—	A.	A.	—
	4	,,	+	—	A.	—	A.	A.	A.	A.	A.	A.	—	A.	A.	—
	5	,,	+	+	A.	—	A.	A.	A.	A.	A.	A.	—	A.	—	—
	6	,,	+	+	A.	—	A.	A.	A.	A.	A.	A.	—	A.	A.	—
	7	,,	+	+	A.	—	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.
	8	,,	+	+	A.	F.	A.	A.	A.	A.	A.	A.	—	A.	A.	—
	9	,,	+	+	A.	—	A.	A.	A.	A.	A.	—	—	A.	—	A.
	10	,,	+	+	A.	—	A.	A.	A.	A.	A.	A.	—	A.	A.	—
	Orig.	Ac. Coag.	+	+	A.	A.	A.	A.	—	A.	A.	A.	—	A.	A.	—
42B/1 *Corynebacterium.*	1	Alk. D.P.	C.S.	+	—	—	A.	A.	—	A.	—	—	—	F.	A.	—
	2	,,	,,	+	—	—	—	—	—	—	—	—	—	—	—	—
	3	,,	,,	+	F.	—	—	—	—	—	—	—	—	—	—	—
	4	D.P.	,,	+	—	—	—	—	—	—	—	A.	—	A.	—	—
	5	,,	,,	+	A.	—	—	—	A.	—	—	—	—	—	—	—
	6	,,	,,	+	—	—	—	—	—	—	—	—	—	—	—	—
	7	,,	,,	+	—	—	—	—	—	—	—	—	—	F.	—	—
	8	,,	,,	+	—	—	—	—	—	—	—	—	—	—	—	—
	9	,,	,,	+	A.	—	—	—	—	—	—	—	—	A.	—	—
	10	,,	,,	+	A.	?	A.	A.	A.	A.	F.	?	?	A.	—	—
	Orig.	,,	,,	+	—	—	—	—	A.	—	—	—	—	—	—	—
24A/1 *Corynebacterium.*	1	Alk. D.P.	C.S.	+	A.	—	—	—	—	—	—	—	—	A.	—	—
	2	,,	,,	+	—	—	A.	A.	F.	A.	—	A.	—	A.	—	—
	3	,,	,,	+	A.	—	—	A.	—	—	—	—	—	A.	?	—
	4	,,	,,	+	—	—	—	—	—	—	—	—	—	—	—	—
	5	,,	,,	+	—	—	—	—	—	A.	—	—	—	A.	—	—
	6	,,	,,	+	—	—	—	—	—	A.	—	—	—	A.	—	—
	7	,,	,,	+	A.	—	—	—	—	—	—	F.	—	—	—	—
	8	D.P.	,,	+	—	F.	—	—	—	—	—	A.	—	—	—	—
	9	Alk. D.P.	,,	+	—	—	—	—	—	—	—	—	—	A.	—	—
	10	,,	,,	+	—	—	—	—	—	—	—	—	—	A.	—	—
	Orig.	,,	,,	+	—	—	—	—	—	A.	A.	—	—	—	—	—

Gelatin: + = liquefaction; C.S. = crateriform softening.
Milk: Peptonized; Ac. = acid; Ac. Coag. = acid with coagulation; Alk. = alkaline.
Carbohydrates: A. = acid; F. = faint acid; ? = faint and transient acid.

a definite differentiation can be made. A similar problem confronts us when we meet a spore-forming aerobe with polar flagella. Is it to be classified as a spore-forming *Pseudomonas* or an aberrant *Bacillus*? As Stanier and van Neil (1941) point out, we can only determine such points by studying the physiological characteristics of the organism for, since we have in *Sporosarcina* a spore-forming coccus, it is logical to accept a spore-forming *Pseudomonas*. The same applies to fluorescent cocci, spore-formers, etc. Until recently I regarded *Proteus* as a distinctive genus on account of its inability to produce gas from lactose and its possession of a urease; but lately a strain which gave only a small quantity of gas in glucose and saccharose after ten days' incubation was isolated in this laboratory; this organism obviously forms a link between *Proteus* and the non-gas-producing Bacteria. Rustigan and Stuart (1943) give as characteristics of *Proteus* that it gives a small quantity of gas in 48 hours and attacks urea rapidly. The extreme morphological variation in *Corynebacterium* intergrading with the Coccaceae is another case in point. One culture isolated here showed only coccoid forms and was classified as *Staphylococcus* until the diffusible red pigment associated with *C. erythrogenes* appeared. Slide cultures showed rod forms with snapping division after 8 to 16 hours, but at 48 hours all forms were coccoid. Van Niel (1946) records similar changes in morphology in *Corynebacterium*, while Burke, Schwartz and Klise (1943) record a *Staphylococcus aureus* strain which regularly showed rod forms in cultures less than 12 hours old. This discounts Rahn's theory that "morphological changes are usually decided changes". Topping (1937) records motile *Corynebacteria* and her findings seem to be accepted. I have also isolated these forms, motile with polar flagella but otherwise identical with *Corynebacterium* species. Pigment production is another unreliable character. Lehmann and Neumann (1930) point out that the Coccaceae often show single colonies with pigmented and non-pigmented sectors and I have frequently found such colonies in the Coccaceae. These authors go on to show the relationship between some of the non-pigmented and pigmented forms, and bearing this in mind, use colour variations as specific characters only. Pigment variation is well known in the *Serratia* group where different shades of red and non-pigmented strains occur. Bergey also accepts this idea for the Coccaceae, though he does not point out the relationships, but uses pigmentation to separate *Achromobacter* from *Flavobacterium* while he is not consistent in retaining this character in the two genera.

In addition, van Niel (1944) has pointed out that the non-sulphur purple bacteria show characters putting sections of them in the Coccaceae, Pseudomonadaceae, etc. He places them in a photosynthetic group parallel with the Eubacteria. He also points out that loss of photosynthetic activity would transfer these bacteria to the heterotrophic bacteria and a similar loss of photosynthetic activity among the primitive algae would make these also indistinguishable from heterotrophic bacteria. The marine micro-flagellates are particularly difficult to separate as some become heterotrophic in absence of light.

The argument as to whether heterotrophs preceded autotrophs or *vice versa* is probably unimportant and it may well be that development in both directions occurred. Luria (1947) points out that in its present stage, bacterial genetics cannot be expected to "bring under control the hornet's nest of bacterial taxonomy but may suggest some useful precautions in approaching it". He shows that "species, genera and even tribes are often separated on the basis of character differences that may be brought about by a single mutational step, for example the tribes Eschericheae and Proteae, the genera *Salmonella* and *Eberthella*, the species *Staphylococcus aureus* and *Staphylococcus albus* in the classification of Bergey's Manual. Genera (for example *Phytomonas*) are separated from closely related groups (*Pseudomonas*) on the basis of plant pathogenicity, a character that may well arise or disappear by mutation. It is obvious that such mutable properties can be used only in practical determinative keys without claim to any taxonomic significance. Even then the greatest caution should be observed, since variable characters may prove too elusive to "permit recognition of organisms of practical importance. In many cases, description of a variability pattern might prove

a better taxonomic criterion than description of any one or more of the variable phenotypic traits themselves" (Luria, 1947). I had already reached the same conclusions from my study of the Corynebacteria and Coccaceae, basing them on purely practical determinative considerations.

With the motile strains of *Corynebacterium*, all, on prolonged culture, lost their motility and then became indistinguishable from the rest of the *Corynebacterium* strains I was investigating. I was unable to induce motility on any medium. As I intended to keep these cultures as type cultures and submit them to the National Collection of Type Cultures at the end of the war, I was concerned at the variation, which is just another example of the danger of placing too much reliance on type cultures.

This means that we have to rely on differentiation mainly on the original description of the species, and, with changing methods over the years, correlation becomes increasingly difficult.

B. THE COMPOSITION OF MEDIA.

It is well known that slight variations in the composition of media cause changes in the observed reactions of bacteria. This becomes important as soon as we begin to use physiological characters for differentiation. For example, if British, American and German peptones give different results it becomes very difficult to assess the reactions in peptone media, while many organisms do not grow well in the absence of peptone. During the war we could not, at this laboratory, get Lab-Lemco meat extract and were forced to use whatever peptone we could buy—we had always used Witte peptone for carbohydrate media—and we had to change from Japanese agar to Australian agar made from *Gracilaria confervoides*, which has a higher setting point and other peculiar properties. We could get no uniformity in the production of green water-soluble pigment from *Pseudomonas* strains, or in the red diffusible pigment of *Corynebacterium erythrogenes*. Even the use of Georgia and Poe's and of Gessard's media was not wholly satisfactory in establishing fluorescence or pyocyanin production in *Pseudomonas*, the trouble apparently lying in the agar. The production of hydrogen sulphide appears to depend on the meat or yeast extract used, Australian "Marmite" giving different results from "Yeastrel". The nitrate reduction test, normally unsatisfactory, becomes more so with chemicals from different sources. In Australia skimmed powdered milk is no longer manufactured and this has given another source of error.

C. STAINING REACTIONS.

Here, too, one finds difficulties. Many Coccaceae and Corynebacteria are Gram positive no matter which of the recognized methods are used, but others are positive by some methods, negative by others. Age of culture and composition of media also interfere and must result in anomalies, unless the worker is familiar with the particular group under study. As an example, *Corynebacterium helvolum* is described by Bergey (5th Edition) as *Flavobacterium helvolum*, and is recorded by him as Gram negative, whereas I have found it Gram positive, as does Jensen (1934).

When one considers the difficulties enunciated above, one sees the futility of the system of specific description and at times of generic description given in Bergey's manual and finds the necessity of weighing Bergey's species in the light of one's knowledge of variation. I have recently used a punched card system for sorting my organisms and have applied it also to descriptions of species by Bergey and his followers, checking back against the full description given. It is surprising to find how many of these species are indistinguishable from one another and for this reason alone cannot stand.

PREVIOUS SCHEMES OF CLASSIFICATION.

Bacteriological literature is full of papers on the classification of the bacteria and this literature has been ably reviewed by a number of authors. "Natural" systems have been proposed based on morphology (including the Gram stain), physiology and function, and these characters frequently have been combined in an arbitrary and at times confused manner with unfortunate results. The morphologists include the earlier

bacteriologists; the physiologists really began with Orla-Jensen; while function, especially pathogenicity, is stressed by Bergey and the Society of American Bacteriologists in such genera as *Phytomonas*, *Erwinia*, *Xanthomonas*, *Corynebacterium*, etc.

A very sound discussion of previous systems is given in Kluyver and van Niel's paper (1936). These authors show the importance of morphology in defining the primary groups and the necessity for careful evaluation of physiological characters in subdivision. They point out that one should not push morphological divisions too far. They do not agree with Prévot (1940) that physiological characters must be confined to the delimitation of species. They discriminate between "incidental" and "genuine" immotility, a distinction which is fundamentally important. For example, the non-motile Gram negative rods morphologically and physiologically resembling the genus *Pseudomonas* should be regarded as "incidentally" non-motile, whereas the Corynebacteria should be regarded as "genuinely" immotile. However, Topping and I have found motile Corynebacteria—which in this case would be classed as "incidentally motile". This shows a relationship between *Corynebacterium* and *Pseudomonas* which is strengthened by the frequency of pleomorphism in both genera and the occasional occurrence of snapping division in the latter genus, which I have observed in slide cultures.

The system of Migula (1895) given in Engler and Prantl follows Cohn (1872) in applying a binomial classification. This system was based on morphology and stressed the flagellation of motile organisms which is nowadays accepted as a highly useful character. Buchanan (1917) developed a system on which was founded the classification now used by the Society of American Bacteriologists in Bergey's Manual. This was a distinct advance, being put forward as a contribution to an expanding science. This system was also primarily morphological though physiological characters were also used extensively. Orla-Jensen (1909) put forward a physiological classification, neglecting morphological characters except flagellation, and assumed that physiological characters were phylogenetic, putting *Streptococcus*, etc., with the Peritrichineae, and *Corynemonas*, *Mycomonas* and *Actinomyces* among the Cephalotrichineae, while *Mycobacterium* and *Corynebacterium* were removed to another family. Kluyver and van Niel (1936) give further criticism of Orla-Jensen's suggestions which is sufficient to show that physiological criteria alone do not provide a satisfactory classification. They point out, however, that these suggestions do emphasize the importance of physiology, an importance which most subsequent writers have been ready to concede. We can follow the line of thought from Migula through Chester (1901), Orla-Jensen and Buchanan to the present Bergey's Manual, but unfortunately the later developments appear to most other writers and to me as unfortunate.

Lehmann and Neumann in Europe produced a textbook of determinative bacteriology, which, while reasonably sound as far as it goes, is too conservative for our present knowledge. Despite this, British and European bacteriologists still prefer Lehmann and Neumann to Bergey. I have found myself forced to abandon Bergey and think it desirable to set out my views in this paper.

Previous Schemes of Classification.

A. Lehmann and Neumann classification.

Lehmann and Neumann's classification (Bacteriologische Diagnostik, 8th ed., Breed's translation, 1930) gives a classification of the Schizomytes which appears to be more reliable than that of Bergey in that they take into full consideration the variability of bacteria. On this account, they are perhaps rather conservative in the number of genera which they accept. Some of these, for example the genus *Bacterium*, become rather unwieldy in size, at the same time including organisms with fairly wide range of characteristics. The number of species in each genus is much less than in Bergey but it is probable that they are more well founded. Jensen has used Lehmann and Neumann's classification in his work on the soil organisms of the Actinomycetales (Jensen, H. L., 1931, 1932, 1934). Lehmann and Neumann's classification is based primarily on morphological characters, thus following the classification of the higher orders of plants and animals. Actually they carry this morphological differentiation

right down to genera, and it is at this point that the system begins to show signs of strain.

In this paper I am primarily concerned with Lehmann and Neumann's two orders, Schizomycetales and Actinomycetales. Kluyver and van Niel point out that the biggest contribution of these two authors was their recognition of the relationship between *Corynebacterium*, *Mycobacterium* and *Actinomyces*, although for physiological reasons Kluyver and van Niel find it difficult to include *C. diphtheriae* with *Corynebacterium*. This is due to lack of knowledge of the variability of the genus in its ability to ferment carbohydrates, and once this variability is recognized the difficulty disappears. One wonders why the editors of Bergey's Manual have been so slow to accept Lehmann and Neumann's suggestion.

Apart from Desmobacteriaceae and Spirochaetaceae with which I am not familiar and which have been dealt with by Kluyver and van Niel, I would, in the main, accept Lehmann and Neumann's families, although I would certainly rearrange some of the genera and subgenera. It is when one reaches a consideration of the genera of this system that the difficulties are realized. Even if we accept their subgenera as genera the classification is still inadequate in some respects. I believe, however, that if this were done the result would be a better classification than Bergey's.

The following quotation shows the appreciation by Lehmann and Neumann of the difficulties caused by variation:

"If this classification is reviewed then it cannot be denied that the families and the genera are frequently connected by intermediate forms. The following may be recalled: The border-line between the Coccaceae and Bacteriaceae is made indistinct by oval and lancet-shaped cocci and by species which sometimes form cocci, then rods (see the later description of *Streptococcus acidi* and *Bacterium melitense*). It is frequently difficult to "distinguish between *Streptococcus* (*'Diplococcus'*) and *Micrococcus* and *Sarcina*. Twisted forms occur in the life cycle of many rods; flagella and endospores occur in quite different species so that it would lead to an entirely artificial grouping if a classification were based exclusively upon flagella or upon endospores.

"Various only relatively acid-fast species form transitions between *Corynebacterium* and *Mycobacterium*. The border between *Mycobacterium* and *Actinomyces* is made very indefinite because of the fragility and the relative acid-fastness of many Actinomycetes. Similar transitions have been found in other parts of the plant and animal system. They cause difficulties in regard to names, but only because the nomenclature of Linné that is still used is founded on hypotheses which prevailed 100 years before Darwin." (Breed's translation.)

B. BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY.

Bergey's Manual of Determinative Bacteriology set out to be a comprehensive list of described species of bacteria and to present these forms in something resembling a natural system. Based on Migula and Chester, it incorporated some of Orla-Jensen's then revolutionary ideas but has since degenerated into a "splitters" paradise of genera and species. This is no doubt the result of trying to reconcile divergent views on classification by a number of eminent bacteriologists who have assisted in editing the various editions. The work has come in for a great deal of criticism in recent years. Rahn (1937), Kluyver and van Niel (1936) and Stanier and van Niel (1941) have been particularly searching in their criticisms.

The arbitrary use of characters in the system is most striking, producing as it does such unfortunate results, as is also "the utter disregard for the significance of mutual relationships between natural groups" (Kluyver and van Niel). Another bad feature is the uncritical recording of species described elsewhere without taking any note of their variation or of their distinguishing features. My main criticism of this part of the Manual is that American bacteriologists as a whole do not realize the variability of bacteria, and have accepted the Manual as a precedent for creating further species on flimsy evidence. I shall reserve my detailed criticism of the Manual till I come to discuss the genera and species. It is obvious that this work will have very limited

usefulness until it is completely revised in the light of the recent criticism and until all genera and species are pruned to a bare minimum. It is usual in classification for schools of "splitters" to be followed by schools of "lumpers", and there are signs that this is about to take place. The papers of Taylor (1938), Topping (1937), Jensen (1943), van Niel (1944), Stanier (1942) are examples of this tendency, and I feel constrained to follow their example by suggesting places where the pruning knife may well be wielded. Each writer has of course his own ideas of pruning, and these ideas are governed by his own experience with regard to his special branch. At the moment, in Great Britain there is a strong move in the direction of "lumping".

C. KLUYVER AND VAN NIEL'S CLASSIFICATION.

Kluyver and van Niel, in their paper of 1936, suggest a classification based on morphological and katabolic grounds, but it would appear to the writer that, in certain cases, they have gone too far in this respect; for example, they cannot fit *Corynebacterium diphtheriae* within the saphrophytic Corynebacteria because of its fermentative capacity. The writer has found that quite a number of Corynebacteria from marine sources are fermentative and there appears to be a continuous gradation between non-fermentative and fermentative strains. As a variation in fermentative capacity can be shown to exist in *Corynebacterium*, it is to be presumed that other genera are likely to show this property. So the use of katabolic activity as a major generic criterion seems to be fraught with some danger. In certain cases, such as the use of photosynthesis, there would seem to be less difficulty, and there are good grounds for basing some genera, e.g., *Clostridium*, on anaerobic growth. In the case of *Streptococcus*, on the other hand, the writer isolated numerous strains of anaerobic streptococci from teeth. In all cases, although the organisms were obligate anaerobes on first isolation, they later grew quite well in aerobic culture. The writer feels that the solution to this problem is, as suggested by Bruce White, along the lines of a study of a group of strains building up from the strain to the variety and from the variety to the species, and thence to the genus, keeping in mind, however, the criteria used in existing classifications. This would entail a great deal of study by bacteriologists working with any given material, but should go a long way to confirming or refuting the work of the determinative school of bacteriologists. The writer does not hold, however, with Bruce White's suggestion of using serology as a panacea for all ills, since although the *Salmonella* group fit well into a serological scheme this applies to very few other groups. One may note the failure of Rustigan and Stuart to obtain serological differentiation among the closely related *Proteus* group. Most bacteriologists have at one time or another tried to solve their classification problems by serological methods, but few of them have been able to get results consistent enough to warrant publication. Thus, Bruce White's implied suggestion that non-medical bacteriologists have ignored the serological method has more apparent than real foundation. As an example of the inapplicability of this method, the genus *Rhizobium* shows serological relationships which cut straight across the host relationships. In such a case, the host relationships must stand and the serological relationships must be discarded in classification. In genera such as *Achromobacter*, where no pathogenic organisms are known, it is difficult to decide just where to start serological investigation. On the other hand, serological tests on the coliform bacteria, *Erwinia* and *Serratia*, suggest a close relationship between these genera which, confirmed by other characters, seems to show that they do not merit generic rank. Kluyver and van Niel have made some very pertinent observations on the desiderata of a natural classification of bacteria. They state "We are of the opinion that Prévot rightly emphasized the priority of morphological over physiological characters, yet his restriction that the application of the latter should be confined to the limitation of species is of quite arbitrary nature. In accepting a taxonomic value for physiological characters, it cannot be understood why it could not also be applied for the demarkation of higher systematic units." Hence it follows in an attempt to subdivide the organisms belonging to one "natural" group of bacteria into species one shall have to create as many species as there are organisms which differ in

"sufficiently fundamental" characters, regardless of the possible existence of intermediate types. It depends entirely upon the "scientific tact" of the investigator which characters will be deemed worthy of the designation "sufficiently fundamental".

D. RAHN'S SYSTEM.

Rahn's system of classification divides the Eubacteriales primarily on spore formation, then on the Gram reaction, relations to oxygen, and lastly flagellation and form. It does not seem sound to me to make two primary divisions so unequal as that based on spore formation. Then the complete separation of *Lactobacillus* from the *Actinomycetales*, which this system implies, seems unnatural, although it still retains them in the same group as the Streptococci. The relationship to oxygen is a character of doubtful value when used for a major division, as there are so many facultative organisms. For these reasons I do not see my way clear to accept Rahn's system.

E. PRINGSHEIM'S SYSTEM.

The system of Pringsheim (1923) is a development of Lehmann and Neumann's system. He separates the purple bacteria as Rhodobacteriales with two families—Rhodobacterinae and Thiorhodinae—and places the much disputed Beggiatoaceae with the Chlamydobacteriaceae in the Chlamydobacteriales. He keeps the order Actinomycetales in the sense of Lehmann and Neumann but alters the name to Mycobacteriales.

In Eubacteriales he has three families, Coccaceae with three genera—*Streptococcus*, *Micrococcus* (retaining the *Staphylococcus* group and the *Gonococcus* group although the *Staphylococcus* group corresponds to the wider sense of *Micrococcus* without *Neisseria*), the *Sarcina*: Bacteriaceae with two genera—*Bacterium* and *Bacillus*, the former including a Gram negative group with three sub-groups—dysentery group, non-liquefying coli group and the liquefying *Proteus* group, and a Gram positive group—the long lactic acid bacteria (obviously out of place here), containing an aerobic and an anaerobic group, and lastly Spirillaceae with three genera, *Pseudomonas*, *Vibrio* and *Spirillum*.

F. STANIER AND VAN NIEL'S CLASSIFICATION.

The most adequate system as far as it goes is that of Stanier and van Niel (1941). The system proposed by Kluyver and van Niel, in which morphology is used for dividing bacteria into tribes, and mode of nutrition for genera, is abandoned and Haeckel's kingdom Monera is divided into two: Myxophyta which represent the photo-synthetic chlorophyll-bearing representatives producing oxygen, and their colorless non-photosynthetic counterparts, and Schizomycetae which are non-photosynthetic or photosynthetic without oxygen production. The Schizomycetae are divided into three classes: Eubacteriae with rigid cell walls, flagellar motility (when motile), reproduction by transverse fission and spores as endospores, cysts or conidia; Myxobacteriae without rigid cell walls, with creeping motility and spores as microcysts or fruiting bodies; and Spirochaetae without a rigid cell wall and with motility by means of an axial filament or modified fibrillar membrane.

The Eubacteriae are divided into three orders:

1. Rhodobacteriales—photosynthetic but not producing oxygen.
2. Eubacteriales—non-photosynthetic unicellular, no conidia.
3. Actinomycetales—non-photosynthetic usually mycelial, with conidia (Jensen's emendation accepted by the authors).

The Myxobacteriae have one order, Myxobacteriales, and the Spirochaetae one order, Spirochaetales.

This scheme has the advantage of having the Rhodobacteriales, Eubacteriales and Actinomycetales (including *Mycobacterium* and *Corynebacterium*) as parallel units, suggesting their probable parallel development. The authors show the probable derivation of the Rhodobacteriales and the Actinomycetales from the Eubacteriales and suggest that the Beggiatoaceae and Myxobacteriae are also derived from the Eubacteriales through the Chroococcales.

G. VAN NIEL'S SUGGESTIONS.

An interesting discussion on the possibility of an adequate bacterial classification is that of van Niel (1946). He now considers that a natural classification is impossible at the present time and suggests that bacteriologists content themselves with working out a series of keys based on different properties, each organism being diagnosed by cross reference to the keys. This seems to me ideal rather than practical, requiring a new approach that will leave the identity of a given organism in doubt until adequate keys are produced. I do not think we can afford to break with the past in the manner suggested, although van Niel's work is too penetrating and founded on too long an experience in bacteriology to be lightly dismissed. I feel that we should compromise by studying existing classifications and abandoning any genera that are not adequately differentiated, while at the same time revising our concept of genus and species.

THE GENUS–SPECIES CONCEPT.

Van Niel shows clearly that Cohn, while creating the idea of a binomial classification of the bacteria, did not necessarily mean to give bacterial genera and species the Linnean significance. The Binomial system was merely a convenience, and as such it appeals to me. The mistake some bacteriologists have made is in tacitly accepting a Linnean significance and in insisting that bacterial genera and species must fall into orders and families and thence assuming that we should aim at a phylogenetic classification. Moreover, some have assumed that a determinative classification is a phylogenetic one. Most of the anomalies we have encountered since have been due to such conceptions. If, as van Niel suggests, we realize this, I think we can find a way out of the dilemma. Our genera and other groupings then are convenient, not absolute, and we can draw our dividing lines at the most convenient point. As for species, these must be still more flexible and must allow for the wide range of variation which has been shown to be possible. Later we may find that some of our genera have phylogenetic significance while others have not. This inequality of status will not be a serious obstacle in determinative work. We thus provide ourselves with a working tool until we improve our knowledge of bacterial genetics to the point where a strictly phylogenetic classification becomes possible.

THE BASES OF CLASSIFICATION.

As van Niel points out, there has in the past been a great deal of discussion on the relative merits of morphological and physiological features for bacterial classification, but it has mainly centred on their taxonomic rather than their determinative importance. The question of their pragmatic importance has not been so carefully considered. It is this that I would stress. Stanier and van Niel use both morphological and physiological characters in their grouping of the bacteria, but they have carefully chosen the characters used as being the most constant characters available. It is that fact which commends their classification to me. They have also kept in view the apparent phylogenetic relationships. Despite van Niel's criticism of the use of the terms "order", "family", "genus" and "species", these are convenient and it is necessary from a practical point of view to make at first broad and then ever-narrowing distinctions. If we regard the groupings as pigeon holes we may find that in dividing our pigeon holes into smaller ones we have a few things left over. We must remember that in every filing system we have our "miscellaneous" file. Van Niel would avoid this by a series of cross indices, but we must always have some sort of label on our files and the genus-species concept does give us a label which is easy to understand: and we must have a system for attaching our labels. We have, then, to choose characters which are constant enough to use as labels. We find a few characters, some morphological and some physiological, which we can use. Ability to photosynthesize under a given set of conditions is a sound character, and so is a tendency towards mycelium formation or branching. These probably have phylogenetic value and are an obvious starting point. Broadly speaking, the Gram stain is allied with resistance to certain bactericides, e.g., the sulpha drugs, and therefore this stain is a useful physiological character. Shape, presence and location of flagella, presence or absence and form of spores are also useful and may in

some cases represent phylogenetic trends. Even range of variation, as with the Actinomycetales (if we retain this order), is a useful character. I have used these in the decisions regarding the validity of genera, but have disregarded others, such as pigmentation, which experience has shown are not so constant. The usual biochemical tests have not, in the organisms I have investigated, stood up to the test. They seem to vary too much within a given strain, and cannot be correlated with one another or worked out on a statistical basis. If used at all, they must, I submit, be confined to specific distinctions. Colour also belongs to this group.

In addition, these characters are, as Luria points out, alterable by a single mutation and such mutations have been shown to occur. The change from an obligate autotrophic or obligate anaerobic existence to a heterotrophic or aerobic existence requires a change in enzyme systems, and is therefore not alterable by a single mutation.

Once we have put a label on our organisms there is no reason why we should not cross-index our organisms on a basis of, say, red pigment or cellulose digestion as van Niel suggests, and I agree with him that there is no reason for keys to follow the classification. I also agree with him that the more keys the better, provided the characters used are reasonably stable. Agar digestion, for example, would be inadmissible, as the property is easily lost on subculture, while cellulose digestion, being relatively stable, is admissible.

The Suggested Classification of the Bacteria.

Even with all these well-considered systems to guide us it does not seem possible to provide a system which will be acceptable to all. The following classification is set out as the one seeming to suit my own conceptions and to fulfil my own needs. It develops the European system in the light of more recent work and deliberately rejects many of the genera set up by the Society of American Bacteriologists as being either ill-founded (those based on pathogenicity or pigment production) or unnecessary. The main requirement of any proposed system at the moment is simplicity, reducing the number of genera and species, with the desideratum that even the genera and species retained be further reduced, if it can be shown that this can be conveniently done. It is felt that a few well-marked genera and species are preferable to a number of vaguely defined ones. It is also strongly urged that new genera and species should be created only when ample evidence for their creation is available and when it is definitely of advantage to the science that they should be created. Frequently, perhaps, we shall have to rely on the "scientific tact" of the bacteriologist in this respect. Every author should, however, when creating new species or genera, define his creations as clearly as possible, including in his definitions the characters which differentiate them from the nearest allied genera or species.

The divisions as far as orders follow Stanier and van Niel (1941), except for the deletion of the order Actinomycetales.

Kingdom Monera Haeckel.

Micro-organisms which do not appear to possess true nuclei or plastids and for which sexual reproduction cannot be readily demonstrated.*

Sub-kingdom myxophyta.

Unicellular or multicellular organisms which, if motile, show creeping motility. The predominant type of metabolism is photosynthetic with oxygen production. The photosynthetic pigments are chlorophylls *a* and *b*, accompanied by phycocyanin and sometimes phycoerythrin. Non-photosynthetic colorless organisms which are clearly recognizable as counterparts of photosynthetic genera are also included.

Sub-kingdom schizomycetae.

Unicellular or mycelial organisms, which, if motile, may creep or may move by means of flagella or an elastic axial filament or fibrillar membrane. Metabolism is

* The possession of true nuclei by bacteria is claimed by some writers, as is a form of sexual reproduction. Some of these claims appear well founded but cannot yet be regarded as indisputable. It is, however, thought necessary to modify Stanier and van Niel's description.

predominantly non-photosynthetic, but, if photosynthetic, is without oxygen production. The photosynthetic members of this division never contain chlorophylls *a* or *b*, phycocyanin or phycoerythrin.

Class EUBACTERIAE.

Unicellular or mycelial organisms. If unicellular, they may be spherical, rod-shaped or spiral. Motility when present always by flagella. Multiplication by transverse fission. Resting stages, if present, may be endospores, cysts or conidia. Two orders, Rhodobacteriales, Eubacteriales,

Class MYXOBACTERIAE.

Unicellular rod-shaped organisms. Always show creeping motility (never flagella). Multiplication by transverse fission. Resting stages, if present, may be microcysts, sometimes contained within larger cysts. The individual microcysts or the larger cysts may be borne on or in fruiting bodies of various shapes. One order, Myxobacteriales.

Class SPIROCHAETAE.

Unicellular spiral organisms. Always motile by means either of an elastic axial filament or of a modified fibrillar membrane. Multiplication by transverse fission. No resting stages known. One order, Spirochaetales.

Order RHODOBACTERIALES. Stanier and van Niel (Pringsheim).

Unicellular organisms. No resting stages known. Photosynthetic, not producing oxygen.

Order EUBACTERIALES. Stanier and van Niel (Buchanan).

Unicellular organisms. Resting stages, if present, may be either endospores, cysts or conidia. Non-photosynthetic.

This paper deals with only one order, Eubacteriales.

The Order EUBACTERIALES.

1. Family Coccaceae—Pribram.

Spores absent or rare, cells approximately spherical, usually Gram-positive.

This classification is in accord with Pringsheim and Lehmann and Neumann, except for *Neisseria*, which, on account of its usual Gram-negative staining, parasitism and difficulty of culture except on serum media, seems to warrant generic rank. While parasitism itself should not in the writer's opinion be used to characterize genera, it may in certain cases be used in conjunction with other considerations. That is why parasitism is accepted here and rejected for *Corynebacterium*.

The genus *Staphylococcus* Rosenbach cannot be adequately separated from the genus *Micrococcus* Cohn, as it differs from the latter genus in being "usually parasitic" and possibly in degree of tolerance to a high pH. As some of the Micrococci are able to be facultative parasites, Bergey's distinction has no foundation. The same applies to the genus *Gaffkya*. Prévot's family Neisseriaceae is suppressed, while *Veillonella* appears to be *Micrococcus* or *Staphylococcus*, the two described species differing markedly in their carbohydrate reactions.

N.B.—It would be interesting to test non-parasitic members of this family to the sulpha drugs, acridines, penicillin and other drugs, sensitivity to which is known to be related to the Gram-staining properties, especially as these drugs attack the Gram-negative as well as the Gram-positive pathogenic forms. One of these drugs might well have determinative significance.

The differentiating character used is the number of planes of division, not because it is definitive in itself, but because it separates *Streptococcus* and *Neisseria*, two usually parasitic genera, from the sometimes parasitic *Staphylococcus* and the non-parasitic *Sarcina*.

A. Genus *Streptococcus* Billroth. Division in one plane. Chains frequent. No flagella. Gram-positive. Aerobes or facultative anaerobes. Closely related to *Neisseria*, *Lactobacillus* and to *Corynebacterium*.

B. Genus *Neisseria* Trevisan. Division in one plane forming diplococci. No flagella. Gram-negative. Aerobes or facultative anaerobes. All parasitic.

C. Genus *Staphylococcus* Rosenbach. Division in two planes. Flagella rare. Gram-positive or Gram-variable. Aerobic or aerophilic. Related to *Sarcina* and possibly to *Corynebacterium*. *Staphylococcus* is preferable to *Micrococcus*, as Cohn's description is hardly sufficient.

D. Genus *Sarcina* Goodsir. Division in three planes on suitable media. Not always distinguishable from *Staphylococcus*. Often in packets. Some species motile. Gram-positive. Usually aerobic or partly anaerobic. Related to *Corynebacterium*.

E. Genus *Sporsarcina* Orla-Jensen. Division in three planes. Motile, peritrichous. Endospores formed.

2. Family Bacteriaceae—Cohn.

Gram-negative (one genus Gram-positive). Rod-shaped organisms. Flagella, when present, peritrichous. Usually fermentative, tendency towards gas production. No endospores.

The families Bacteriaceae and Pseudomonadaceae are differentiated primarily on flagellation, which in itself would be dubious procedure. However, it normally agrees with power to ferment carbohydrates, pigment production and other characters which seem to most bacteriologists to justify the separation of these two families.

The Bacteriaceae are probably the most difficult group to classify adequately. Bergey gives a number of families which I consider should be reunited.

The Parvobacteriaceae of Rahn appears to form a doubtful family based as it is on size and growth in body fluids. Most authors are dubious about size as a characteristic, but the three tribes of organisms appear to have some common characters. *Pasteurella* and *Malleomyces* (*Pfeifferella*) are differentiated on reaction on milk and should probably constitute only one genus, *Pasteurella*. The next tribe has one genus, *Brucella*, and the next tribe, Haemophileae, three genera, *Haemophilus*, *Noguchia* and *Dialister*, of which Rahn, I feel correctly, recognizes only *Haemophilus*. We have thus three genera, *Pasteurella*, *Brucella* and *Haemophilus*, which I consider fit well into the Bacteriaceae and are characterized by their small size and a tendency to bipolar staining.

Rahn (1937) follows Orla-Jensen in creating a family, Lactobacteriaceae, to include the Streptococci, while most morphologists include these with the Coccaceae. If we follow Orla-Jensen's classification we are faced with the desirability of including also *Corynebacterium*, which seems to be closely related to *Lactobacillus* but shows affinities also with *Sarcina* and possibly *Streptococcus* and *Staphylococcus*. It seems best, therefore, for the time being to follow the morphologists by including *Streptococcus* with the Coccaceae, though Cowan and Gibson (private communications) would include this genus with *Lactobacillus*, with which it has a great deal in common. The correct position of this genus is admittedly difficult. This leaves us with the genera *Lactobacillus* and *Propionibacterium*, both of which are Gram-positive, pleomorphic, non-motile rods with a tendency towards metachromatic staining. For this reason they do not fit into the Bacteriaceae.

The family, as I envisage it, includes Rahn's Enterobacteriaceae and some of Cohn's Bacteriaceae. *Listerella* and *Microbacterium* are not included in the organisms of this group. *Klebsiella* should be suppressed as being insufficiently differentiated from *Escherischia* and *Aerobacter*, while these two genera show so many intergrading forms that they can hardly be sustained as separate genera. *Erwinia* is not admitted, as it is based only on pathogenicity for plants, while *Serratia* can only with difficulty be sustained on pigment production, especially as this is not constant. The last two are shown by various authors to be similar in reactions to the coli-aerogenes group. I would include all these under the name *Enterobacter* Rahn, emend.

Of Cohn's Bacteriaceae as given by Bergey, *Cellulomonas*, as being obviously a hotch-potch of organisms with one common factor, decomposition of cellulose, must be suppressed. It would be as logical to form a genus on the basis of agar or chitin

digestion or the decomposition of mucin. *Achromobacter* and *Flavobacterium* separated on pigmentation must be combined—I suggest as the genus *Bacterium* Migula emend. Species of the genus *Actinobacillus*, being non-motile and pleomorphic and occurring in lesions resembling actinomycosis, may be Gram-negative *Proactinomyces* or *Actinomyces* or may be better classed as a genus of the *Pseudomonadaceae*. Their acid fastness is not recorded. *Bacteroides* is regarded by Rahn as a mixed genus, and I would follow him in suppressing it. *Fusobacterium* appears to be closely related to *Corynebacterium* as a Gram-negative anaerobic branch and has actually been assigned to this genus by Lehmann and Neumann. This genus and *Actinobacillus* obviously do not belong here. *Alcaligenes*, incorporated by Bergey with *Rhizobium* in the Rhizobiaceae, is a mixture of *Corynebacterium* and *Bacterium* (*Achromobacter* and *Flavobacterium*), although one type culture is stated by Conn (1942) to have polar flagella and is therefore *Pseudomonas*.

We are thus left with the following genera:

A. Genus *Pasteurella* Trevisan. Small Gram-negative rods showing bipolar staining, microaerophilic, haemophilic. Usually grow on ordinary media.

B. Genus *Brucella* Meyer & Shaw. Small Gram-negative rods, no marked bipolar staining, microaerophilic. Grow on ordinary media.

C. Genus *Haemophilus* Winslow *et al.* Small Gram-negative rods, often showing bipolar staining. Microaerophilic to anaerobic. Haemophilic. Do not grow on ordinary media.

Note.—It appears rather difficult to retain these three genera and perhaps they should be united. There is some indication of serological affinity between them.

D. Genus *Enterobacter* Rahn emend. Gram-negative rods and filaments, motile with peritrichous flagella or non-motile. Acid and gas produced from glucose and lactose. Members appear to be antigenically related.

E. Genus *Proteus* Hauser. Gram-negative pleomorphic rods and filaments, motile with peritrichous flagella. Acid and gas produced from glucose rapidly but in small quantities. No gas from lactose. Usually characteristic spreading growth. Decompose urea rapidly. Serological groups difficult to separate.

F. Genus *Salmonella* Lignières. Gram-negative rods, motile with peritrichous flagella or non-motile. Acid and often gas produced from carbohydrates. Gas not normally produced from lactose or saccharose. An antigenically related group.

G. Genus *Shigella* Castellani Chalmers. Gram-negative non-motile rods producing acid but no gas from carbohydrates. Do not liquefy gelatin. Living in intestinal tract of warm-blooded animals. Often pathogenic.

H. Genus *Kurthia* Trevisan. Gram-positive rods. Peritrichous flagella. No acid from carbohydrates.

I. Genus *Bacterium* Migula emend. Gram-negative rods, motile with peritrichous flagella or non-motile. Gas not formed from carbohydrates.

3. Family Pseudomonodaceae—Winslow *et al.*

Gram-negative rods, straight or curved. Motile with polar flagella or non-motile. May show snapping division. Poor fermentation of carbohydrates is usual; no gas formed. No endospores.

The differentiation of non-motile *Bacterium* from non-motile *Pseudomonas* is difficult and may at times be impossible. In such cases yellow to orange cell pigment suggests *Bacterium*; greenish diffusible pigment, *Pseudomonas*; fermentation of glucose and no other carbohydrates, production of ammonia from urea, asparagin and peptone suggest *Pseudomonas*.

This group seems related to the motile Staphylococci, some of which are feebly Gram-positive and through the Gram-positive motile *Corynebacterium* forms described by Topping and myself to the Actinomycetaceae. The non-sulphur purple bacteria are a photosynthetic offshoot of this group and the autotrophic *Nitrosomonas* forms are another branch. *Hydrogenomonas* and *Methanomonas* are probably normal Pseudo-

monadaceae, as few of these organisms have been tested under the conditions under which the two last-mentioned genera were isolated.

Of the genera listed in Bergey's Manual, the two differentiated on the basis of cellulose digestion, *Cellvibrio* and *Cellfalcicula*, should be suppressed, as suggested by Stanier (1941). *Cellfalcicula* is obviously synonymous with *Pseudomonas*, though, owing to its metachromatic granules and pleomorphism, it has some affinities with *Corynebacterium* (cf. *Corynebacterium fimi* and the motile polar flagellate Corynebacteria isolated by Topping and myself). *Phytomonas* distinguished by pathogenicity for plants should be suppressed, as the genus *Pseudomonas* as a whole shows a tendency towards pathogenicity under certain circumstances. Instances are the records of occasional pathogenicity for fish of *Pseudomonas* strains normally inhabiting water. *Protaminobacter* appears to be merely a group of *Pseudomonas* strains with a rather specialized enzyme structure and has close affinities with the Gram-negative, pleomorphic organisms which I have isolated from sea-water and which, apart from their Gram reaction, closely resemble *Corynebacterium*. Jensen (1931) points out the relationship between *Mycoplana* and the Actinomycetaecae. It is possibly best to retain the genus *Mycoplana*, dropping the physiological characterization of Gray and Thornton and using it to denote all Gram-negative highly pleomorphic forms which appear to bridge the gap between *Pseudomonas* and the Actinomycetaecae. Both motile and non-motile forms would be included in this genus. *Actinobacillus* appears to be related to these organisms, while *Fusobacterium* may be the anaerobic representative, although Sanarelli (1927) considers it to be related to the Spirochaetes rather than to the Actinomycetaceae.

We are left with the following genera:

A. Genus *Vibrio* Müller. Gram-negative non-sporing curved rods with only a single curve. Polar flagella.

B. Genus *Spirillum* Ehrenberg. Gram-negative non-sporing spiral forms with polar flagella.

C. Genus *Pseudomonas* Migula. Gram-negative non-sporing straight rods. Non-motile or motile with polar flagella. Usually produce acid in glucose broth. May produce a green fluorescent pigment.

D. Genus *Mycoplana* Gray and Thornton emend. Gram-negative highly pleomorphic rods with granules. Non-motile or motile by polar flagella. Carbohydrates not readily fermented.

E. Genus *Actinobacillus* Brumpt. Gram-negative pleomorphic non-motile rods. Carbohydrates usually fermented.

F. Genus *Fusobacterium* Knorr. Gram-negative rods, fusiform, non-motile. Anaerobic. (The place of this genus is considered doubtful. Cowan (verb. com.) places it with the Mycobacteriaceae.)

4. Family Bacillaceae—Fischer.

Rods producing endospores: usually Gram-positive. Flagella, when present, generally peritrichous.

Bergey's Manual gives two genera:

A. Genus *Bacillus*—aerobic forms.

B. Genus *Clostridium*—obligate anaerobic forms.

Imsenecki (1945) has shown that these two genera show cytological differences and for this reason they appear to be soundly based. Bergey's separation of the genus *Bacillus* into species groups is also sound. I have found that certain physiological characters are more constant for one group, e.g., sugar fermentation in Staphylococci than for another, e.g., the same reactions in *Corynebacterium*. This means that there is justification for using different physiological characters to separate different groups.

The spore-forming genus *Bacillus* contains types (*Aerobacillus* group) which in other respects resemble *Enterobacter* in the production of acid and gas from carbohydrates, *Pseudomonas* (*B. serusitidis* with polar flagella and only weakly Gram-positive

or Gram-variable), *Bacterium* (*B. subtilis* with peritrichous flagella thus resembling *Bacterium*), so that we may regard spore formation as a possible phylogenetic end point of development for each group. Some recent work has shown that asporogenous strains of spore formers do exist. The genus *Aerobacillus* may also be soundly based. This idea is strengthened by the existence of a sporing coccus with peritrichous flagella—*Sporosarcina ureae*. As well as the Gram-positive forms there exist Gram-negative species with often a tendency towards pleomorphism—the *Bacillus fusiformis* group which suggests a relationship with the *Fusobacterium–Actinobacillus* group. I have recently isolated from marine muds spore-forming pleomorphic *Bacillus* strains which suggest spore-forming types of *Corynebacterium*, *Mycobacterium* or *Actinomyces*.

This leaves two genera—*Acetobacter* and *Azotobacter*—which Bergey raises to the status of monogeneric families. Both these genera seem to have relationships with the *Pseudomonas–Mycoplana–Corynebacterium* group. They should, I think, be left as genera *incertae sedis* or placed in a family with the other Gram-negative pleomorphic organisms.

These Gram-negative pleomorphic forms—*Mycoplana*, *Actinobacillus*, *Fusobacterium*, *Azotobacter* and *Acetobacter*, should probably be separated from the *Pseudomonadaceae* as a separate family, the *Mycoplanaceae*, but I would not stress the point at this stage.

5. Family *Actinomycetaceae*—Buchanan.

I do not think that future bacteriologists will be able to accept the order Actinomycetales as separate from the Eubacteriales owing to the obvious links between the Coccaceae, *Lactobacillus* and *Corynebacterium* on the one hand and *Pseudomonas*, the Gram negative pleomorphic bacteria and *Corynebacterium* on the other. I have included *Lactobacillus* and *Propionibacterium* with the *Actinomycetaceae*. If this is done, *Streptococcus* seems out of place here. H. L. Jensen (1931–1934) has shown the transition between the non-acid-fast Corynebacteria and *Proactinomyces* and the acid-fast *Mycobacterium*, *Proactinomyces* and *Actinomyces*. It is possibly a question whether the line should be drawn separating the group, as is done in this paper, on the basis of the Gram stain, regarding the non-sporing Gram negative bacteria as Pseudomonadaceae and the non-sporing Gram positive bacteria as Actinomycetaceae or whether *Lactobacillus* and *Propionibacterium* should be separated from *Corynebacterium* and included with *Streptococcus* in a separate family Lactobacillaceae. One could justify including *Corynebacterium* with the Lactobacilli on account of the polar flagellate members of this genus found by Topping and myself, and one could also justify the inclusion in the Actinomycetaceae of the pleomorphic granular Gram-negative forms. Transitional forms among the bacteria are so frequent that it seems to me that no indisputable line can be drawn at all.

Cohn and Dimmick (1947) seem in error when they state that *Mycobacterium* has been extended to include "all acid-fast forms whether or not branching occurs" or "many branching forms whether or not they are acid-fast". Jensen has created the genus *Proactinomyces* to include the branched forms, one group of which are acid-fast, the other non-acid-fast.

The occurrence of snapping cell division appears to me as to Jensen a valid character for differentiating *Corynebacterium* as it can easily be demonstrated by using the agar slide culture described by Topping. In such cases there is no inference, although the presence of V. and N. forms, if they predominate, can safely be regarded as strong evidence. In my study of marine Corynebacteria I have encountered all types from those showing branching to forms normally coccoid, and have found from agar culture that they are in the young stages rods with true snapping division. They range from forms morphologically identical with *mitis* strains of *C. diphtheriae* to diphtheroid forms. The sugar reactions cannot be regarded as diagnostic, as although I agree with Cohn and Dimmick that they are characteristically poor fermenters, variation in the direction of strong fermentation does occur. I cannot therefore accept their genus *Arthrobacter*.

6. Family *Actinomycetaceae*—Lehmann and Neumann (1927).

Gram positive rods, frequently pleomorphic. Non-motile or rarely motile by means of polar flagella. No endospores. Conidia may be formed.

A. Genus *Leuconostoc.* Gram positive, non-motile, coccoid forms, rods may occur. Ferments carbohydrates producing lactic acid.

B. Genus *Lactobacillus* Cohn. Gram positive, non-motile rods frequently forming chains, tendency towards pleomorphism and irregular staining. Aerobic. Ferments carbohydrates producing lactic acid. Not acid-fast. Normal division.

C. Genus *Propionibacterium.* Gram positive, non-motile rods. Pleomorphic. Metachromatic granules usual. Microaerophilic. Carbohydrates actively fermented producing propionic and acetic acids and carbon dioxide. Not acid-fast. Normal division, or at least no record of snapping division.

D. Genus *Corynebacterium* Lehmann and Neumann emend Jensen. Gram positive rods, usually non-motile; when motile, flagella polar. Pleomorphic, with club-shaped and coccoid forms frequent, some branching. Cystites common. Metachromatic granules or irregular staining characteristic. Carbohydrate fermentation usually poor. Acid formation normally weak. Not acid-fast. Snapping cell division, giving V, N and palisade arrangement.

E. Genus *Mycobacterium* Lehmann and Neumann emend Jensen. Rods, staining with difficulty. Acid-fast. Pleomorphism including branched forms frequent. Slipping cell division giving characteristic bundles.

F. Genus *Proactinomyces* Jensen. Gram positive rods and mycelial forms. Frequently acid-fast. Pleomorphic, conidio-spores. No spores on aerial mycelium.

G. Genus *Actinomyces* Harz. Mycelial forms. Conidial spores in aerial mycelium.

H. Genus *Micromonospora* Ørskov. Mycelial forms. Spores single, terminal or on branches of vegetative mycelium.

The group is characterized, as Jensen points out, by a wide range of variation and the genera are in practice difficult to separate. The variation probably shows their phylogenetic relationships. Jensen (1931–32) points out that some species show characteristics of both *Corynebacterium* and *Mycobacterium* and others of *Mycobacterium* and *Proactinomyces*, and that it is therefore impossible to draw a hard and fast line between the genera. Other species, however, are not so variable and show the validity of the genera. The anomalous species may best be regarded as bridging forms.

I have found in *Corynebacterium* an almost continuous gradation of types from those fermenting no carbohydrates to those fermenting many. The fermentation is as a rule slow and a high degree of acidity is not reached (M.R. negative). Experiments have shown that fermenting strains may appear in non-fermenting cultures, so that sugar fermentations cannot be relied upon for specific diagnosis in this genus. There seem, however, to be good grounds for separating *Corynebacterium* from *Lactobacillus* and *Propionibacterium* on the basis of carbohydrate fermentation and snapping cell division. I am not certain, however, whether *Propionibacterium* should be retained as a separate genus.

Discussion.

This paper is intended to outline a classification of the Bacteria which removes a good many of the objections raised by other authors of the two classifications most generally in use, that of Lehmann and Neumann, and Bergey *et al.*, and which also smooths out some of the difficulties I have found as a bacteriologist in classifying organisms that I have encountered during twenty years of bacteriological research, first in plant pathology, then in medical and water bacteriology and latterly in marine bacteriology.

It brings out some relationships which seem apparent to me and which I do not think other writers have recorded.

Finally, it is intended to provoke thought among bacteriologists, especially among those bacteriologists who have in the past been prone to ignore the problems of classification. I wish to stress the point that adequate studies of the relationships of bacterial

assemblages cannot be made until taxonomy has become more orderly than it is at present.

In presenting this paper I realize that I am laying myself open to criticism. I am doing so deliberately in order to provoke discussion, hoping that science will gain by such criticism. I also hope that some of the suggestions I have made will be accepted, for example the strong connection between *Pseudomonas*, the Gram-negative pleomorphic organisms and the *Actinomycetaceae*.

SUMMARY.

An attempt is made in this paper to provide a determinative classification of the Bacteria which will be of greater practical use than those of Lehmann and Neumann and of Bergey *et al.* It follows Stanier and van Niel and then defines genera mainly on morphological grounds. The classification is not phylogenetic, as the author realizes that we do not know enough about the genetical relationships of bacteria to create a natural classification. For this reason it must be clearly understood that the divisions are not used in Linnean sense but are convenient groupings for determination.

The bias of the paper is admittedly towards general rather than medical bacteriology because the writer feels that the field of general bacteriology has been sadly neglected.

It is impossible for any determinative classification to accommodate satisfactorily all the organisms that a research worker may encounter, so that there will be times when intensive study of a particular group of organisms will be necessary in order to allot a generic name to a given organism.

This paper does not go beyond genera, because although the writer has at one time or another encountered most of the genera, he has studied only a few of them in sufficient detail to discuss specific relationships.

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AUSTRALIAN POLYPORACEAE IN HERBARIA OF ROYAL BOTANIC GARDENS, KEW, AND BRITISH MUSEUM OF NATURAL HISTORY.

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(*Communicated by N. A. Burges.*)

[Read 26th July, 1950.]

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Introduction.

Several months were spent in England in 1948 studying collections of Polyporaceae from Australia, Tasmania and New Guinea in the herbarium of the Royal Botanic Gardens, Kew; and the Berkeley and Broome types in the herbarium of the British Museum of Natural History, South Kensington. This paper provides a complete list of species and their synonyms, together with collections from this region available for study in these herbaria.

I am grateful to Sir Edward Salisbury, F.R.S., Director of the Royal Botanic Gardens, and Miss E. M. Wakefield, Deputy Keeper of the herbarium, for allowing me to examine the Kew collections; to Dr. J. Ramsbottom, Keeper of the herbarium of the British Museum of Natural History, and Mrs. Balfour Brown, who kindly made available for study specimens in the herbarium of that institution.

Valid species are set in capitals, synonyms in italics and incidental species, misdeterminations, etc., in capitals and lower case. Literature references were checked in the library at Kew, so that these are accurate both for types and synonyms. I have added the Australian States to locality references, since these were seldom inserted on the sheets at Kew.

1. ABIETINA, DAEDALEA Fr., Syst. Myc., Vol. 1, p. 334, 1821.

abietina, Lenzites Fr., Epicrisis, p. 407, 1838.

The following collections from Australia are under the cover of *Lenzites abietina* at Kew: "Rockhampton, Q., Mrs. Thozet", "Port Denison, Q., Shann", and "Australia, S.12, S.65".

acervatus, Polyporus Lloyd = *Polyporus grammocephalus.*

2. ACUPUNCTATA, COLTRICIA (Berk.), nov. comb.

latus, Polyporus Berk., Ann. Nat. Hist., Vol. 3, p. 325, 1839, type ex Van Diemen's Land, Gunn.; non Fries, Syst., p. 384, 1821.

acupunctata, Trametes Berk., Jour. Linn. Soc., Vol. 13, p. 164, 1873, type ex Lord Howe Island, J. P. Fallagher.

aratus, Polyporus Berk., Jour. Linn. Soc., Vol. 16, p. 53, 1878, type was based on the same collection as *T. acupunctata.*

Other collections at Kew are: "Tasmania", "Endeavour River, Q., Persietz", "Daintree River, Q., Pentzcke, No. 143", all being filed under *P. latus*; "Brisbane, Q., Bailey", which is under the cover of *P. aratus.*

acuta, Trametes Cke. = *Trametes floccosa.*

adami, Polystictus Cke. = ? *Polyporus obovatus.*

adusta, Lenzites Lloyd = *Daedalea subferruginea.*

3. ADUSTUS, POLYPORUS Fr., Syst. Myc., Vol. 1, p. 363, 1821.

demissus, Polyporus Berk., Lond. Jour. Bot., Vol. 4, p. 52, 1845, type ex Swan River, W. Aus., Drummond, No. 50.

ochraceo-stuppeus, Polystictus Lloyd, Letter 63, p. 11, 1916, type ex Petersham, N.S.W., E. Cheel.

Additional collections at Kew are: "Narranoom, Vic., Stranger", filed under *P. adustus*; "Daintree River, Q., Pentzcke", placed by Cooke under *Polyporus pallescens* Fr.

aequus, Polystictus Lloyd = *Coriolus versicolor.*

albertinii, Polyporus Lloyd = *Coltricia schweinitzii.*

albida, Hexagona Berk. = *Hexagona vespacea.*

4. ALBIDUS, POLYPORUS (Trog.) Fr., Epicrisis, p. 475, 1838.

Under the cover of *Polyporus fragilis* is a specimen ex "Richmond River, N.S.W., Camara" which on the sheet was referred by Cooke to *P. destructor.* It is of *P. albidus* in the sense that this species is now interpreted in northern Europe.

albo-fuscus, Polyporus Lloyd = *Polyporus portentosus.*

albo-niger, Polyporus Lloyd = *Polyporus atro-maculus.*

5. ALBO-VESTIDUS, CORIOLUS (Lloyd), nov. comb.

albo-vestidus, Polystictus Lloyd, Myc. Notes, No. 69, p. 1192, 1923, type ex Adelaide, S. Aus., J. B. Cleland, No. 752.

6. ALVEOLARIS, HEXAGONA (Fr.) Murr., Bull. Torrey Bot. Club, Vol. 31, p. 327, 1904.

alveolaris, Cantharellus Fr., Syst. Myc., Vol. 1, p. 322, 1821.

ohiensis, Favolus Berk. & Mont., in Mont. Syll., p. 171, 1856, type ex Ohio, U.S.A.

hispidulus, Favolus Berk. & Curt., Jour. Linn. Soc., Vol. 10, p. 321, 1865, type ex Cuba.

Under *Favolus hispidulus* is filed one specimen ex "Australia" which matches North American collections of *H. alveolaris*; a second, under the same cover, ex "N.Z., Colenso", is a fragment of *Polyporus colensoi.* Under *Favolus ohiensis* is filed one collection ex "Endeavour River, Q., Persieh, No. 202".

alveolaris, Polyporus (Bosc) Fr. Cooke referred *P. collybioides*, type ex "Richmond River, N.S.W.", to this species. It does not agree with other collections in the cover at Kew, but is a poorly preserved specimen of *Polyporus arcularius.*

7. AMBIGUUS, CORIOLUS (Berk.), nov. comb.

ambigua, Daedalea Berk., Lond. Jour. Bot., Vol. 4, p. 305, 1845, type ex Ohio.

lactea, Trametes Fr., Nov. Symb., p. 96, 1851, type ex Europe.

aesculi, Daedalea (Fr.) Murr., N. Am. Fl., Vol. 9, p. 126, 1908.

Specimens at Kew are "Brisbane, Q.", labelled *Daedalea aesculi* by Lloyd; and "Clarence River, N.S.W., Wilcox". Both are filed under *Trametes ambigua* Fr., which is a different species.

amboinensis, Polyporus Fr. Two specimens so named from Australia are of *Ganoderma lucidum.*

angustus, Polyporus Berk. = *Polyporus tephronotus.*

8. ANNOSA, FOMITOPSIS (Fr.) Karst., Rev. Myc., Vol. 3, p. 18, 1886.
annosus, Polyporus Fr., Syst. Myc., Vol. 1, p. 373, 1821.

A specimen ex "Richmond River, N.S.W." so referred by Cooke is resupinate and cannot be identified with certainty. It possesses the same microstructure as *F. annosa.*

anthracophilus, Polyporus Cke. = *Polyporus campylus.*

applanata, Daedalea Kl. = *Lenzites palisoti.*

9. APPLANATUS, FOMES (Pers. ex Wallr.) Gill., Champ., p. 686, 1878.
applanatus, Polyporus (Pers.) Wallr., Fl. Krypt. Germ., Vol. 4, p. 591, 1833.
incrassatus, Polyporus Berk., Jour. Linn. Soc., Vol. 16, p. 41, 1878, type ex Cape York Peninsula, Q., Challenger Expedition.
scansilis, Polyporus Berk., Jour. Linn. Soc., Vol. 16, p. 53, 1878, type ex Juan Fernandez Islands, Challenger Expedition.

Other collections at Kew are: "Upper Hunter River, N.S.W., Miss Carter" and "Endeavour River, Q., Persieh", both filed under *Fomes marginatus.* The types of *Polyporus incrassatus* and *P. scansilis* possess the same type of cuticle, context and spores, so are obviously synonyms. One other collection, filed under *P. incrassatus,* is ex "New Guinea, Capt. Armit".

aprica, Polyporus Berk., *Fl. Tas.*, Vol. 2, p. 254, 1860. No specimen is at Kew, so that the species cannot now be identified.

arata, Hexagona Berk. = *Inonotus setiporus.*

aratus, Polyporus Berk. = *Coltricia acupunctata.*

archeri, Polyporus Berk. = *Polyporus merulius.*

10. ARCULARIUS, POLYPORUS Fr., Syst. Myc., Vol. 1, p. 342, 1821.
similis, Polyporus Berk., Lond. Jour. Bot., Vol. 2, p. 635, 1843, type ex Brazil.
squamiger, Favolus Berk., Jour. Linn. Soc., Vol. 13, p. 166, 1873, type ex New England, N.S.W.
armitii, Polyporus Muell. & Kalch., ex Cke., Grev., Vol. 10, p. 94, 1882, type ex Dunrobin, Q.
collybioides, Polyporus Kalch., ex Cke., Grev., Vol. 10, p. 94, 1882, type ex Richmond River, N.S.W.

The following Australian collections are at Kew: "Brisbane, Q., F. M. Bailey, No. 708", "Botanic Gardens, Sydney, N.S.W., E. Cheel", and "Illawarra, N.S.W., Kirton, No. 145". Filed under the cover of *P. lentus* are "Guntawang, N.S.W., Hamilton, No. 6" and "Govt. Domain, near Melbourne, F.v.M."; under *P. similis* "Endeavour River, Q., Persieh" and "Goode Island, Torres Strait, Powell"; and under *Favolus squamiger* are "Australia" and "New England, N.S.W.".

argentatus, Polyporus Cke. = *Coriolus paleaceus.*

armitii, Polyporus Muell. & Kalch. = *Polyporus arcularius.*

ascoboloides, Polyporus Berk., *Jour. Linn. Soc.*, Vol. 13, p. 162, 1873. The type ex "Australia" is an unidentifiable mycelial fragment growing upon debris from the forest floor.

11. ASPERA, LENZITES (Kl.) Fr., Epicrisis, p. 405, 1838.
aspera, Daedalea Kl., Linnaea, Vol. 8, p. 480, 1833.
nivea, Lenzites Cke., Grev., Vol. 15, p. 94, 1887, type ex Russell River, Q., Sayer, No. 50.

The following collections at Kew agree with the type ex Mauritius—"Brisbane, F. M. Bailey, No. 165", "Daintree River, Q., Pentzcke", "Dunk Island, Q., W. Cottrell-Dormer, No. 31", "Richmond River, N.S.W., Camara, White", and "New Guinea, Jola River, W. E. Armit"; filed under *L. nivea* is "Bellenden Ker, Q., No. 835"; and placed by Massee under *Daedalea subsulcata* is "Sunday Island, Kermadecs, W. R. B. Oliver".

atro-hispidus, Polyporus Lloyd = *Polyporus pelliculosus.*

12. ATRO-MACULUS, POLYPORUS Lloyd, Myc. Notes, No. 67, p. 1162, 1922 (name only); Stevenson and Cash, Bull. Lloyd Library, No. 35, p. 98, 1936.
albo-niger, Polyporus Lloyd, in herb. Kew.

Part of the type ex "L. Rodway, Tasmania" is at Kew; as is also a second specimen labelled *P. albo-niger* Lloyd ex "Hobart, Tasmania, L. Rodway". In *Mycological Notes,* p. 1162, Lloyd recorded the species but did not publish a description. He filed one

with the specimens in his herbarium and after Lloyd's death this was published by Stevenson and Cash.

13. ATRO-VINOSA, PORIA Cke., Grev., Vol. 14, p. 110, 1886.

atro-vinosus, Polyporus Cke., Grev., Vol. 10, p. 131, 1882.

The type ex "Clarence River, N.S.W., Wilcox" and a second collection ex "Mt. Napier, Vic." are the only specimens at Kew.

atypus, Polyporus Lev. Two specimens ex "Strickland River, New Guinea, Armit", filed under this cover by Cooke, were referred by Lloyd to *Polyporus brunneolus* Berk. They are of *Coriolus paleaceus.*

aulacophylla, Daedalea Berk. = *Lenzites tenuis.*

aureus, Merulius Fr. Cooke (*Hdbk.*, p. 168, 1892) recorded the species from Queensland. No specimens are at Kew from this region.

14. AUSTRALIENSIS, POLYPORUS Wakef., Kew Bull. Misc. Inf., p. 157, 1914.

The following collections from Australia are at Kew: Type ex "Coomera River, Q., C. T. White"; "Victoria, F. Campbell", named *P. retiporus* by Cooke; "Geographe Bay, W. Aus.", referred to *P. stypticus* by Cooke; "Toowoomba, Q." and "Grampian Mts., Vic., Sullivan", placed under *P. portentosus* by Cooke; "Johnstone River, Q.", filed by Cooke under *P. nidulans* Fr.

15. AUSTRALIS, FOMES (Fr.) Cke., Grev., Vol. 14, p. 18, 1885.

australis, Polyporus Fr., Elench., p. 108, 1828.

brownii, Elfvingia Murr., Western Polypores, p. 39, 1915.

The type, which was described from a specimen collected on a tree trunk on a Pacific Ocean island, no longer exists. At Kew, however, is a plant labelled *P. australis* in Fries' handwriting, which may be regarded as the neotype. It is imperfect in that the cuticle is wanting; but in microstructure, size of spores and pores, etc., matches numerous collections from this region. As the spores are the largest known of the ganoderma type, the species may be identified readily by this feature alone. A co-type specimen of *Elfvingia brownii* at Kew, ex "California, Brown", is of the same species.

awhitu, Fomes G. H. Cunn. = *Fomes endapalus.*

16. AZUREUS, CORIOLUS (Fr.) G. H. Cunn., Plant Diseases Division Bull, 75. p. 7, 1948.

azureus, Polystictus Fr., Nov. Symb., p. 93, 1851, type ex Mirador, Mexico, Dr. Liebman.

The species is common in New Zealand, collections from this region matching part of the type at Kew. One collection from Australia is at Kew, ex "Zoological Gardens, Perth, W. Aus., W. N. Cheesman", filed under *Polystictus versicolor.*

badio-lutescens, Polyporus Kalch. = *Coriolus occidentalis.*

baileyi, Merulius Berk. & Br., Trans. Linn. Soc., Ser. II, Vol. 2, p. 62, 1883.

The type, ex "Brisbane, Q., F. M. Bailey, No. 171", is an agaric, which Bresadola, on the type sheet, noted as being "Paxilli sp.—Pax. pannoidi Fr. valde affinis". On the sheet the specific name is spelled "baileie", and in Saccardo's Sylloge Fungorum, Vol. 6, p. 414, 1888, was published as "baylei".

17. BECKLERI, CORIOLUS (Berk.), nov. comb.

beckleri, Polyporus Berk., Jour. Linn. Soc., Vol. 13, p. 162, 1873.

Only the type collection is at Kew, ex "Clarence River, N.S.W., Dr. Beckler, No. 16". On the type sheet it had also been labelled *P. scobinaceus* Berk.

berkeleyi, Lenzites Lev. = *Lenzites betulina.*

18. BECKLERI, LENZITES Berk., Jour. Linn. Soc., Vol. 13, p. 161, 1873.

torrida, Lenzites Kalch., ex Cke., Grev., Vol. 8, p. 154, 1880, type ex Richmond River, N.S.W.

guilfoylei, Lenzites Berk., ex Cke., Grev., Vol. 10, p. 64, 1881, type ex Tweed River, N.S.W., Guilfoyle.

Other Australian collections at Kew are: the type ex "Clarence River, N.S.W., Dr. Beckler, No. 10" and "Trinity Bay, Q., M. Sayer, No. 36"; under *L. faventina* Cald. Cooke filed "Brisbane, Q., No. 559" and "Brisbane, Q., No. 202"; "Goode Island, Torres Strait" was misnamed *Daedalea subsulcata* B. & Br. by Cooke. No specimen of *Lenzites torrida* is at Kew; but the illustration in Grevillea, Vol. 10, Pl. 144, f. 21, shows it to have been based on *L. beckleri.*

19. BERKELEYI, POLYPORUS Fr., Nov. Symb., p. 56, 1851.

retiporus, Polyporus Cke., Grev., Vol. 12, p. 15, 1883, type ex Daintree River, Q., T. Pentzcke.

zelandicus, Polyporus Cke., Grev., Vol. 16, p. 113, 1888, type ex New Zealand, T. Kirk, No. 309.

One other specimen is at Kew, ex "Illawarra, N.S.W., Camara", which was by Cooke referred to *P. retiporus*.

20. BETULINA, LENZITES Fr., Epicrisis, p. 405, 1838.

betulina, Daedalea Fr., Syst. Myc., Vol. 1, p. 333, 1821.

flaccida, Lenzites Fr., Epicrisis, p. 406, 1838.

berkeleyi, Lenzites Lev., Ann. Sci. Nat., Ser. III, Vol. 5, p. 122, 1846.

Collections at Kew are "Western Point, Vic.", "Richmond River, N.S.W., Camara, No. 89" and "Sugar Loaf Mt., N.S.W., F.v.M.", the two last being filed under *L. flaccida*, which is merely a thin form of the species. In Handbook, p. 101, 1892, Cooke recorded the species also from Queensland, but there are no specimens at Kew from that State.

betulinus, Polyporus Fr. A specimen so named by Cooke, ex "Toowoomba, Q.", is of *Polyporus portentosus*.

bicolor, Hexagona McAlp. = *Hexagona gunnii*.

biennis, Polyporus Fr. Cooke (*Hdbk.*, p. 114, 1892) recorded the species from Queensland. There is no specimen at Kew from this region.

bifasciata, Lenzites Cke. & Mass. = *Daedalea subferruginea*.

biformis, Polyporus Fr. A specimen so labelled by Cooke, ex "Near Melbourne, Vic., F. Reader", is of *P. elongatus*.

21. BINOMINATUS, MERULIUS Mass., Kew Bull. Misc. Inf., p. 104, 1913. The only specimen at Kew is the type ex "Brisbane, Q., F. M. Bailey, Nov., 1912".

bireflexus, Polyporus Berk. & Br. = *Coriolus zonatus*.

biretum, Polyporus Kalch. = *Coltricia fruticum*.

bistratosus, Polyporus Berk. & Cke. The species, a *Fuscoporia*, type ex "Paricatuba", is not in this region, Australian records being based on such species as *Poria vincta* (= *P. radula*), *Poria medulla-panis* and *Poria calcicola*.

blepharistoma, Poria (Berk. & Br.) Cke. The only Australian collection under this cover at Kew, ex "Toowoomba, Q.", is an immature specimen of *Poria mucida*.

22. BLUMEI, CORIOLUS (Lev.), nov. comb.

blumei, Polyporus Lev., Ann. Sci. Nat., Ser. III, Vol. 2, p. 185, 1844, type ex Java.

gallopavonis, Polyporus Berk. & Br., Trans. Linn. Soc., Ser. II, Vol. 2, p. 59, 1883, type ex Brisbane, Q., Bailey.

sub-zonalis, Polyporus Cke., Grev., Vol. 19, p. 44, 1890, type ex Daintree River, Q., Pentzcke.

Other collections at Kew are "Christmas Island, Kermadecs, H. W. Rietz", named *P. sub-zonalis* by Cooke; "Endeavour River, Q., Persietz", filed under *Polyporus versicutus* Berk. & Curt. There is also a specimen ex "Samoa, C. G. Lloyd" which is filed under *P. gallopavonis*.

23. BOREALIS, POLYPORUS Fr., Syst. Myc., Vol. 1, p. 366, 1821.

Two collections are at Kew from Australia: "Loddon River, Vic., Wallace" and "Melbourne, Vic., Reader".

24. BOWMANI, CORIOLUS (Berk.), nov. comb.

bowmani, Daedalea Berk., Jour. Linn. Soc., Vol. 13, p. 166, 1873.

The only specimen at Kew is the type ex "Herbert's Creek, Q., E. M. Bowman". It is white, almost resupinate, but not daedaloid. Cooke (*Hdbk.*, p. 163, 1892) misspelled the name *D. bowmanni*.

breviporus, Polyporus Cke. = *Fomes scruposus*.

brisbanensis, Polyporus Berk. & Br. A herbarium name applied to specimens ex "Brisbane, Q., Nos. 136, 138", filed under *Polyporus havannensis* Berk. & Curt. They are of *Fomitopsis ochroleuca*.

brownii, Elfvingia Murr. = *Fomes australis*.

broomei, Polyporus Rabh. = *Poria undata*.

brumalis, Polyporus Fr. Though recorded by Cooke (*Hdbk.*, p. 112, 1892) from Queensland, no specimens from this region are at Kew. Obviously the record was based on specimens of *P. arcularius*.

brunneo-albus, Polyporus Fr. Cooke (*Hdbk.*, p. 148, 1892) referred *P. brunneo-leucus* as a synonym of this species. The former name cannot be used, since it was erected by Fries (*Nov. Symb.*, p. 94, 1851) without a diagnosis or reference to a type, and was published after *P. brunneo-leucus*.

25. BRUNNEO-LEUCA, COLTRICIA (Berk.), nov. comb.

brunneo-leucus, Polyporus Berk., Lond. Jour. Bot., Vol. 5, p. 4, 1846.
corrivalis, Polyporus Berk., Jour. Linn. Soc., Vol. 13, p. 162, 1873, type ex Adelaide, S. Aus., Dr. Schomburgk, No. 47.
illudens, Daedalea Cke. & Mass., Grev., Vol. 21, p. 37, 1892, type ex Kurrumburra, Vic., Mrs. Martin, Nos. 1066, 1026, 1037.

Types of the synonyms listed match the type of *P. brunneo-leucus* ex "Van Diemen's Land, R. Gunn, Esq.". No other specimens are at Kew from this region.

brunneolus, Polyporus Berk. One specimen so referred by Cooke, ex "Daintree River, Q., T. Pentzcke", is too imperfect for identification, being partly destroyed by insects. No other collection from this region is at Kew.

bulbipes, Polyporus Fr. Specimens of the type of *P. cladonia* (= *Coltricia oblectans*) were referred by Cooke to *P. bulbipes*, but do not resemble others under that cover.

byrsinus, Polyporus Mont. Cooke (*Hdbk.*, p. 150, 1892) recorded the species from Queensland, but there are no specimens at Kew from this region. His record was probably based on a specimen of *C. occidentalis*.

26. CALCICOLA, PORIA Sacc. & Syd., Syll. Fung., Vol. 14, p. 192, 1899.

calceus, Polyporus Berk. & Br., Jour. Linn. Soc., Vol. 14, p. 55, 1875, non Schw., 1834.

Though several collections from Australia are at Kew, none is filed under this cover. A specimen ex "New Guinea, Strickland River, Bauerlen" is filed under *Fomes bistratosus*; "Richmond River, N.S.W." is under *Poria calcea*; "Brisbane, Q., ex herb. Broome" is under *Poria medulla-panis*; "Daintree River, Q., Pentzcke" is in the cover of *Poria fatiscens*; "Milsom Island, Hawkesbury River, N.S.W., J. B. Cleland, No. 17" is under *Poria selecta*; "Clarence River, N.S.W., Wilcox" and "Toowoomba, Q., Hartmann" are filed under *Poria victoriae*.

callosa, Poria (Fr.) Cke. A collection ex "Endeavour River, Q., Persieh" was filed under this species by Cooke but does not agree with other specimens in this cover at Kew.

27. CAMPYLUS, POLYPORUS Berk., Fl. Tas., Vol. 2, p. 252, 1860.

gunnii, Polyporus Berk., Fl. Tas., Vol. 2, p. 253, 1860, type ex Back River Gully, Tasmania.
anthracophilus, Polyporus Cke., Grev., Vol. 12, p. 16, 1883, type ex Rockhampton, Q., Mrs. Thozet.
laurencii, Polyporus Berk., in herb. Kew.
rosettus, Polyporus Lloyd, Myc. Notes, No. 43, p. 601, 1916, type ex Australia, W. A. Scarfe.
wilsonianus, Polyporus Lloyd, Myc. Notes, No. 55, p. 787, 1918, type ex Victoria, Jas. Wilson.

Types of the synonyms match the type of *P. campylus*, ex "Tasmania, Archer". Other collections at Kew are "North Gippsland, Vic., Dr. Tisdale", referred to *P. gunnii*; "Port Phillip, Vic.", "Daylesford, Wallace", "Gippsland, Vic., Murray", "Melbourne, Vic., F. Reader", "S.W. Australia, T. Muir" and "Australia", filed under *P. anthracophilus*; under *P. tephronotus* is "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition"; and under *P. pelliculosus* is "Gippsland, Vic., Webb".

caperatus, Polyporus Berk. Two specimens so referred by Cooke, ex "Richmond River, N.S.W., Camara", are of *Coriolus corrugatus*. Under the cover of *P. lanatus* is a plant ex "Port Denison, Q., Shann" which Lars Romell on the sheet referred to *P. caperatus*. It is a specimen of *C. occidentalis*.

carneo-fulvus, Fomes (Berk.) Cke. Two collections placed under this cover by Cooke, ex "Tropical Queensland, Bailey" and "Bellenden Ker, Q." are of *Fomes conchatus* or *F. gilvus*.

28. CARNEO-LUTEA, PORIA Rodw. & Clel., Papers and Proc. Roy. Soc. Tas. for 1929, p. 18, 1929.

Represented at Kew by a co-type specimen ex "Bullahdelah, N.S.W., J. B. Cleland, No. 10".

29. CARNEO-NIGER, CORIOLUS (Berk.), nov. comb.

carneo-niger, Polyporus Berk. ex Cke., Grev., Vol. 12, p. 15, 1883.

Three specimens are mounted on the type sheet at Kew, ex "Daintree River, Q., Pentzcke". One is typical and may be regarded as the holotype.

carneus, Polyporus Nees. Australian collections at Kew filed under this cover are "Melbourne, Vic., G. LeFevre", "Narranoom, Vic., Stranger", and "Brisbane, Q.". These are collections of *Trametes lilacino-gilva.* A specimen ex "Endeavour River, Q., Persieh" filed under this cover = *Coriolus paleaceus*; and a collection ex "Bot. Gardens, Sydney, N.S.W., A. Grant" is of *Fomes gilvus.*

carteri, Trametes Berk., ex Sacc. & Trott., *Syll. Fung.*, Vol. 9, p. 196, 1891. The type ex "Bombay, H. J. Carter" and a collection ex "National Park, Adelaide, S. Aus., W. N. Cheesman, No. 3" are of *Trametes protea.*

cartilagineus, Polyporus Berk. & Br. = *Fomes durus.*

30. CARYOPHYLLACEUS, FOMES (Berk. & Curt.) Cke., Grev., Vol. 15, p. 22, 1886.

caryophyllaceus, Polystictus Berk. & Curt., ex Cke., Grev., Vol. 14, p. 78, 1886.

One collection from Australia, ex "Dunk Island, Q., W. Cottrell-Dormer, No. 38" matches the type, ex Venezuela.

chilensis, Polyporus Fr. Recorded by Cooke, as *Fomes chilensis* (*Hdbk.*, p. 130, 1892) from Queensland. The only specimen from Australia in this cover at Kew, ex "Australia, F.v.M.", is of *Fomes mastoporus.*

chioneus, Polyporus Fr. Though not uncommon in New Zealand, there are no collections at Kew from Australia. A collection filed by Cooke under this cover, ex "Richmond River, N.S.W., Camara" = *Polyporus lacteus*; those ex "Melbourne, Vic., Reader" and "Trinity Bay, Q., Sayer, No. 7" = *Fomitopsis ochroleuca.*

chlorina, Poria Mass. = *Poria versipora.*

chrysoleucus, Polyporus Kalch. = *Trametes protea.*

cichoraceus, Polyporus Berk. = *Inonotus setiporus.*

cinereo-fuscus, Fomes Currey. Though recorded by Cooke (*Hdbk.*, p. 136, 1892) from Queensland, there is no specimen at Kew from this region.

31. CINGULATUS, CORIOLUS (Fr.), nov. comb.

cingulatus, Polyporus Fr., Linnaea, Vol. 5, p. 518, 1830.

The following collections are at Kew: "Lower Ardur River, Gulf of Carpentaria, Q.", "Dunk Island, Q., W. Cottrell-Dormer, No. 2", "Daintree River, Q., Pentzcke" and "Endeavour River, Q., Persieh". The last two were filed by Cooke under *P. cubensis.*

cinnabarinus, Polyporus Fr. = *Coriolus sanguineus.*

cinnamomeo-squamulosus, Polyporus P. Henn. = *Polyporus russiceps.*

32. CITREUS, POLYPORUS Berk., Jour. Linn. Soc., Vol. 13, p. 162, 1873.

Only the type is at Kew, ex "Clarence River, N.S.W., Dr. Beckler, No. 3".

cladonia, Polyporus Berk. = *Coltricia oblectans.*

33. CLELANDII, FOMES Lloyd, Letter 60, p. 11, 1915; Myc. Notes, No. 40, p. 550, 1916.

The species is represented at Kew by part of the type ex "Tupperak, N.S.W., J. B. Cleland, No. 52".

clelandii, Lenzites Lloyd = *Daedalea trabea.*

cognatus, Polyporus Kalch. in herb. Kew. Referred by Cooke (*Hdbk.*, p. 140, 1892) as a synonym of *Polystictus stereinus*, which is, in turn, a synonym of *Polyporus liebmannii.*

34. COLENSOI, POLYPORUS Berk., Fl. N.Z., Vol. 2, p. 178, 1855.

multiplex, Polyporus Berk., in herb. Kew.

Australian collections at Kew are: "Victoria" and "Australia, Mueller teste Massee" (a note on the sheet to this effect by Lloyd), the latter being labelled *P. multiplex* Berk. It is merely a pallid form with identical microstructure.

colliculosa, Trametes Berk. A specimen from Australia under this cover at Kew ex "Port Denison, Q., Shann" is not the same as other collections included therein, the pores being much too small. Unfortunately I was unable to identify the plant.

collybioides, Polyporus Kalch. = *Polyporus arcularius.*

compressus, Polyporus Berk. = *Fomitopsis ochroleuca.*

35. CONCAVA, FOMITOPSIS (Cke.), nov. comb.

concavus, Fomes Cke., Grev., Vol. 19, p. 44, 1890.

Two collections are at Kew, the type ex "Johnstone River, Q., Berthoud" and "Brisbane, Q., F. M. Bailey". On the type sheet Bresadola referred specimens to *P. ferreus* Berk. and *P. dochmius* Berk. The latter is a synonym of *Trametes ferrea*, which is distinct from *Fomitopsis concava.*

36. CONCHATUS, FOMES (Pers. ex Fr.) Cke., Grev., Vol. 14, p. 18, 1885.

conchatus, Polyporus (Pers.) Fr., Syst. Myc., Vol. 1, p. 376, 1821.

One collection ex "Endeavour River, Q., Persieh" is filed under this cover at Kew. A second, probably of this species, ex "Broger's Creek, N.S.W., Bauerlen", was filed by Cooke under *P. murinus.*

conchoides, Polyporus (Mont.) Lloyd = *Polyporus thelephoroides.*

confluens, Polyporus Fr. One collection referred to this species by Cooke, ex "Lord Howe Island, Camara" I was unable to identify; a second ex "Port Denison, Q., Shann" is of *Polyporus fusco-lineatus.*

37. CONNATA, FOMITOPSIS (Fr.), nov. comb.

connatus, Polyporus Fr., Elench., p. 92, 1828.

One Australian collection at Kew, ex "Moreton Bay, Q., F. M. Bailey", agrees with European specimens in the herbarium.

contigua, Fuscoporia (Pers. ex Fr.) G. H. Cunn. Under the cover of *Poria contigua* is part of a specimen ex "Swan River, W. Aus., Drummond" labelled on the sheet *Poria ferrugineus*; the other portion is filed under *Poria ferruginosa.* The plant is *Fuscoporia punctata.*

contrarius, Fomes (Berk. & Curt.) Cke. The species was recorded from Queensland by Cooke (*Hdbk.*, p. 132, 1892), but there are no specimens from this region at Kew.

cookei, Hexagona Sacc. = *Hexagona vespacea.*

cookei, Trametes Sacc. = *Trametes floccosa.*

38. CORIUM, MERULIUS Fr., Elench., p. 58, 1828

pelliculosus, Merulius Cke., Grev., Vol. 19, p. 109, 1881, type ex Victoria, Mrs. Martin, No. 762.

eriophorus, Polyporus Berk. & Br., Trans. Linn. Soc., Ser. II, Vol. 2, p. 60, 1883, type ex Brisbane, Q., F. M. Bailey, No. 419.

Other Australian collections at Kew are "Adelaide, S. Aus., W. N. Cheesman", "Tasmania" (seven specimens named by Berkeley) and "Swan River, W. Aus., Nos. 249, 253".

corrivalis, Polyporus Berk. = *Coltricia brunneo-leuca.*

39. CORRUGATUS, CORIOLUS (Pers.), nov. comb.

corrugatus, Polyporus Pers., in Gaud. Voy. Freyc. Bot., p. 172, 1826.

sanguinea, Daedalea Kl., Linnaea, Vol. 8, p. 481, 1833.

persoonii, Polystictus Cke., Grev., Vol. 14, p. 85, 1886.

The following collections are at Kew filed under the cover of *Polystictus persoonii*: "Goode Island", "Upper Daintree River, Q., Harris, No. 35", "Tropical Queensland, Bailey", "Trinity Bay, Q., Sayer", "Brisbane, Q., ex herb. Broome", "New Guinea, Armit", "Tweed River, N.S.W., Guilfoyle" and "Gippsland, Victoria". A ninth collection ex "Richmond River, N.S.W., Camara" is filed under *P. caperatus.* In the cover some sheets are labelled *Daedalea sanguinea*, others *Polyporus corrugatus*, both synonyms of *C. corrugatus.*

corticola, Poria (Fr.) Cke. Under this cover are two collections. One, ex "Australia", is a resupinate fragment of *Trametes protea*; the other, ex "N.Z., Colenso, No. 923", is of *Polyporus merulius.*

cretaceus, Polyporus Lloyd = *Polyporus portentosus.*

crinigera, Hexagona Fr. Though recorded by Cooke (*Hdbk.*, p. 164, 1892) from Queensland, there are no specimens from this region at Kew or the British Museum of Natural History.

40. CRISTATA, TRAMETES Cke., Grev., Vol. 10, p. 132, 1882.

Australian collections at Kew are the type ex "Port Denison, Q., Shann, No. 26", "Russell River, Q., W. A. Sayer, No. 47", "Brisbane, Q., ex herb. Broome, No. 190" and "Clarence River, N.S.W., Wilcox". The last two were filed by Cooke under *Trametes curreyi*.

cryptarum, Fomes (Fr.) Cke. The two collections from Australia under this cover are of different species. "Brisbane, Q., No. 978" is a contorted specimen of *Fomitopsis scutellata*; "Victoria, No. 1014" is a *Poria*, probably *P. vaporaria*.

cubensis, Polyporus Mont. Two collections so named by Cooke, ex "Daintree River, Q., Pentzcke" and "Endeavour River, Q., Persieh" are of *Coriolus cingulatus*.

cupreo-nitens, Polyporus Kalch. = *Coriolus xanthopus*.

cupreo-roseus, Polyporus Berk. One specimen from Australia filed under this cover = *Trametes lilacino-gilva*.

cupuliformis, Polyporus Berk. & Curt. = *Polyporus pusillus*.

curreyi, Polyporus Berk. = *Fomes strigatus*.

curreyi, Trametes Cke. Of the Australian collections filed under this cover two, "Clarence River, N.S.W., Wilcox" and "Brisbane, Q., ex herb. Broome, No. 190", are of *Trametes cristata*; one, ex "Upper Hunter River, Miss Carter", is of *T. drummondii*; and one, ex "Adelaide, W. N. Cheesman", is either *T. drummondii* or *T. protea*, probably the latter.

cyclodes, Polyporus Fr. A specimen ex "Australia", labelled by Berkeley *Trametes occidentalis* (to which species it was correctly referred) was filed by Cooke under *P. cyclodes*.

daedalioides, Trametes Berk., *Ann. Nat. Hist.*, Vol. 3, p. 325, 1839.

tasmanica, Daedalea Sacc., Syll. Fung., Vol. 6, p. 384, 1886.

The type, ex "Van Diemen's Land, Messrs. Gunn and Laurence, ex herb. Hooker", is a resupinate specimen of some *Trametes*. Saccardo arbitrarily changed the name to *D. tasmanica* on Cooke's statement (Grev., Vol. 15, p. 54, 1886) that the species was a *Daedalea*. It is poroid and a *Trametes* as the genus is now defined.

decipiens, Hexagona Berk. = *Trametes drummondii*.

demissus, Polyporus Berk. = *Polyporus adustus*.

deplanata, Daedalea Fr. = *Lenzites palisoti*.

destructor, Polyporus Fr. A specimen ex "Richmond River, N.S.W., Camara", filed under the cover of *P. fragilis*, was on the sheet referred by Cooke to *P. destructor*. It is the plant which is now known in northern Europe as *P. albidus*.

41. DEVEXA, TRAMETES Berk., Jour. Linn. Soc., Vol. 13, p. 165, 1873.

On the sheet of the type ex "Tweed River, N.S.W., Dr. Guilfoyle" Bresadola had written: "= *Polystictus occidentalis* Kl., forma obesa, juvenilis, hymenio nondum evoluto". It is a distinct species, nevertheless. Other Australian collections at Kew are: "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition", "Toowoomba, Q., Hartmann" and "North Gippsland, Vic., H. Tisdall". The last is filed under *Trametes heteromalla*, and on the sheet Lars Romell had noted "= *T. devexa* Berk.".

42. DICHROUS, POLYPORUS Fr., Syst. Myc., Vol. 1, p. 364, 1821.

Australian specimens at Kew are: "Enoggera, near Brisbane, Q., W. N. Cheesman" and "Macedon, Vic., Mrs. Martin, No. 435". Others from this region under the cover of *P. dichrous* are of *P. thelephoroides*.

43. DICTYOPORA, PORIA Cke., Grev., Vol. 14, p. 114, 1886.

dictyoporus, Polyporus Cke., Grev., Vol. 12, p. 17, 1883.

The type ex "Toowoomba, Q., Hartmann" is the only authentic collection under this cover at Kew.

44. DICTYOPUS, POLYPORUS Mont., Ann. Sci. Nat., Ser. II, Vol. 3, p. 349, 1835.

Though the species is abundant in New Zealand there are no collections at Kew

from Australia. A specimen so named by Berkeley, filed under this cover, ex "Parramatta, Challenger Expedition", is of *P. melanopus.*

dielsii, Polyporus P. Henn. = *Polyporus portentosus.*

diminutus, Polyporus Mass. = *Polyporus rhipidium.*

discolor, Hexagona Fr., *Nov. Symb.*, p. 102, 1851. Named by Fries from Western Australia (as *Favolus discolor* Fr., *Pl. Preiss*, Vol. 2, p. 136, 1847). No type specimen is known, and as the description is inadequate the name should be deleted.

dispar, Polyporus Kalch. ex Cke., *Grev.*, Vol. 10, p. 101, 1882. The type was from "Victoria". No specimens are at Kew or the British Museum of Natural History.

dochmius, Polyporus Berk. & Br. = *Trametes ferrea.*

45. DORCADIDEA, HEXAGONA (Berk. & Br.), nov. comb.

dorcadideus, Polyporus Berk. & Br., Trans. Linn. Soc., Ser. II, Vol. 2, p. 57, 1883.

The type, ex "Enoggera Creek, near Brisbane, Q., No. 374, herb. Broome, No. 155", is in the British Museum of Natural History. No specimens are at Kew.

46. DRUMMONDII, TRAMETES, nov. nom.

decipiens, Hexagona Berk., Lond. Jour. Bot., Vol. 4, p. 57, 1845.

Type is ex "Swan River, W. Aus., Drummond, Nos. 151, 152". Other collections at Kew are "Lower Ardur River, N.Q.", "Moruya, N.S.W., W. N. Cheesman", "Snowy River, Bauerlen, No. 230", "Melbourne, LeFevre, Nos. 202, 204, 209", "Dimboola, Vic., No. 65", "Murray River, Vic., C. French" and "Upper Hunter River, N.S.W., Miss Carter". The last was filed by Cooke under *Trametes curreyi.*

Though described as a *Hexagona*, the species is a *Trametes.* As the combination *T. decipiens* is preoccupied (Bres., *Ann. Myc.*, Vol. 18, p. 40, 1920) I have renamed the species, which is not the same as that described by Bresadola, after the collector of the type, James Drummond, who sent to Berkeley many collections from Western Australia.

47. DRYADEUS, POLYPORUS Fr., Syst. Myc., Vol. 1, p. 374, 1821.

At Kew there is one collection ex "Beenak, Vic., J. H. Willis".

durissima, Hexagona Berk. & Br. Cooke (*Hdbk.*, p. 164, 1892) recorded the species from Victoria. As there are no specimens at Kew from this region, it is probable he based the record on a collection of *Hexagona gunnii*, which the species resembles closely.

48. DURUS, FOMES (Jungh.), nov. comb.

durus, Polyporus Jungh., Ann. Sci. Nat., Ser. II, Vol. 16, p. 315, 1841.

cartilagineus, Polyporus Berk. & Br., Jour. Linn. Soc., Vol. 14, p. 49, 1875, type ex Dolosbagey, Ceylon.

testudo, Polyporus Berk. & Br., Trans. Linn. Soc., Ser. II, Vol. 2, p. 59, 1883, type ex Brisbane, Q., F. M. Bailey, No. 323, in British Museum of Natural History.

The following collections are at Kew: "Fishery Falls, Q., G. W. Priest", "Dunk Island, Q., W. Cottrell-Dormer, No. 29", the latter being filed under *Polyporus durus*, "Trinity Bay, Q., Sayer", "Daintree River, Q., Pentzcke" and "Johnstone River, Q., Berthoud" Cooke filed under *P. cartilagineus.*

49. ELEGANS, POLYPORUS Fr., Epicrisis, p. 440, 1838.

guilfoylei, Polyporus Berk. & Br., Trans. Linn. Soc., Ser. II, Vol. 2, p. 58, 1883, type ex Tweed River, N.S.W., Dr. Guilfoyle.

Collections at Kew are: "Endeavour River, Q., Persieh"; "Brisbane, Q., No. 168, herb. Broome", filed under *P. picipes*; "Tumbulgum, R. T. Burke", filed under *P. melanopus*; and under *P. guilfoylei*, which is merely a spathulate form, are "Australia", "Samoa, C. G. Lloyd" and "Baridi, Papua, C. E. Carr". On the type sheet of this synonym Bresadola had noted: "Ne pas specifiquement distincte de *Polyporus elegans.*"

50. ELONGATUS, CORIOLUS (Berk.) Pat., Essai tax., p. 94, 1900.

elongatus, Polyporus Berk., Lond. Jour. Bot., Vol. 1, p. 149, 1842.

hodgkinsoniae, Polyporus Kalch., ex Cke., Grev., Vol. 10, p. 96, 1882, type ex Richmond River, N.S.W., Maria Hodgkinson.

Australian collections at Kew are: "Tropical Queensland, Bailey", "Trinity Bay, Q., Sayer", "Bloomfield River, Miss Bauer", "Shoalhaven, N.S.W., Bauerlen", "Sugar Loaf Mt., N.S.W.", "New England, N.S.W.", "Port Phillip, Vic.", "New Guinea, Armit"

and "Van Diemen's Land, Gunn", this last being filed under *Polyporus friesii*. Under *P. biformis* is placed a specimen ex "Near Melbourne, Vic., F. Reader".

emerici, Polyporus Berk. = *Polyporus fusco-lineatus.*

51. ENDAPALUS, FOMES (Berk.) Cke., Grev., Vol. 14, p. 20, 1885.

endapalus, Polyporus Berk., Jour. Linn. Soc., Vol. 13, p. 163, 1873.

fusco-dresdensis, Polyporus Lloyd, Myc. Notes, No. 66, p. 1112, 1922, type ex Tasmania, L. Rodway.

awhitu, Fomes G. H. Cunn., Plant Diseases Divn. Bull. 79, p. 16, 1948, type ex Awhitu Peninsula, Auckland, N.Z.

Types of *P. fusco-dresdensis* and *F. awhitu* match the type of *F. endapalus* ex "Tweed River, N.S.W., Dr. Guilfoyle".

epileucus, Polyporus Fr. A specimen so named by Cooke, ex "Australia, Reader", is of *Polyporus tephroleucus.*

52. EPITEPHRUS, CORIOLUS (Berk.), nov. comb.

epitephra, Trametes Berk., Jour. Linn. Soc., Vol. 13, p. 165, 1873.

The type is ex "Adelaide, S. Aus., Schomburgk, No. 26". Other collections at Kew are "St. Vincent Gulf, N.S.W.", labelled *Trametes dealbata* Kalch.; and "Moruya, N.S.W., W. N. Cheesman, No. 43". A specimen under this cover, ex "N.Z., Colenso, No. 525", is a somewhat distorted plant of *Trametes protea.*

eriophorus, Polyporus Berk. & Br. = *Merulius corium.*

eucalypti, Polyporus Kalch. = *Coriolus paleaceus.*

eucalyptorum, Polyporus Fr. = *Polyporus portentosus.*

53. EUPORA, PORIA (Karst.) Cke., Grev., Vol. 14, p. 110, 1886.

euporus, Polyporus Karst., Not. Sallsk. Faun. Fl. Fenn., Vol. 9, p. 360, 1868.

One collection is at Kew, ex "Moruya, N.S.W., W. N. Cheesman, No. 111".

exotephrus, Fomes (Berk.) Cke. Under this cover is filed a small specimen, ex "Near Trinity Bay, Q., W. A. Sayer", which is of *Fomitopsis hemitephra.*

extensus, Polystictus Berk. = *Coriolus occidentalis.*

fasciatus, Fomes (Fr.) Cke. A specimen filed under this cover, ex "Barron River, Q.", is of *Fomitopsis hemitephra.*

fatiscens, Poria (Berk. & Rav.) Cke. The plant so named by Cooke, ex "Daintree River, Q., Pentzcke", is of *Poria calcicola.*

faventina, Lenzites Cald. Two collections filed under this cover by Cooke, ex "Brisbane, Q., F. M. Bailey, Nos. 202, 559", are thin specimens of *Lenzites beckleri.*

favoloides, Hexagona Cke. = *Hexagona vespacea.*

54. FEEI, TRAMETES (Fr.) Lloyd, Syn. gen. Fomes, p. 226, 1915.

feei, Polyporus Fr., Linnaea, Vol. 5, p. 518, 1830.

The following collections agree with specimens at Kew from Brazil (type locality) and Cayenne, ex herb. Paris: "Trinity Bay, Q., Sayer", "Queensland", "Mosman River, Barnard" and "Astrolabe, New Guinea, Armit".

55. FERREA, TRAMETES (Berk.), nov. comb.

ferreus, Polyporus Berk., Lond. Jour. Bot., Vol. 6, p. 502, 1847.

dochmius, Polyporus Berk. & Br., Jour. Linn. Soc., Vol. 14, p. 50, 1875, type ex Ceylon.

seriatus, Polyporus Kalch., ex Cke., Grev., Vol. 10, p. 102, 1882, type ex Victoria.

Australian collections at Kew which match the type ex "Hautane Range, Ceylon" are filed under *Fomes ferreus* (Berk.) Cke. They are "Port Denison, Q., Shann", "Daintree River, Q.", "Johnstone River, Q., Berthoud", "Richmond River, N.S.W., Camara", "Tweed River, N.S.W., Camara" and "New Guinea, Armit". Under *Fomes oblinitus* (Berk.) Cke. is an additional collection ex "Clarence River, N.S.W., Moreton".

ferrugineus, Poria (Berk.) Cke. Under the cover of *Poria contigua* is part of a specimen ex "Swan River, W. Aus., Drummond" which Berkeley had named as above. The other part is filed under *Poria ferruginosa.* The plant is *Fuscoporia punctata.*

56. FERRUGINOSA, FUSCOPORIA (Schrad. ex Fr.) Murr., N. Am. Fl., Vol. 9, p. 5, 1907.

ferruginosus, Polyporus (Schrad.) Fr., Syst. Myc., Vol. 1, p. 378, 1821.

obliquus, Polyporus Fr., Syst. Myc., Vol. 1, p. 378, 1821.

One specimen, ex "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition", is filed under *Fomes obliquus.* Other Australian collections under the cover of *F. obliquus*

are of several species. "Clarence River, N.S.W., Wilcox" = *Fuscoporia laevigata*; "Swan River, W. Aus., Drummond" = *Fuscoporia punctata*. One ex "Melbourne, F. Reader, No. 24" is now merely a fragment of brown mycelium.

57. FLABELLIFORMIS, CORIOLUS (Kl.), nov. comb.

flabelliformis, Polyporus Kl., Linnaea, Vol. 8, p. 483, 1833.
murinus, Polyporus Kalch. ex Thuem., Grev., Vol. 4, p. 72, 1875.
glirinus, Polystictus Kalch. ex Cke., Grev., Vol. 14, p. 83, 1886, type ex South Africa, McOwan.
rufo-rigidus, Polystictus Lloyd, Myc. Notes, No. 73, p. 1330, 1924, type ex Melbourne, C. C. Brittlebank.

Collections at Kew are: "Port Denison, Q., Shann", "Johnstone River, Q., Berthoud", "Dunk Island, Q., W. Cottrell-Dormer", "Brisbane, Q., Bailey", "Tweed River, N.S.W., Guilfoyle", "Clarence River, N.S.W., Camara, Thorneton", "Clarence River, N.S.W., Dr. Beckler" (the last filed under *Polystictus versicolor*), "New Guinea, Armit", "Strickland River, New Guinea" and "S.E. New Guinea, Capt. Armit" (the last is under *P. pictilis*), "Suva" (under *Polystictus comptus*), "South Australia, J. B. Cleland" (one of three specimens filed under *Polystictus versicolor*).

flaccida, Lenzites Fr. = *Lenzites betulina*.

58. FLOCCOSA, TRAMETES (Jungh.), nov. comb.

floccosus, Polyporus Jungh., Ann. Sci. Nat., Ser. II, Vol. 16, p. 313, 1841.
acuta, Trametes Cke., Grev., Vol. 10, p. 132, 1882, type ex Richmond River, N.S.W., Camara, No. 733.
cookei, Trametes Sacc., Syll. Fung., Vol. 6, p. 342, 1888.

One collection is under this cover at Kew, ex "Moruya, N.S.W., W. N. Cheesman".

floridanus, Polyporus Berk. Cooke (*Hdbk.*, p. 146, 1892) recorded the species from Queensland, but there are no specimens from this region at Kew.

59. FOEDATA, TRAMETES (Berk.), nov. comb.

foedatus, Polyporus Berk., Jour. Linn. Soc., Vol. 16, p. 41, 1878.

The type ex "Cape York, Q., Challenger Expedition" consists of two plants in good condition.

fomentarius, Fomes (Fr.) Kickx. The species is not present in this region. Collections filed under this cover at Kew are of several species. "Port Jackson, N.S.W., Hewland" and "Twofold Bay, N.S.W., White" = *Fomes setulosus*; "King George Sound, W. Aus., Harris" = *Fomes scruposus*; "N.Z., Ohaiwai, Dr. Berggren" = *Fomes mastoporus*.

fragilis, Polyporus Fr. One specimen filed under this cover, ex "Richmond River, N.S.W., Camara" = *Polyporus albidus*; a second, ex "Gippsland, Vic., Webb", may be the same but is too damaged for accurate identification.

friesii, Polyporus Kl. Two plants so named by Berkeley, ex "Van Diemen's Land" = *Coriolus elongatus*.

frondosus, Polyporus Fr. Cooke (*Hdbk.*, p. 118, 1892) recorded the species from Tasmania, but there are no specimens at Kew from this region.

60. FRUTICUM, COLTRICIA (Berk. & Curt.), nov. comb.

fruticum, Polyporus Berk. & Curt., Jour. Linn. Soc., Vol. 10, p. 310, 1869.
biretum, Polyporus Kalch., Hedw., Vol. 15, p. 114, 1876, type ex Clarence River, N.S.W.

Two Australian collections match the type ex Cuba. One, "Mt. Williams, Bellingen River, N.S.W.", was filed under *Fomes inflexibilis*; the second, ex "Endeavour River, Q., Persieh", was placed by Cooke under *Polyporus pubescens* Fr.

fulvus, Fomes (Fr.) Cke. Australian collections placed under this cover at Kew are of two species. "King George Sound, W. Aus., Harris" and "Port Denison, Q., Shann" = *Fomes setulosus*; "Toowoomba, Q., Hartmann" = *Fomitopsis ochroleuca*.

funalis, Polyporus Fr. = *Coriolus leoninus*.

fusco-dresdensis, Polyporus Lloyd = *Fomes endapalus*.

61. FUSCO-LINEATUS, POLYPORUS Berk. & Br., Trans. Linn. Soc., Ser. II, Vol. 1, p. 401, 1879.

platotis, Polyporus Berk. & Br., Trans. Linn. Soc., Ser. II, Vol. 1, p. 401, 1879, type ex Brisbane, Q., C. E. Broome.

emerici, Polyporus Berk. ex Cke., Grev., Vol. 10, p. 96, 1882, type ex Brisbane, Q., F. M. Bailey, No. 204.
macroter, Polyporus Berk. & Br., in herb. Kew.
magnoporus, Polyporus Lloyd, Myc. Notes, No. 66, p. 1111, 1922, type ex Australia, Jas. Wilson, No. 6.

The type, ex "Brisbane, F. M. Bailey, Nos. 80, 204", consisting of two specimens, is in the herbarium of the British Museum of Natural History. One specimen is a duplicate of the type of *P. emerici* at Kew. Other collections at Kew are "Endeavour River, Q., Persieh", filed under *Polyporus nilgheriensis*, and "Port Denison, Q., Shann", placed by Cooke under *P. confluens*.

fusco-maculatus, Polyporus Bres. & Pat. = *Polyporus udus*.

galactinus, Polyporus Berk. A specimen so referred by Cooke, ex "Endeavour River, Q., Pentzcke" = *Polyporus tephroleucus*.

gallopavonis, Polyporus Berk. & Br. = *Coriolus blumei*.

gausapatus, Polyporus Kalch. = *Trametes protea*.

gilvo-rigidus, Polyporus Lloyd = *Inonotus tabacinus*.

62. GILVUS, FOMES (Fr.) Lloyd, Letter 42, p. 6, 1912.
gilvus, Polyporus Fr., Elench., p. 104, 1828.
rubiginosus, Polyporus Berk., Ann. Nat. Hist., Vol. 3, p. 324, 1839, type ex Mauritius.
laurencii, Polyporus Berk., Fl. Tas., Vol. 2, p. 254, 1860, type ex Van Diemen's Land, Lawrence.
scabrosus, Polyporus Berk., in herb. Kew.

Australian collections at Kew are: "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition" (four specimens, two being of *F. gilvus*, one *F. scruposus* and one the form known as *F. hookeri*); "Clarence River, N.S.W., Wilcox", filed under *P. laurencii*; "Brisbane, Q.", under *Polyporus plebius*; and "New Guinea", named *P. holosclerus*.

glabra, Lenzites Lloyd = *Lenzites glabrescens*.

glabratus, Polyporus Kalch. The type collection was from "Clarence River, N.S.W.". No specimens are at Kew from this region.

63. GLABRESCENS, LENZITES (Berk.), nov. comb.
glabrescens, Daedalea Berk., Jour. Linn. Soc., Vol. 16, p. 39, 1878.
glabra, Lenzites Lloyd, Myc. Notes, No. 56, p. 811, 1918, type ex Russell Islands, Solomons, E. Cheel.

Only the type collection is at Kew, ex "Parramatta River, N.S.W., Challenger Expedition". On the sheet Bresadola had written: "Tout à fait identique au *Lenzites platyphylla* Lev." Specimens of the latter are not at Kew, so I have been unable to compare the two species.

glabro-tabacinus, Polystictus Lloyd = *Inonotus tabacinus*.

glirinus, Polystictus Kalch. = *Coriolus flabelliformis*

gourliaei, Polyporus Berk. = *Trametes protea*.

64. GRAMMOCEPHALUS, POLYPORUS Berk., Lond. Jour. Bot., Vol. 1, p. 148, 1842.
muelleri, Polyporus Kalch. ex Cke., Grev., Vol. 10, p. 97, 1882, type ex Richmond River, N.S.W.
acervatus, Polyporus Lloyd, Myc. Notes, No. 64, p. 1006, 1920, type ex Singapore.

The following Australian collections match the type ex Philippine Islands: "Daintree River, Q., Pentzcke", "Endeavour River, Q., Persieh", "Port Denison, Q., Shann", "Brisbane, Q., Bailey, No. 808", "Cape Direction, Q., D. Thomson", "Richmond River, N.S.W., Camara" and "Lake Barine, H. Flecker" are under the cover of *P. grammocephalus*. "Brisbane, Q., C. E. Broome" is labelled *P. macroter*, a herbarium name for *P. fusco-lineatus*; and under *Favolus spathulatus* is filed a branched specimen ex "Dunk Island, Q., W. Cottrell-Dormer, No. 41".

gryphaeformis, Polyporus Berk., *Lond. Jour. Bot.*, Vol. 4, p. 54, 1845. The type was from "Swan River, W. Aus., Drummond, No. 149". No cover is at Kew or British Museum of Natural History.

guilfoylei, Lenzites Berk. = *Lenzites beckleri*.
guilfoylei, Polyporus Berk. & Br. = *Polyporus elegans*.

65. GUNNII, HEXAGONA Fr., Ann. Nat. Hist., Vol. 7, p. 452, 1841.

vesparius, Polyporus Berk., Ann. Nat. Hist., Vol. 3, p. 323, 1839.
bicolor, Hexagona McAlp., Proc. Linn. Soc. N.S.W., p. 123, 1904.
olivacea, Hexagona Lloyd, Letter 53, p. 11, 1914, type ex Victoria, Jas. Wilson.
praetervisa, Trametes Bres., Ann. Myc., Vol. 18, p. 39, 1920, type ex Seven Hills, Australia, Torrend.

The name was changed by Fries, who held that *P. vesparius* was too close to *P. vespaceus* Pers. Four collections from Australia are at Kew: the type ex "Van Diemen's Land, Gunn", "Van Diemen's Land, Lawrence", "Grampian Mts., Vic., Sullivan" and "Melbourne, Vic., LeFevre, No. 201".

gunnii, Polyporus Berk. = *Polyporus campylus.*

66. HARTMANNII, POLYPORUS Cke., Grev., Vol. 12, p. 14, 1883.

The type, which is excellently preserved, was from "Toowoomba, Q., Hartmann, No. 10". Other collections from the region are "Gladesville, Parramatta River, N.S.W., Miss Margaret Flocton" and "Brisbane, Q., Bailey".

67. HASKARLII, COLTRICIA (Lev.), nov. comb.

haskarlii, Polyporus Lev., Ann. Sci. Nat., Ser. III, Vol. 2, p. 190, 1844.

The following collections are at Kew: "Upper Daintree River, Q., Harris", "Bellenden Ker, Q., Bailey", "Toowoomba, Q., Hartmann", "Clarence River, N.S.W., Camara, No. 70" and "Strickland River, New Guinea, Bauerlen".

hemileucus, Fomes (Berk. & Curt.) Cke. Cooke (*Hdbk.*, p. 132, 1892) recorded the species from Queensland. As there are no specimens from this region at Kew it is probable that the record was based on a specimen of *Fomitopsis hemitephra.*

68. HEMITEPHRA, FOMITOPSIS (Berk.) G. H. Cunn., Plant Diseases Division Bull. 76, p. 2, 1948.

hemitephrus, Polyporus Berk., Fl. N.Z., Vol. 2, p. 179, 1855.
hypopolius, Polyporus Kalch. ex Cke., Grev., Vol. 10, p. 99, 1882, type ex Rockhampton, Q., Mrs. Thozet.

Australian collections which match the type ex "N.Z., Colenso" are: "Brisbane, Q., W. N. Cheesman, No. 47", "Trinity Bay, Q., W. A. Sayer" (the latter is filed under *Polyporus exotephrus*); "Barron River, Q.", placed by Cooke under *Fomes fasciatus*; "Moruya, Blue Mountains, N.S.W., W. N. Cheesman"; and "Sydney, N.S.W., W. Froggatt", misnamed *Fomes martius* by Lloyd.

heteromalla, Trametes Cke. = *Coriolus occidentalis.*

69. HIRSUTUS, CORIOLUS (Fr.) Quel., Fl. Myc. Fr., p. 389, 1888.

hirsutus, Polyporus Fr., Syst. Myc., Vol. 1, p. 367, 1821.
pisiformis, Polyporus Kalch. ex Cke., Grev., Vol. 10, p. 98, 1882, type ex Richmond River, N.S.W., Camara.

Australian collections at Kew are: "Endeavour River, Q., Persietz", "Rockhampton, Q., Mrs. Thozet", "Toowoomba, Q., Hartmann", "Trinity Bay, Q., Karsten", "Sunnybrook, Q., C. G. Greenham", "Toowoomba, Q., Hartmann" (this last filed under *P. glirinus*), "Illawarra, N.S.W., Karsten", "Nat. Park, Sydney, N.S.W., W. N. Cheesman", "Clarence River, N.S.W., Beckler", "North Gippsland, Vic., H. Tisdale"; and "Tasmania, Dr. Milligan".

hispidulus, Favolus Berk. & Curt. = *Hexagona alveolaris.*

hispidus, Polyporus Fr. Cooke (*Hdbk.*, p. 123, 1892) recorded the species from Queensland, but there are no specimens from the region at Kew.

hodgkinsoniae, Polyporus Kalch. = *Coriolus elongatus.*

hololeuca, Trametes Kalch. = *Polyporus portentosus.*

holosclerus, Fomes (Berk.) Cke. One specimen filed under this cover, ex "New Guinea, Capt. Armit" = *Fomes gilvus*; a second, ex "Endeavour River, Q., Persietz" = *F. scruposus.*

70. HYALINA, PORIA (Berk.) Cke., Grev., Vol. 14, p. 109, 1886.

hyalinus, Polyporus Berk., Fl. Tas., Vol. 2, p. 255, 1860.

The type was from "Tasmania, Archer, growing on naked wood of an eucalypt". A second collection at Kew is from "Orange, N.S.W., J. B. Cleland, No. 18".

71. HYPOMELANUS, POLYPORUS Berk. ex Cke., Grev., Vol. 15, p. 51, 1886.

Under the cover of *Polyporus melanopus* are two Australian collections which match the type ex "N.Z., Grey River". They are ex "Toowoomba, Q., Hartmann" and "Melbourne, Vic., Dr. Berggren".

hypopolius, Polyporus Kalch. = *Fomitopsis hemitephra.*

72. HYPOSCLERA, PORIA (Berk.) Cke., Grev., Vol. 14, p. 110, 1886.

hyposclerus, Polyporus Berk. ex Cke., Grev., Vol. 10, p. 103, 1882.

The following Australian collections at Kew match the type from Tahiti: "Brisbane, Q., C. E. Broome", "Trinity Bay, Q., Sayer, No. 9", "Kin Kin, Q., No. 3850", "Katoomba, Blue Mts., N.S.W., W. N. Cheesman" and "Clarence River, N.S.W., Wilcox". One collection, filed under the cover by Cooke, ex "Daintree River, Q., Pentzcke" = *Poria undata*; a second ex "Dunk Island, Q., W. Cottrell-Dormer, No. 14" = *Poria radula.*

hypothejus, Polyporus Kalch. ex Cke., *Grev.*, Vol. 10, p. 102, 1882. The type was from Richmond River, N.S.W. No specimens are at Kew from this region.

hystriculus, Polyporus Cke. = *Polyporus pelliculosus.*

igniarius, Fomes (Fr.) Kickx. The species has not been found in this region. Australian collections at Kew, filed under this cover, are of the following species: "Daintree River, Q., T. Pentzcke" = *Fomes senex*; "Port Denison, Q., Shann" = *Fomes setulosus*; "N.Z., Ohaiwai, Dr. Berggren" = *F. australis.*

illotus, Polyporus Kalch. = *Trametes protea.*

illudens, Daedalea Cke. & Mass. = *Coltricia brunneo-leuca.*

imbricatus, Polystictus Lloyd, *Myc. Notes*, No. 55, p. 791, 1918. The co-type specimen at Kew, ex "Sydney, N.S.W., J. H. Maiden" is too fragmentary to name. Lloyd's figure (f. 1191) and description suggest that the species may have been based on *P. berkeleyi.*

incompta, Daedalea Berk., *Trans. Linn. Soc.*, Ser. II, Vol. 2, p. 61, 1883, type ex Port Douglas, Q., J. E. T. Woods.

No specimens are at Kew or the British Museum of Natural History.

inconcinna, Daedalea Berk. = *Hexagona vespacea.*

incrassatus, Polyporus Berk. = *Fomes applanatus.*

73. INFERNALIS, POLYPORUS Berk., Lond. Jour. Bot., Vol. 2, p. 637, 1843.

Specimens which match the type, ex "Arrial das Merces, South America" are: "Gippsland, Vic., Webb", "Moonan Brook, Vic., Miss Carter", "New Caledonia, Dr. F. Sarasin" (the last filed under *Polyporus blanchetianus* Mont.) and "New Guinea, Fly River, Bauerlen", which is under the cover of *P. russiceps.*

inflexibilis, Fomes (Berk.) Cke. The Australian record is based on two collections ex "Mt. Williams, Bellingen River, N.S.W.". They are of two species, *Coltricia fruticum* and *Fomes scruposus.*

intermedia, Daedalea Berk. = *Hexagona vespacea.*

intonsus, Polyporus Berk., *Fl. Tas.*, Vol. 2, p. 252, 1860, type ex Tasmania, Archer. No cover is at Kew, and the description is too incomplete to allow of identification.

intybaceus, Polyporus Berk. = *Inonotus setiporus.*

laceratus, Polyporus Berk. = *Coriolus pargamenus.*

lactea, Trametes Fr. = *Coriolus ambiguus.*

74. LACTEUS, POLYPORUS Fr., Syst. Myc., Vol. 1, p. 359, 1821. One collection, ex "Richmond River, N.S.W., Camara" is at Kew, filed by Cooke under *Polyporus chioneus.*

75. LACTINEUS, CORIOLUS (Berk.), nov. comb.

lactineus, Polyporus Berk., Ann. Nat. Hist., Vol. 10, p. 373, 1842.

levis, Trametes Berk., Lond. Jour. Bot., Vol. 6, p. 507, 1847, type ex Ceylon.

Under this cover at Kew are the following collections: "Dunk Island, Q., W. Cottrell-Dormer, No. 5" (intermediate between this species and *C. sprucei*), "Katoomba, Blue Mts., N.S.W., W. N. Cheesman", "Lilyvale, N.S.W., A. A. Hamilton", "Mt. Dandenong, Vic., comm. E. McLennan" and "Strickland River, New Guinea, Bauerlen". A specimen ex "Australia" was by Cooke placed under *Trametes hololeuca.* Under *Trametes levis* are filed: "Port Denison, Q., Shann", "Toowoomba, Q., Hartmann", "Richmond River, N.S.W., Camara" and "Parramatta River, N.S.W., L. Weber".

76. LAETA, COLTRICIA (Cke.) G. H. Cunn., Plant Diseases Division Bull. 77, p. 8, 1948.
laetus, *Polyporus* Cke., Grev., Vol. 12, p. 16, 1883.
lateritius, *Polyporus* Lloyd, Letter 67, p. 12, 1918.

Collections at Kew from Australia agree with the type ex "Upper Owens River, Vic., Mrs. McCann". They are "Guntawang, N.S.W., Hamilton", "Victoria, Dr. Winter" and "Gippsland, Vic., Mrs. Murray".

77. LAEVIGATA, FUSCOPORIA (Fr.) G. H. Cunn., Plant Diseases Division Bull. 73, p. 9, 1948.

laevigatus, *Polyporus* Fr., Hym. Eur., p. 571, 1874.

Under the cover of *Poria ferruginosa* is a collection ex "Clarence River, N.S.W., Wilcox"; a second, ex "Australia", is filed under *Poria rufitincta* (Berk. & Curt.) Cke.

lanatus, Polyporus Fr. A specimen ex "Port Denison, Q., Shann" filed under this cover by Cooke is of *Coriolus occidentalis*.

78. LATISSIMA, DAEDALEA Fr., Syst. Myc., Vol. 1, p. 340, 1821.

Under *Daedalea microsinulosa* Kl. (= *D. sinulosa* Fr.) are filed two Australian collections, ex "Endeavour River, Q., Persietz" and "Port Denison, Q., Shann". A specimen ex "Daintree River, Q., Pentzcke", placed under this cover by Cooke, is of *Poria medulla-panis*.

lateritius, *Polyporus* Lloyd = *Coltricia laeta*.

latus, *Polyporus* Berk. = *Coltricia acupunctata*.

laurencii, *Polyporus* Berk. = *Fomes gilvus*, and also a herbarium name for *P. campylus*.

lenis, Polyporus Lev. Under the cover of *Polystictus trizonatus* is a specimen ex "Upper Yarra, Vic., Lucas" to which Bresadola had attached a note "= *Polyporus lenis* Lev. = form of *P. occidentalis* Kl., old, naked". The plant is a slightly discoloured specimen of *Coriolus zonatus*.

lentus, Polyporus Berk. Two collections from Australia, placed by Cooke under this cover, are of *Polyporus arcularius*. They were from the following localities: "Guntawang, N.S.W., Hamilton, No. 6" and "Govt. Domain, Vic.".

79. LEONINUS, CORIOLUS (Kl.), nov. comb.
leoninus, *Polyporus* Kl., Linnaea, Vol. 8, p. 486, 1833.
funalis, *Polyporus* Fr., Epicrisis, p. 459, 1838.
mons-veneris, *Polyporus* Jungh., Batav. Genootsch. Verhand., Vol. 17, p. 61, 1839.

One specimen from Australia, ex "Lower Ardur River, Gulf of Carpentaria, Q.", is under the cover of *Polyporus leoninus*. A second, ex "Endeavour River, Q., Persieh", is filed under *P. funalis*.

leonotis, Polyporus Kalch. ex Thuem., *Grev.*, Vol. 4, p. 73, 1875. The type was ex "Australia". No specimens are at Kew or British Museum of Natural History.

leucocreas, *Polyporus* Cke. = *Polyporus portentosus*.

80. LEUCOPLACA, PORIA (Berk.) Cke., Grev., Vol. 14, p. 113, 1886.
leucoplacus, *Polyporus* Berk., Fl. N.Z., Vol. 2, p. 180, 1855.
macrospora, *Poria* Rodw. & Clel., Papers and Proc. Roy. Soc. Tas. for 1929, p. 81, 1929.

The type is from New Zealand, ex "N.Z., Colenso", and is matched by the type of *P. macrospora* ex "National Park, S. Aus.". An Australian specimen ex "Kurrumburra, Vic., No. 1044" filed under the cover at Kew is not the same species.

levis, *Trametes* Berk. = *Coriolus lactineus*.

libum, Polyporus Berk., *Jour. Linn. Soc.*, Vol. 13, p. 163, 1873. The type ex "Tweed River, N.S.W., Guilfoyle" is merely a fragment which has been so damaged by insects as to be unrecognizable.

81. LIEBMANNII, POLYPORUS Fr., Nov. Symb., p. 59, 1851.
mutabilis, *Polyporus* Berk. & Curt., Ann. Mag. Nat. Hist., Ser. II, Vol. 12, p. 433, 1853.
sterinus, *Polyporus* Berk. & Curt., Jour. Linn. Soc., Vol. 10, p. 308, 1869.
rigescens, *Polyporus* Cke., Grev., Vol. 14, p. 12, 1885.
stercoides, *Polystictus* Berk. & Curt. ex Cke., Grev., Vol. 14, p. 78, 1885.
cognatus, *Polyporus* Kalch., in herb. Kew.

Specimens from this region at Kew are "Clarence River, N.S.W.", filed under *P. luteus*, and on the sheet referred by Lloyd to *P. mutabilis*; "Aloha, Papua, C. E. Carr"; and "Samoa, C. G. Lloyd", which was identified by Bresadola as *P. liebmannii*.

82. LILACINO-GILVA, TRAMETES (Berk.) Lloyd, Syn. gen. Fomes, p. 226, 1915.

lilacino-gilvus, Polyporus Berk., Ann. Nat. Hist., Ser. I, Vol. 3, p. 324, 1839.
rosea, Trametes Lloyd, Letter 59, p. 5, 1915, type ex Australia, J. B. Cleland.
stowardii, Trametes Lloyd, Myc. Notes, No. 48, p. 683, 1917, type ex W. Australia, Dr. F. Stoward.

Australian collections at Kew are: "Enoggera, Brisbane, Q., W. N. Cheesman, No. 77", "Daintree River, Q., Pentzcke, No. 219", "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition", "Tilba Tilba, N.S.W., Miss Bates", "Gippsland, Vic., Murray, Webb", "Loddon River, Vic., Wallace", "Ballarat, Vic., C. French", "S.W. Aus., T. Muir", "Van Diemen's Land, Gunn" and "Australia", this last being filed under *Polyporus cupreo-roseus* Berk. Under the cover of *Polyporus carneus* are "Brisbane, Q.", "Melbourne, Vic., G. LeFevre, No. 212" and "Narranoom, Vic., Stranger".

limbatus, Polyporus Fr. Cooke (*Hdbk.*, p. 148, 1892) recorded the species from Victoria, but there are no specimens from this region at Kew.

lineato-scaber, Fomes Berk. & Br. = *Fomes strigatus*.

83. LIVIDUS, FOMES (Kalch.) Sacc., Syll. Fung., Vol. 6, p. 206, 1888.

lividus, Polyporus Kalch., ex Cke., Grev., Vol. 10, p. 103, 1882.
luridus, Fomes (Kalch.) Cke., Grev., Vol. 14, p. 21, 1885.

Collections from Australia at Kew are the type ex "Richmond River, N.S.W.", "Clarence River, N.S.W., Thorneton", "Imbil State Forest, Q., J. B. Cleland, No. 14", "Hawkesbury River, N.S.W., J. B. Cleland" and "Lismore, N.S.W., J. B. Cleland".

84. LIVIDA, PORIA Cke., Grev., Vol. 10, p. 131, 1882.

The type ex "Clarence River, N.S.W., Wilcox" is probably a resupinate form of *Fomes lividus*.

85. LLOYDII, FOMES Cleland, Toadstools . . . of S. Aus., p. 200, 1935.

The type was from "National Park, S. Aus., J. B. Cleland". No specimens are at Kew.

86. LUCIDUM, GANODERMA (Ley. ex Fr.) Karst., Rev. Myc., Vol. 3, p. 17, 1881.

lucidus, Polyporus (Leyss.) Fr., Syst. Myc., Vol. 1, p. 353, 1821.

Under the cover of *Ganoderma lucidum* are filed two collections ex "Stradbroke Island, Q., C. E. Hubbard" and "Queensland, Bailey". Under *Fomes lucidus* (Fr.) Cke. are "Lower Ardur River, Gulf of Carpentaria, Q.", "Dunk Island, Q., W. Cottrell-Dormer, No. 11", "Brisbane, F. M. Bailey", "Lord Howe Island, Camara", "Port Denison, Q., Fitzalan, Shann", "Endeavour River, Q., Persieh", "Trinity Bay, Q., Karsten". Filed under *Polyporus amboinensis* are "Trinity Bay, Q." and "Johnstone River, Q.".

luridus, Fomes (Kalch.) Cke. = *Fomes lividus*.

luteo-nitidus, Polyporus Berk. One specimen so referred, ex "Brisbane, Q.", does not agree with the type, but is a form of *Polyporus zonalis* with a brief lateral stem.

luteo-olivaceus, Polyporus Berk. & Br. = *Coltricia placodes*.

luteus, Polyporus Nees. A specimen ex "Clarence River, N.S.W., Wilcox" referred here by Cooke does not resemble collections under the cover named by Fries. Lloyd referred it—correctly, I believe—to *P. mutabilis* (= *P. liebmannii*).

macrospora, Poria Rodw. & Clel. = *Poria leucoplaca*.

macroter, Polyporus Berk. & Br. = *Polyporus fusco-lineatus*.

maculatissimus, Polyporus Lloyd = *Polyporus portentosus*.

magnoporus, Polyporus Lloyd = *Polyporus fusco-lineatus*.

marginatus, Fomes (Fr.) Gill. The species has not been found in this region, collections at Kew from Australia being of the following species: "North Queensland", "Endeavour River, Q., Persieh" and "Mt. Macdonald" = *Fomitopsis ochroleuca*; "Endeavour River, Q., Persieh" and "Upper Hunter River, N.S.W., Miss Carter" = *Fomes applanatus*; "N.Z., Hokianga, Dr. Berggren" = *Fomitopsis hemitephra*.

martius, Fomes (Berk.) Cke. Lloyd so named a specimen of *Fomitopsis hemitephra* ex "Sydney, N.S.W., W. Froggatt".

87. MASTOPORUS, FOMES (Lev.) Cke., Grev., Vol. 13, p. 118, 1885.

mastoporus, Polyporus Lev., Ann. Sci. Nat., Ser. III, Vol. 2, p. 182, 1844.
ponderosus, Polyporus Kalch. ex Cke., Grev., Vol. 10, p. 99, 1882.

Two specimens are at Kew from this region. One, ex "Australia, F.v.M.", is filed under *F. chilensis*; the other, ex "N.Z., Ohaiwai, Dr. Berggren", had been placed under *F. fomentarius*.

88. MEDULLA-PANIS, PORIA (Fr.) Cke., Grev., Vol. 14, p. 109, 1886.

medulla-panis, Polyporus Fr., Syst. Myc., Vol. 1, p. 380, 1821.

Collections at Kew are: "Dunk Island, Q., W. Cottrell-Dormer, No. 18", "Daintree River, Q., Pentzcke" (the latter labelled *Daedalea latissima*), "Mt. Lofty, S. Aus., J. B. Cleland", "Sydney, N.S.W., J. B. Cleland", "Hawkesbury River, N.S.W., J. B. Cleland", "Richmond River, N.S.W., Mrs. Hodgkinson", "Melbourne, Vic., F. M. Reader, No. 384" (the last filed by Cooke under *Poria tarda*) and "Strickland River, New Guinea, Bauerlen, No. 48" (filed under *Fomes bistratosus*).

megaloporus, Polyporus Lloyd = *Polyporus russiceps.*

megaloporus, Polyporus Mont. A collection ex "Coomera River, Q., C. T. White" filed under this cover appears to be of *Polyporus russiceps.*

89. MELANOPUS, POLYPORUS Fr., Syst. Myc., Vol. 1, p. 347, 1821.

Only one authentic specimen is at Kew from this region; this, ex "Parramatta River, N.S.W., Challenger Expedition", was filed by Cooke under *Polyporus dictyopus.* Collections under *P. melanopus* ex "Toowoomba, Q., Hartmann" and "Melbourne, Vic., Dr. Berggren" are of *P. hypomelanus*; that ex "Tumbulgum, R. T. Burke" = *P. elegans.*

membranicinctus, Polyporus Berk. = *Polyporus merulius.*

menziesii, Trametes Berk. = *Trametes protea.*

90. MERULIUS, POLYPORUS Berk., Fl. Tas., Vol. 2, p. 254, 1860.

archeri, Polyporus Berk., Fl. Tas., Vol. 2, p. 255, 1860.
membranicinctus, Polyporus Berk. ex Cke., Grev., Vol. 15, p. 26, 1886.
tasmaniae, Polyporus Berk., in herb. Kew.

Three collections from Tasmania are at Kew, the type ex "Tasmania, Archer", type of *P. archeri* ex "Tasmania, Archer" and type of *P. membranicinctus* ex "Tasmania, No. 1379". Two collections from New Zealand are also at Kew, "N.Z., Colenso, No. 136", filed under *Poria fusco-carnea* Pers., and "N.Z. Colenso, No. 923" placed under the cover of *Poria corticola.*

91. MEYENII, CORIOLUS (Kl.), nov. comb.

meyenii, Polyporus Kl., Nova Acta Acad. Leop.-Carol., suppl. 19, p. 236, 1843.
obstinatus, Trametes Cke., Grev., Vol. 12, p. 17, 1883.

Collections at Kew are: "Tropical Queensland, Bailey", "Endeavour River, Q., Persieh" (type of *T. obstinatus*), "Baridi and Yodda River, Papua", "Strickland River, New Guinea, Bauerlen" and "Samoa, C. G. Lloyd".

mirabilis, Hexagona Lloyd = *Hexagona thwaitesii.*

mollis, Trametes Fr. Cooke (*Hdbk.*, p. 160, 1892) recorded the species from New South Wales, but no specimens from Australia are at Kew.

mollusca, Poria (Fr.) Cke. One specimen from Australia, ex "Endeavour River, Q., Persieh", is filed under this cover at Kew. It is not *P. mollusca*, but a much-decayed pileate species, too damaged to identify.

mons-veneris, Polyporus Jungh. = *Coriolus leoninus.*

92. MUCIDA, PORIA (Pers. ex Fr.) Cke., Grev., Vol. 14, p. 109, 1886.

mucidus, Polyporus (Pers.) Fr., Syst. Myc., Vol. 1, p. 382, 1821.

A specimen ex "Toowoomba, Q.", filed by Cooke under *Poria blepharistoma*, is an immature plant of *Poria mucida.*

muelleri, Daedalea Berk. = *Lenzites palisoti.*

muelleri, Hexagona Berk. = *Hexagona tenuis.*

muelleri, Polyporus Kalch. = *Polyporus grammocephalus.*

muelleri, Trametes Berk. = *Coriolus paleaceus.*

multilobus, Polyporus Kalch. ex Cke., *Grev.*, Vol. 10, p. 95, 1882. The type was from "Richmond River, N.S.W.". No specimens are at Kew.

multiplex, *Polyporus* Berk. = *Polyporus colensoi.*

multisetosus, *Polyporus* Lloyd = *Fomes setulosus.*

murinus, *Polyporus* Kalch. = *Coriolus flabelliformis.*

murinus, Polyporus Lev. The collection from "Broger's Creek, N.S.W., Bauerlen" does not resemble other specimens under this cover at Kew. It is probably of *Fomes pectinatus*, but of this I am not certain, as spores were not found.

mutabilis, *Polyporus* Berk. & Curt. = *Polyporus liebmannii.*

myelodes, Polyporus Kalch. ex Thuem., *Grev.*, Vol. 4, p. 73, 1875. No specimens are at Kew or British Museum of Natural History.

93. MYLITTAE, POLYPORUS Cke. & Mass., Grev., Vol. 21, p. 37, 1892.

australis, Mylitta Berk., Ann. Mag. Nat. Hist., Vol. 3, p. 326, 1839.

Only sclerotia are at Kew, ex "Beechworth, Vic., J. W. Howard", which were named *Mylitta australis* by Berkeley. Cooke (*Hdbk.*, p. 249, 1892) placed *M. australis* under the Ascomycetes.

nanus, *Polyporus* Mass. = *Polyporus rhipidium.*

nidulans, Polyporus Fr. The specimen on which this record was based, ex "Johnstone River, Q.", is of *P. australiensis.*

nigripes, Fomes (Fr.) Cke. Cooke (*Hdbk.*, p. 127, 1892) recorded the species from New South Wales, but there are no specimens from this region at Kew.

niphodes, Poria (Berk. & Br.) Cke. Cooke (*Hdbk.*, p. 154, 1892) recorded the species from New South Wales. No specimens from this region are at Kew, the record probably being based on a specimen of *Poria calcicola.*

nivea, *Lenzites* Cke. = *Lenzites aspera.*

novo-guineensis, *Polyporus* P. Henn. = *Polyporus virgatus.*

94. OBLECTANS, COLTRICIA (Berk.) G. H. Cunn., Plant Diseases Division Bull. 77, p. 3, 1948.

oblectans, *Polyporus* Berk., Lond. Jour. Bot., Vol. 4, p. 51, 1845.
cladonia, *Polyporus* Berk., Lond. Jour. Bot., Vol. 4, p. 51, 1845.
perdurans, *Polyporus* Kalch. ex Cke., Grev., Vol. 9, p. 1, 1880.

The following Australian collections match the type at Kew, ex "Swan River, W. Aus., Drummond, No. 157": "Swan River, W. Aust., Drummond, No. 220", the type of *P. cladonia* which is filed under the cover of *P. bulbipes*; "Gaisford, Q., E. Bowden", "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition", "Sydney, North Shore, N.S.W., Whitelegge, No. 7", "Oweo, J. Sterling", "Melbourne, Vic., F. M. Reader", "Tasmania, W. Archer", "Van Diemen's Land, Mr. Gunn" and "S.W. Aus., T. Muir, Nos. 110, 122" (No. 122 is filed under *P. parvulus*).

oblinitus, Fomes (Berk.) Cke. A specimen ex "Clarence River, N.S.W., Moreton" so referred by Cooke = *Trametes ferrea.*

obliquus, *Polyporus* Fr. = *Fuscoporia ferruginosa.*

obovatus, Polyporus Jungh., *Ann. Sci. Nat.*, Ser. II, Vol. 16, p. 316, 1841. Cooke (*Hdbk.*, p. 139, 1892) recorded the species from Queensland and New South Wales under the name *Polystictus adami.* No collections from these localities are at Kew, the only specimen being from New Guinea, also filed under *P. adami.* Bresadola referred this plant to *P. obovatus.*

obstinatus, *Trametes* Cke. = *Coriolus meyenii.*

95. OCCIDENTALIS, CORIOLUS (Kl.), nov. comb.

occidentalis, Polyporus Kl., Linnaea, Vol. 8, p. 486, 1833.
occidentalis, Trametes (Kl.) Fr., Epicrisis, p. 491, 1838.
heteromalla, Trametes Cke., Grev., Vol. 10, p. 132, 1882, type ex Mt. Dromedary, N.S.W., Miss Bates.
extensus, Polystictus Berk. ex Cke., Grev., Vol. 14, p. 82, 1886, type ex Daintree River, Q., Persietz.
badio-lutescens, Polyporus Kalch., in herb. Kew.
subcongener, Daedalea Berk. ex Cke., Grev., Vol. 19, p. 93, 1892, type ex Australia.

Additional Australian collections at Kew are: "Trinity Bay, Q., Karsten", "Endeavour River, Q., Persietz", "Daintree River, Q., Pentzcke", "Port Denison, Q., Shann" (the last filed by Cooke under *P. lanatus* and by Lars Romell referred to

P. caperatus), "Australia" (referred by Cooke to *P. cyclodes*), "Richmond River, N.S.W., Camara", "Tarwin, Gippsland, Vic.", "Moe Swamp, Gippsland, Vic.", "North Gippsland, Vic., H. Tisdall". The last three are filed under *T. heteromalla*, and the specimen from North Gippsland was by Lars Romell referred to *T. devexa*.

ochraceo-stuppeus, Polystictus Lloyd = *Polyporus adustus*.

96. OCHROLEUCA, FOMITOPSIS (Berk.) G. H. Cunn., Plant Diseases Division, Bull 76, p. 5, 1948.

ochroleucus, Polyporus Berk., Lond. Jour. Bot., Vol. 4, p. 53, 1845.
compressus, Polyporus Berk., Lond. Jour. Bot., Vol. 4, p. 53, 1845, type ex Swan River, W. Aus., Drummond, No. 141.
brisbanensis, Polyporus Berk. & Br., in herb. Kew.
ungulata, Trametes Berk., Jour. Linn. Soc., Vol. 13, p. 165, 1873, type ex Adelaide, S. Aus., Schomburgk, No. 2.
scrobiculata, Trametes Berk., Grev., Vol. 6, p. 70, 1877, type ex Melbourne, Vic., LeFevre, No. 212.
varia, Trametes Lloyd, Myc. Notes, No. 66, p. 1114, 1922, type ex Tasmania, L. Rodway.

The type is from "Swan River, W. Aus., Drummond, Nos. 248, 285". Additional collections from Australia are: "Quiedong, Q., No. 454" (filed under *P. ostraeformis*), "Brisbane, Q., Nos. 136, 138" (filed under *P. havannensis* and on the sheet labelled *P. brisbanensis*), "Trinity Bay, Q., Sayer, No. 7" (placed under the cover of *P. chioneus*). "Endeavour River, Q., Persieh" and "Mt. Macdonald" (filed under *Fomes marginatus*), "Toowoomba, Q., Hartmann" (under cover of *Fomes fulvus*), "Nambour, Blackball Range, Q., W. N. Cheesman, No. 59", "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition", "Gippsland, Vic., Mrs. Campbell", "Melbourne, Vic., F. M. Reader" and "W. Aus.".

ochro-flava, Trametes Cke. Cooke (*Hdbk.*, p. 159, 1892) recorded the species from Queensland, but there are no specimens from this region at Kew.

odontoporus, Polyporus Kalch. in herb. Kew. A specimen labelled *Poria odontoporia* by Cooke, ex "Guntawang, N.S.W., Hamilton, No. 4", is of *Poria versipora* growing over the surface of an irregular piece of wood.

ohiensis, Favolus Berk. & Mont. = *Hexagona alveolaris*.

olivacea, Hexagona Lloyd = *Hexagona gunnii*.

orbicularis, Polyporus Berk., Ann. Nat. Hist., Vol. 3, p. 324, 1839. The type, ex "Van Diemen's Land, Gunn, No. 16, on living bark", is, as Bresadola had noted on the type sheet, a species of *Septobasidium*.

orbiformis, Fomes (Fr.) Cke. Cooke (*Hdbk.*, p. 130, 1892) recorded the species from Victoria, but there are no specimens from this region at Kew.

ornithorhynchi, Polyporus Kalch. ex Cke., *Grev.*, Vol. 10, p. 96, 1882. The type was from "Richmond River, N.S.W., Maria Hodgkinson". No cover for this species is at Kew.

ostraeformis, Polyporus Berk. One specimen ex "Quiedong, Q., No. 454" is filed under this cover at Kew. As Bresadola had noted on the sheet, it is of *Fomitopsis ochroleuca*.

ovinus, Polyporus Fr. A specimen so named by Cooke, ex "Queensland, Campbell, No. 96" = *Amauroderma rudis*.

97. PALEACEUS, CORIOLUS (Fr.), nov. comb.

paleacea, Trametes Fr., Nov. Symb., p. 97, 1851.
muelleri, Trametes Berk., Jour. Linn. Soc., Vol. 10, p. 320, 1869, type ex Victoria River, Q., F.v.M.
eucalypti, Polyporus Kalch. ex Thuem., Grev., Vol. 4, p. 73, 1875, type ex Rockhampton, Q., Mrs. Thozet.
picta, Trametes Berk., Trans. Linn. Soc., Ser. II, Vol. 2, p. 61, 1883, type ex Moreton Bay, Q., F. M. Bailey, Nos. 11, 14.
argentatus, Polyporus Cke., Grev., Vol. 15, p. 20, 1886, type ex Narranoom, Vic., Stranger.

Additional collections at Kew are: "North Queensland", "Brisbane" and "Dunk Island, Q., W. Cottrell-Dormer, No. 28", which are filed under *Polyporus rudis* Berk.; "Bellenden Ker, Q., Bailey, Nos. 820, 821" and "Victoria, Mrs. Martin, No. 634", placed

under *Polyporus eucalypti*; "Endeavour River, Q., Persietz", "Daintree River, Pentzcke" and "New Guinea, Armit", filed under *Trametes muelleri*; one specimen ex "Endeavour River, Q., Persietz" is filed under *Trametes picta*, a second from the same source under *Polyporus carneus*; "Yerrigong, Nowra, N.S.W., F. A. Rodway" and "Dunk Island, Q., W. Cottrell-Dormer, No. 13" are filed under *Trametes lilacino-gilva*; "Trinity Bay, Q., Karsten, No. 2", "Loddon River, Vic., No. 6", "Toowoomba, Q., Hartmann" and "Narranoom, Vic., Stranger" are under *Polyporus pallisieri.*

98. PALISOTI, LENZITES Fr., Epicrisis, p. 404, 1838.

palisoti, Daedalea Fr., Syst. Myc., Vol. 1, p. 335, 1821.
laevis, Daedalea Hook., in Kuenth., Syn., p. 9, 1822.
deplanata, Daedalea Fr., Linnaea, Vol. 5, p. 513, 1830.
polita, Daedalea Fr., Linnaea, Vol. 5, p. 514, 1830.
applanata, Daedalea Kl., Linnaea, Vol. 8, p. 481, 1833.
repanda, Lenzites (Mont.) Fr., Epicrisis, p. 404, 1838.
pallida, Lenzites Berk., Lond. Jour. Bot., Vol. 1, p. 146, 1842.
muelleri, Daedalea Berk. ex Cke., Grev., Vol. 19, p. 93, 1891.

Collections of species listed above, and other combinations, though varying somewhat in size and colour, possess the same microstructure and cannot be separated on any constant feature. Australian collections at Kew are: "Stannary Hills, Q., Dr. T. L. Bancroft, No. 2039", filed under *Lenzites deplanata*; "Toowoomba, Q., Hartmann", under the cover of *Lenzites repanda*; "Toowoomba, Q.", type of *Daedalea muelleri.* On the sheet of this last Bresadola had noted "= *Tr. ludificans* Ces. = forma abortiva *L. applanata*".

99. PALLENS, MERULIUS Berk., Outlines, p. 256, 1860.

Collections from Australia at Kew filed under this cover are: "Victoria, No. 430", "Tasmania" (three collections) and "Tarwin, No. 21".

pallescens, Polyporus Fr. Specimens ex "Daintree River, Q., Pentzcke" are thick plants of *Polyporus adustus.*

pallida, Lenzites Berk. = *Lenzites palisoti.*

pallisieri, Polyporus Berk. ex Cke., Grev., Vol. 10, p. 98, 1882. The type, ex Saskatchewan, Canada, was referred to *Fomes subroseus* (Weir) Ov., in Rhodora, Vol. 25, 1923. It is not the same as *Fomes pallisieri* (Berk.) Cke., Grev., Vol. 14, p. 21, 1885, which is based on the species later renamed by Cooke *Polyporus argentatus*, a synonym of *Coriolus paleaceus.* Collections from Australia at Kew so referred by Cooke are of *Coriolus paleaceus.*

100. PARGAMENUS, CORIOLUS (Fr.), nov. comb.

pargamenus, Polyporus Fr., Epicrisis, p. 480, 1838.
laceratus, Polyporus Berk., Ann. Nat. Hist., Vol. 2, p. 392, 1839.

Collections at Kew from Australia are filed under *P. laceratus.* They are: "Brisbane, Q., Bailey" and "Illawarra, N.S.W., Karsten, No. 43". Another specimen ex "Adelaide, S. Aus., W. N. Cheesman" may be the same but is too imperfect for accurate diagnosis.

parilis, Polyporus Fr., *Pl. Preiss.*, Vol. 2, p. 136, 1847. No cover is at Kew. The species was described from material collected in Australia, but the description is too incomplete to enable it to be recognized.

parvulus, Polyporus Kl. A specimen from Australia, ex "S.W. Aus., T. Muir, No. 110", filed under this cover = *Coltricia oblectans.* Bresadola noted on the sheet that it is a synonym of *C. cinnamomea.*

101. PECTINATUS, FOMES (Kl.) Cke., Grev., Vol. 10, p. 20, 1885.

pectinatus, Polyporus Kl., Linnaea, Vol. 8, p. 485, 1833.

The following collections from this region are at Kew: "Campbell's Creek, Q., H. Fletcher", "Condamine River, Q., Hartmann, No. 513" (the latter filed under *Fomes spadiceus* and referred by Cooke to *P. substygius* Berk., by Bresadola to *P. nilgheriensis*), "New Guinea, Capt. Armit, No. 18" and "N.Z., Bay of Islands, No. 334".

pelles, Polyporus Lloyd = *Polyporus pelliculosus.*
pelliculosus, Merulius Cke. = *Merulius corium.*

102. PELLICULOSUS, POLYPORUS Berk., Lond. Jour. Bot., Vol. 7, p. 575, 1848.

hystriculus, *Polyporus* Cke., Grev., Vol. 15, p. 16, 1886, type ex Melbourne, Vic., F. M. Reader, No. 13.

pelles, *Polyporus* Lloyd, Syn. sect. Apus Polyporus, p. 327, 1915, type ex Queensland, E. Jarvis.

atro-hispidus, *Polyporus* Lloyd, Myc. Notes, No. 57, p. 823, 1919, type ex Victoria, Jas. Wilson.

strigoso-albus, *Polyporus* Lloyd, Myc. Notes, No. 73, p. 1329, 1924, type ex Australia, J. B. Cleland.

The type ex Penguite is not at Kew; but two specimens, ex "Tasmania, Archer" so labelled by Berkeley may be regarded as neotypes. The following collections agree with these. "Upper Yarra, Vic., Lucas" and "Western Port, Vic., Musgrave". One specimen so referred by Cooke, ex "Gippsland, Vic., Webb" = *Polyporus campylus*.

pendula, *Daedalea* Berk. = *Lenzites unicolor*.

pentzckei, Polyporus Kalch., PROC. LINN. Soc. N.S.W., Vol. 8, p. 175, 1884. The type was from "Daintree River, Q., Pentzcke". No specimens are at Kew.

103. PERADENIAE, CORIOLUS (Berk. & Br.), nov. comb.

peradeniae, *Polyporus* Berk. & Br., Jour. Linn. Soc., Vol. 14, p. 51, 1875.

Bresadola referred the species to *P. cervino-gilvus* Jungh., Crypt. Java, p. 45, 1838; Petch in Lloyd's Mycological Notes, No. 67, p. 1163, 1922, to *P. zeylandicus* Berk. with as synonyms *P. diversiporus* and *P. personatus* Berk. Both were published on later pages of the same periodical in which *Polyporus peradeniae* was described. Filed under *P. peradeniae* are the following collections from Australia: "Dunk Island, Q., W. Cottrell-Dormer, No. 44", "Nambour, Blackball Range, Q., W. N. Cheesman", "Daintree River, Q., Pentzcke", "Clarence River, N.S.W., Thorneton", "Richmond River, N.S.W., Camara", and "Creswick, near Ballarat, Vic., W. N. Cheesman".

perdurans, *Polyporus* Kalch. = *Coltricia oblectans*.

peroxydatus, Polyporus Berk., *Jour. Linn. Soc.*, Vol. 16, p. 39, 1878. The type was ex "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition". No specimens are at Kew or British Museum of Natural History.

persooni, *Polystictus* Cke. = *Coriolus corrugatus*.

104. PES-CAPRAE, POLYPORUS (Pers.) Fr., Syst. Myc., Vol. 1, p. 354, 1821.

tasmanicus, *Polyporus* Mass., Kew Bull. Misc. Inf., p. 179, 1899.

Both collections, ex "Australia" and "Tasmania, L. Rodway", type of *P. tasmanicus*, resemble European specimens of *P. pes-caprae*. Massee's name cannot be used, since it is preoccupied by *P. tasmanicus* Berk., 1860.

phellina, Trametes Berk., *Jour. Linn. Soc.*, Vol. 13, p. 164, 1873. The type was from "New England, Australia". No cover is at Kew.

picipes, Polyporus Fr. Portion of a specimen ex "Brisbane, Q., No. 168, ex herb. Currey" is under this cover at Kew. The specimen is of *P. elegans*.

picta, *Trametes* Berk. = *Coriolus paleaceus*.

pictilis, Polyporus Berk. A specimen at Kew, so labelled by Cooke, ex "S.E. New Guinea, Capt. Armit" = *Coriolus flabelliformis*.

105. PINSITUS, CORIOLUS (Fr.) Pat., Tax. hymen., p. 94, 1900.

pinsitus, *Polyporus* Fr., Elench., p. 95, 1828.

Collections at Kew are "Port Denison, Q., Shann", "Dunk Island, Q., W. Cottrell-Dormer, No. 39", "Endeavour River, Q., Persieh", "Daintree River, Q., Pentzcke" (the last filed by Cooke under *P. vellereus*), "Clarence River, N.S.W., Wilcox" (placed under *P. rufescens*). A specimen so named by Massee, ex "N.Z., Colenso, No. 1427" = *Coriolus hirsutus*.

pisiformis, *Polyporus* Kalch. = *Coriolus hirsutus*. Type specimens ex "Richmond River, N.S.W." are immature and about the size of a grain of wheat. Their microstructure is that of *C. hirsutus*.

106. PLACODES, COLTRICIA (Kalch.), nov. comb.

placodes, *Polyporus* Kalch. ex Thuem., Grev., Vol. 4, p. 73, 1875.

luteo-olivaceus, *Polyporus* Berk. & Br., Trans. Linn. Soc., Ser. II, Vol. 1, p. 402, 1879.

The type of *P. luteo-olivaceus* ex "Brisbane, Q., Bailey" matches that of *P. placodes* ex "Rockhampton, Q., Mrs. Thozet", the prior name. Other collections at Kew, filed

under *P. luteo-olivaceus* are: "Port Denison, Q., Shann, No. 19", "Endeavour River, Persieh", "Toowoomba, Q., Hartmann", "Enoggera, Q., C. T. White", "Toowoomba, Q.", "Clarence River, N.S.W., Wilcox", "Richmond River, N.S.W., Camara" and "Moonan Brook, Vic., Miss Carter".

platotis, Polyporus Berk. & Br. = *Polyporus fusco-lineatus.*

plebius, Polyporus Berk. A specimen ex "Brisbane, Q., herb. Broome" so referred is of *Fomes gilvus.* No others are at Kew from this region.

pocula, Polyporus (Schw.) Berk. & Curt. = *Polyporus pusillus.*

polita, Daedalea Fr. = *Lenzites palisoti.*

107. POLYGRAMMA, HEXAGONA (Mont.) Fr., Epicrisis, p. 497, 1838.

polygrammus, Polyporus Mont., Ann. Sci. Nat., Ser. II, Vol. 8, p. 365, 1837.

rigida, Hexagona Berk., Jour. Linn. Soc., Vol. 16, p. 54, 1878, type ex Lord Howe Island, J. P. Fallagher.

subtenuis, Hexagona Berk. ex Cke., Grev., Vol. 19, p. 103, 1891, type ex Rockhampton, Q., Mrs. Thozet.

Additional collections at Kew are: "Australia, S.15, S.19", "Queensland, Walter", "Brisbane, Q., ex herb. Broome", "Goode Island, Torres Strait, Powell", "Toowoomba, Q., Hartmann", "Tweed River, N.S.W., Camara" and "Illawarra, N.S.W., Johnson". Under *Hexagona rigida* are filed "Lord Howe Island, C. French, Jr.", "Kin Kin, Q., W. D. Francis", "Dunk Island, Q., W. Cottrell-Dormer, No. 35", and "New Guinea, Capt. W. Armit". Under the cover of *H. nitida* is one collection ex "Samoa, W. Powell" which Lloyd on the sheet referred to *H. tenuis.* Under *H. subtenuis* are "Queensland, Bailey", "Port Denison, Q., Shann" and "Botanic Gardens, Sydney, N.S.W., A. Grant".

polymorphus, Polyporus Rostk. Under this cover Cooke placed four fragments ex "Endeavour River, Q., Persieh". They are too fragmentary to name, but do not resemble European specimens in the cover.

ponderosus, Polyporus Kalch. = *Fomes mastoporus.*

108. PORPHYRITES, POLYPORUS Berk., Hook. Jour. Bot., Vol. 8, p. 196, 1856.

Specimens under this cover at Kew are: "Tropical Queensland, Bailey" and "Johnstone River, Q., Berthoud".

109. PORTENTOSUS, POLYPORUS Berk., Lond. Jour. Bot., Vol. 3, p. 188, 1844.

eucalyptorum, Polyporus Fr., Pl. Preiss., Vol. 2, p. 135, 1847, type ex Australia.

hololeuca, Trametes Kalch., Hedw., Vol. 15, p. 114, 1876, type ex Victoria.

leucocreas, Polyporus Cke., Grev., Vol. 8, p. 55, 1879, type ex N.Z., Coromandel, No. 311.

dielsii, Polyporus P. Henn., Hedw., Vol. 42, p. 75, 1903, type ex Western Australia.

cretaceus, Polyporus Lloyd, Syn. sect. Apus Polyporus, p. 302, 1915, type ex Tasmania, L. Rodway.

maculatissimus, Polyporus Lloyd, Myc. Notes, No. 66, p. 1113, 1922, type ex Tasmania, L. Rodway, No. 1037.

albo-fuscus, Polyporus Lloyd, Myc. Notes, No. 73, p. 1318, 1924, type ex South Australia, J. B. Cleland, No. 873.

Though the type ex "Swan River, W. Aus., Drummond, No. 125" is now somewhat fragmentary, the microstructure is so characteristic that identification is possible on this feature alone. Other collections at Kew are: "Toowoomba, Q., No. 11", "Toowoomba" (the latter filed under *P. betulinus*), "North Queensland", "Parramatta River, N.S.W., Challenger Expedition", "Melbourne, Vic., F. M. Reader", "Tasmania", "New Guinea, Capt. Armit" and "N.Z., York Bay, G.H.C.", the last identified by Lloyd as *P. eucalyptorum.*

praetervisa, Trametes Bres. = *Hexagona gunnii.*

110. PROTEA, TRAMETES (Berk.), nov. comb.

proteus, Polyporus Berk., Lond. Jour. Bot., Vol. 2, p. 514, 1843.

rigida, Trametes Berk. & Mont., Ann. Sci. Nat., Ser. III, Vol. 11, p. 240, 1849, type ex Bahia, Brazil.

gourliaei, Polyporus Berk., Fl. Tas., Vol. 2, p. 253, 1860, type ex Van Diemen's Land, ex Wm. Gourlie.

menziesii, Trametes Berk., in herb. Kew.

chrysoleucus, Polyporus Kalch. ex Thuem., Grev., Vol. 4, p. 72, 1875, type ex Rockhampton, Q., Thozet, No. 768.

semidigitaliformis, Polyporus Berk., Jour. Linn. Soc., Vol. 16, p. 39, 1878, type ex Pennant Hills, Parramatta River, N.S.W.
gausapatus, Polyporus Kalch. ex Cke., Grev., Vol. 10, p. 102, 1882, type ex Richmond River, N.S.W.
illotus, Polyporus Kalch. ex Cke., Grev., Vol. 10, p. 102, 1882, type ex Richmond River, N.S.W.
carteri, Trametes Berk., ex Sacc., Syll. Fung., Vol. 9, p. 196, 1891.

Additional collections at Kew from this region are: "Toowoomba, Q., Hartmann", "Moreton Bay, Q., Bailey", "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition" and "Tweed River, N.S.W., Guilfoyle", filed under *T. protea.* Under *Polystictus versicolor* is a collection ex "South Australia, J. B. Cleland"; under *Trametes carteri* is filed "Adelaide, National Park, S. Aus., W. N. Cheesman, No. 3". A resupinate specimen ex "Clarence River, N.S.W., Wilcox" is filed under *Poria corium* Kze.; a second, ex "Australia", is under *Poria calcicola.* Under *T. gausapata,* labelled on the sheet *T. menziesii,* is placed "Kurrumburra, Vic., No. 1039"; a second collection under this cover is "Endeavour River, Q., Persietz". Under *T. versiformis* Cooke placed "Rockhampton, Q., Mrs. Thozet, No. 131"; and "New Caledonia, F. J. Roberts" is filed under *T. gibbosa.*

111. PROTEIFORMA, TRAMETES (Cke.), nov. comb.

proteus, Polyporus Kalch. ex Cke., Grev., Vol. 10, p. 102, 1882, non Berk., 1843.
proteiformis, Polystictus Cke., Grev., Vol. 14, p. 81, 1886.

The only specimen at Kew is the type ex "Melbourne, Vic., F. v. Mueller". The name was changed by Cooke since *Polyporus proteus* was preoccupied.

112. PROTEIPORUS, POLYPORUS Cke., Grev., Vol. 12, p. 15, 1883.

The type ex "Toowoomba, Q., Hartmann" and "Gippsland, Vic." are the only collections at Kew. Lloyd (*Syn. stip. Polyp.*, p. 162, 1912) referred the species as a synonym of *P. rufescens*; but it is doubtful if the latter occurs in the region.

proteus, Polyporus Kalch. = *Trametes proteiforma.*

protracta, Lenzites Fr. = *Daedalea trabea.*

pubescens, Polyporus Fr. A collection from Australia, ex "Endeavour River Q., Persieh", so referred by Cooke, consists of two plants of *Coltricia fruticum.*

pullagerii, Polyporus Berk., *Jour. Linn. Soc.*, Vol. 16, p. 54, 1878. No specimens are at Kew or British Museum of Natural History.

113. PULLATUS, AMAURODERMA (Berk.), nov. comb.

pullatus, Polyporus Berk. ex Cke., Grev., Vol. 13, p. 117, 1885, nomen nudum.
pullatus, Fomes Berk. ex Cke., Grev., Vol. 15, p. 21, 1886.

Specimens from Australia, ex "Brisbane, Q., F. M. Bailey, Nos. 730, 764, 766" and "Trinity Bay, Q., Karsten", match the type at Kew ex Hong Kong.

pullus, Fomes (Berk. & Mont.) Cke. Though recorded from Queensland by Cooke (*Hdbk.*, p. 133, 1892) there are no specimens from this region at Kew.

114. PUNCTATA, FUSCOPORIA (Fr.) G. H. Cunn., Plant Diseases Division, Bull. 73, p. 11, 1948.

punctatus, Polyporus Fr., Hym. Eur., p. 572, 1874.

One specimen of the species, ex "Swan River, W. Aus., Drummond", is filed under *Poria contigua* and on the sheet labelled *Poria ferrugineus.* Part of the same specimen is also under the cover of *Poria ferruginosa.*

115. PUSILLUS, POLYPORUS (Fr.), nov. comb.

pusillus, Favolus Fr., Linnaea, Vol. 5, p. 511, 1830.
cupuliformis, Polyporus Berk. & Curt., Hook. Jour. Bot., Vol. 1, p. 103, 1849.
pocula, Polyporus (Schw.) Berk. & Curt., Proc. Am. Acad. Arts and Sci., Vol. 4, p. 122, 1858.

Specimens from this region, ex "Melbourne, Vic., Mrs. Martin, No. 606" (filed under *P. cupuliformis*) and "N.Z., Bay of Islands, No. 236" (filed under *Favolus rhipidium*), match part of the type ex Brazil. A collection at Kew named *Favolus pusillus,* ex "Van Diemen's Land, Gunn" = *Polyporus rhipidium.*

116. PYRRHOCREAS, FOMES Cke., Grev., Vol. 14, p. 11, 1885.

Represented at Kew by the type collection, ex "New Guinea, Capt. Armit". On the sheet Bresadola had noted: "= *Polyporus albo-marginatus* Lev. = *laeticolor* Berk. = *kermes* Berk." Lloyd, on the type sheet, referred the species to *P. croceus* Pers.

117. PYRRHOCREAS, TRAMETES Berk., Jour. Linn. Soc., Vol. 13, p. 164, 1873.

The type consists of two specimens ex "Herbert's Creek, Q.". Other collections which match the type are "Brisbane, Q., F. M. Bailey, No. 337", "Daintree River, Q., Pentzcke", "Guntawang, N.S.W., Hamilton, No. 26", "Clarence River, N.S.W., Wilcox" and "Samoa, C. G. Lloyd", the last being filed under *P. proteus* Berk.

quadrans, Polyporus Berk. & Br., *Trans. Linn. Soc.*, Ser. II, Vol. 1, p. 400, 1879. No specimens are at Kew or British Museum of Natural History.

quercina, Daedalea Fr. A specimen so referred, ex "Bot. Gardens, Adelaide, S. Aus., W. N. Cheesman", is a weathered plant of *Lenzites aspera.*

radiato-rugosis, Polyporus Berk., *Ann. Nat. Hist.*, Vol. 3, p. 323, 1839. No specimens are at Kew or British Museum of Natural History.

118. RADULA, PORIA (Pers. ex Fr.) Cke., Grev., Vol. 14, p. 111, 1886.

radulus, Polyporus (Pers.) Fr., Syst. Myc., Vol. 1, p. 383, 1821.

vinctus, Polyporus Berk., Ann. Mag. Nat. Hist., Ser. II, Vol. 9, p. 196, 1852.

Specimens ex "Daintree River, Q., Pentzcke" are filed under *Poria vincta*; "Dunk Island, Q., W. Cottrell-Dormer, No. 14" is under *Poria hyposclera*; and "Bellenden Ker, Q., Karsten" Cooke placed under *Fomes bistratosus.*

rasipes, Polyporus Berk. = *Polyporus vernicifluus.*

repanda, Lenzites (Mont.) Fr. = *Lenzites palisoti.*

retiporus, Polyporus Cke. = *Polyporus berkeleyi.*

119. RHINOCEPHALUS, POLYPORUS Berk., Fl. Tas., Vol. 2, p. 253, 1860.

The type, ex "Tasmania, Archer", is the only collection at Kew.

120. RHIPIDIUM, POLYPORUS Berk., Lond. Jour. Bot., Vol. 6, p. 319, 1847.

rhipidium, Favolus (Berk.) Cke., Grev., Vol. 15, p. 58, 1886.

diminutus, Polyporus Mass., Jour. Bot., Vol. 34, p. 153, 1896.

nanus, Polyporus Mass., in herb. Kew.

Filed under *Favolus rhipidium* are the following collections: "Sunday Island, Kermadecs, W. R. B. Oliver", "Raoul Island, Kermadecs, McGilivray, voyage of H.M.S. Herald", "Illawarra, N.S.W., Kirton, No. 44", "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition", "Victoria, Dr. Winter" and "Melbourne, Vic., F. M. Reader". Under *P. nanus* is a collection ex "Port Phillip, Vic., F. M. Reader, No. 31"; and under *Favolus pusillus* is filed one ex "Van Diemen's Land, Gunn".

rigescens, Polyporus Cke. = *Polyporus liebmannii.*

rigida, Hexagona Berk. = *Hexagona polygramma.*

rigida, Trametes Berk. & Mont. = *Trametes protea.*

rigidus, Polyporus Lloyd = *Inonotus tabacinus.*

121. RIMOSUS, FOMES (Berk.) Cke., Grev., Vol. 14, p. 18, 1885.

Under this cover at Kew the type collection is given as "Van Diemen's Land, Lawrence"; but in the published description it was stated to be "Swan River, W. Aus., Drummond, No. 144". One other specimen under the cover, ex "Daintree River, Q., Pentzcke" = *Fomes setulosus.*

rosea, Trametes Lloyd = *Trametes lilacino-gilva.*

rosettus, Polyporus Lloyd = *Polyporus campylus.*

rubidus, Polyporus Berk. Three specimens so referred, ex "North Queensland", "Brisbane, Q." and "Dunk Island, Q., W. Cottrell-Dormer, No. 28", are of *Coriolus paleaceus.*

rubiginosus, Polyporus Berk. = *Fomes gilvus.*

122. RUDIS, AMAURODERMA (Berk.), nov. comb.

rudis, Polyporus Berk., Ann. Nat. Hist., Vol. 3, p. 323, 1839.

Specimens at Kew match the type ex "Tasmania, herb. Sir W. Hooker". These are: "Queensland, F. M. Bailey", "Queensland, Campbell, No. 96" (the latter filed under *Polyporus ovinus*), "Melbourne, Vic., F. Reader, No. 40" and "New Caledonia, J. F. Roberts". One specimen so labelled, ex "N.Z., Colenso", = *P. xerophyllus.*

rufa, Poria (Fr.) Cke. Though recorded from Victoria by Cooke (*Hdbk.*, p. 155, 1892), there are no specimens from this region at Kew.

rufescens, Polyporus Fr. Collections from Australia do not resemble those from India on the same sheet. One, "Clarence River, N.S.W., Wilcox" = *Coriolus pinsitus*;

a second, ex "New Guinea", was on the sheet referred by Boedijn to *T. corrugata*; two others, ex "Pennant Hills, Parramatta River, N.S.W." and "Lake Muir, S.W. Aus., T. Muir", are unrecognizable.

rufi-tincta, Poria (Berk. & Curt.) Cke. A specimen so referred by Cooke, ex "Australia, herb. Kalchbrenner" = *Fuscoporia laevigata*.

rufo-lateritius, Polyporus Kalch. ex Cke., *Grev.*, Vol. 10, p. 104, 1882. No specimens are at Kew. The description of the type ex "Richmond River, N.S.W." suggests the species was based on *Polyporus merulius*.

rufo-rigidus, *Polystictus* Lloyd = *Coriolus flabelliformis*.

rufo-rugosus, *Polyporus* Lloyd = *Coriolus zonatus*.

123. RUGICEPS, POLYPORUS Lloyd, Myc. Notes, No. 73, p. 1329, 1924.

Part of the type is at Kew, ex "Victoria, Jas. Wilson".

124. RUGOSUS, AMAURODERMA (Fr.), nov. comb.

rugosus, *Polyporus* Fr., Elench., p. 74, 1828, non Nees, 1826.

The following collections from this region are at Kew: "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition", "S.E. New Guinea, Capt. Armit" and "Mt. Bedford, New Guinea, Capt. Armit".

125. RUSSICEPS, POLYPORUS Berk. & Br., Jour. Linn. Soc., Vol. 14, p. 48, 1875.

cinnamomeo-squamulosus, *Polyporus* P. Henn., Engl. Bot. Jahrb., Vol. 30, p. 43, 1901.
megaloporus, *Polyporus* Lloyd, Myc. Notes, No. 48, p. 684, 1917.

The only authentic collection at Kew from this region is ex "Coomera River, Q., C. T. White", filed under *Favolus megaloporus* (Mont.) Bres. One collection, ex "New Guinea, Fly River, Bauerlen" = *Polyporus infernalis*; a second, ex "New Guinea, Strickland River, Bauerlen" = *P. xerophyllus*.

126. SAEPIARIA, DAEDALEA Fr., Syst. Myc., Vol. 1, p. 333, 1821.

saepiaria, *Lenzites* Fr., Epicrisis, p. 407, 1838.

A collection ex "National Park, Adelaide, S. Aus., W. N. Cheesman, No. 35", filed under *Lenzites saepiaria*, agrees with specimens from Upsala, so named by Fries.

salicinus, Fomes (Fr.) Cke. The specimen so referred, ex "Trinity Bay, Q., Sayer, No. 40" = *Coltricia weberiana*.

sanguinarius, Polyporus Kl. Bresadola referred to this species, on the sheet, a specimen ex "Clarence River, N.S.W., Moreton" which Cooke had filed under *P. oblinitus* Berk. It is *Trametes ferrea*.

sanguinea, *Daedalea* Kl. = *Coriolus corrugatus*.

127. SANGUINEUS, CORIOLUS (Fr.), nov. comb.

sanguineus, *Polyporus* Fr., Syst. Myc., Vol. 1, p. 371, 1821.
cinnabarinus, *Polyporus* Fr., Syst. Myc., Vol. 1, p. 371, 1821.
coccineus, *Polyporus* Fr., Nov. Symb., p. 67, 1851.
puniceus, *Polyporus* Kalch., Rev. Myc., Vol. 4, p. 95, 1882.
semi-sanguineus, *Polystictus* Lloyd, Letter 39, p. 8, 1912.

In Kew herbarium collections are placed under *Polystictus sanguineus* and *P. cinnabarinus*. In some instances the collection has been divided and placed under both. There is no constant feature upon which two species may be maintained, consequently collections should be merged under *C. sanguineus*, since this precedes *P. cinnabarinus* on the same page of Systema.

Australian collections at Kew are: "Trinity Bay, Q., W. A. Sayer", "Endeavour River, Q., Persieh" (under both covers), "Daintree River, Q., Pentzcke", "Condamine River, Q.", "Port Denison, Q., Shann", "Herberton, Q.", "Twofold Bay, Q., White", "Britie Island, Q., C. E. Hubbard", "Stadbroke Island, Q., C. E. Hubbard", "Moreton Bay, Q., F. M. Bailey", "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition" (in both covers), "Sydney, N.S.W., J. B. Cleland", "Mt. Dromedary, N.S.W., Miss Bates", Richmond River, N.S.W., Camara", "Narranoom, Vic., Stranger", "Melbourne, Vic., G. LeFevre", "North Gippsland, Vic., H. Tisdall", "W. Aus.", "King George Sound, Harris", "Tasmania, L. Rodway, No. 1379".

128. SCABER, FOMES (Berk.) Lloyd, Syn. genus Fomes, p. 249, 1915.

igniarius var. *scaber*, *Polyporus* Berk., Ann. Nat. Hist., Vol. 3, p. 324, 1840.
tepperi, *Fomes* Lloyd, Syn. genus Fomes, p. 256, 1915.

Lloyd erected the species upon a specimen ex "Tasmania" which at Kew was filed under *Fomes rimosus*, though published as *Polyporus igniarius* var. *scaber*. A second specimen is at Kew ex "Victoria, ex herb. Lloyd". Filed under *Fomes tepperi* is part of the type ex "Norwood, S. Aus., J. G. O. Tepper" and in addition "Sydney, N.S.W., J. B. Cleland, No. 291". These two collections match the type of *F. scaber*.

scabriusculus, Polyporus Berk., *Jour. Linn. Soc.*, Vol. 18, p. 384, 1881. No specimens are at Kew or British Museum of Natural History.

scabrosus, *Polyporus* Berk. = *Fomes gilvus*, pro parte.

129. SCALARIS, LENZITES (Berk. & Br.), nov. comb.

scalaris, Daedalea Berk. & Br., Trans. Linn. Soc., Ser. II, Vol. 2, p. 61, 1883.

The type, ex "Brisbane, Q., Bailey, No. 429" is in the British Museum of Natural History.

scansilis, Polyporus Berk. = *Fomes applanatus.*

schomburgkii, Daedalea Berk. = *Lenzites tenuis.*

130. SCHWEINITZII, COLTRICIA (Fr.) G. H. Cunn., Plant Diseases Division, Bull. 77, p. 7, 1948.

schweinitzii, Polyporus Fr., Syst. Myc., Vol. 1, p. 351, 1821.
tabulaeformis, Polyporus Berk., Lond. Jour. Bot., Vol. 4, p. 302, 1845.
albertinii, Polyporus Lloyd, Syn. stip. Poly., p. 160, 1912.

Two specimens are at Kew from Australia, "Brisbane, Q., F. M. Bailey, No. 763", filed under *P. tabulaeformis*; and "Endeavour River, Q., Persieh", which Lloyd erected as *P. albertinii* because the spores were slightly coloured, a condition not uncommon in old specimens.

131. SCOPULOSUS, POLYPORUS Berk., Hook. Jour. Bot., Vol. 6, p. 143, 1854.

A specimen ex "Daintree River, Q., Pentzcke" resembles the type, ex Sikkim, India.

scorteus, Polyporus Fr. Recorded by Cooke (*Hdbk.*, p. 149, 1892) from New South Wales. No specimens are at Kew from this region. The species is in any event a synonym of *Coriolus occidentalis.*

scrobiculata, Trametes Berk. = *Fomitopsis ochroleuca.*

132. SCRUPOSUS, FOMES (Fr.) G. H. Cunn., Plant Diseases Division, Bull. 79, p. 11, 1948.

scruposus, Polyporus Fr., Epicrisis, p. 473, 1838.
breviporus, Polyporus Cke., Grev., Vol. 12, p. 17, 1883.

Australian collections at Kew are filed under several species. In the cover of *Fomes gilvus* are "Enoggera, Q., F. M. Bailey", "Toowoomba, Q., Hartmann", "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition" and "Port Albert, Vic.". Under *Fomes fomentarius* is "King George Sound, W. Aus., Harris". "Bellingen River, N.S.W." is filed under *Polyporus fruticum*. Under *Fomes holosclereus* is "Endeavour River, Q., Persietz". Filed under *Fomes inflexibilis* is "Mt. Williams, Bellingen River, N.S.W.". Under *Polyporus laurencii* is "Gippsland, Vic., Miss Campbell". The type of *P. breviporus* is ex "Endeavour River, Q., Persietz".

133. SCUTELLATA, FOMITOPSIS (Schw.) G. H. Cunn., Plant Diseases Division, Bull. 76, p. 6, 1948.

scutellatus, Polyporus Schw., Trans. Am. Phil. Soc., Ser. II, Vol. 4, p. 157, 1832.
atro-albus, Fomes P. Henn., Monsunia, Vol. 1, p. 144, 1899.
yoshinagae, Polyporus Lloyd, Letter 54, p. 5, 1915.

Collections from this region, especially those from New Zealand, match part of the type at Kew, "Bethlehem, U.S.A., ex herb. Schweinitz". Only one specimen from Australia is at Kew, ex "Brisbane, Q., No. 978", filed under *Fomes cryptarum.*

selecta, Poria Karst. A specimen so filed, ex "Milsom Island, Hawkesbury River, N.S.W., J. B. Cleland, No. 17" = *Poria calcicola.*

semi-digitaliformis, Polyporus Berk. = *Trametes protea.*

134. SENEX, FOMES (Nees & Mont.) Cke., Grev., Vol. 13, p. 118, 1885.

senex, Polyporus Nees & Mont., Ann. Sci. Nat., Ser. II, Vol. 5, p. 70, 1836.

Two collections are at Kew: "Daintree River, Q., T. Pentzcke" (filed under *Fomes igniarius*) and "Dunk Island, Q., W. Cottrell-Dormer, No. 8".

seriatus, Polyporus Kalch. = *Trametes ferrea.*

sericea, Hexagona Fr. Though recorded by Cooke (*Hdbk.*, p. 164, 1892) from Queensland, there are no specimens at Kew from Australia.

serpens, Merulius Fr. Cooke (*Hdbk.*, p. 169, 1892) recorded the species from Queensland, but there are no specimens from the region at Kew.

135. SERPENS, TRAMETES Fr., Hym. Eur., p. 586, 1874.

serpens, Daedalea Fr., Syst. Myc., Vol. 1, p. 340, 1821.
stephensii, Polyporus Fr. ex Berk., Outlines, p. 252, 1860.

Two collections are at Kew from Australia, "Queensland, C. Lumholtz" and "Pennant Hills, Parramatta River, N.S.W., Challenger Expedition".

136. SETIPORUS, INONOTUS (Berk.), nov. comb.

intybaceus, Polyporus Berk., Lond. Jour. Bot., Vol. 1, p. 149, 1842.
setiporus, Polyporus Berk., Lond. Jour. Bot., Vol. 6, p. 505, 1847.
cichoraceus, Polyporus Berk. ex Fr., Nov. Symb., p. 92, 1851.
tabacina, Hexagona Lev., in Zoll. Syst. Ind. Arch., p. 15, 1854.
arata, Hexagona Berk., Jour. Linn. Soc., Vol. 16, p. 43, 1877.

The species was first named *P. intybaceus*; but as this was preoccupied, Fries changed the name to *P. cichoraceus* Berk. Meanwhile Berkeley had again named it *P. setiporus*, so this becomes the valid specific name. The following collections are at Kew, all filed under *P. cichoraceus*: "Trinity Bay, Q., Sayer, No. 2", "North Queensland", "Brisbane, Moreton Bay, Q., Bailey", "Morunga, N.S.W., W. N. Cheesman", "Sugarloaf Mt., N.S.W., F.v.M.", "Tweed River, N.S.W., Camara, No. 86", "Clarence River, N.S.W., Wilcox", "Box Hill, Vic., Mrs. Martin, No. 446" and "Sealer's Cove, No. 143". The type of *P. setiporus* was from Ceylon, of *P. intybaceus* from Philippines, and of *H. arata* from Aru Island.

137. SETULOSUS, FOMES Lloyd, Syn. gen. Fomes, p. 243, 1915.

multisetosus, Polyporus Lloyd, Myc. Notes, No. 63, p. 976, 1920, type ex Ararat, Vic., E. J. Semmens, No. 5.

Collections from this region match part of the type at Kew ex Ceylon. They have been filed under several species: "Twofold Bay, Q., White", "Port Jackson, N.S.W., Hewland" and "N.Z., Colenso, No. 25" are under the cover of *Fomes fomentarius*; "Port Denison, Q., Shann" and "King George Sound, W. Aus., Harris" are under *Fomes fulvus*; "Port Denison, Q., Shann" is also under *Fomes igniarius*; "Daintree River, Q., Pentzcke" is filed under *Fomes rimosus*.

138. SIMILIS, HEXAGONA Berk., Lond. Jour. Bot., Vol. 5, p. 4, 1846.

Two collections are filed under this cover at Kew, the type ex "Australia—gathered by one of the officers of the Beagle" and "Australia".

similis, Polyporus Berk. = *Polyporus arcularius*.

139. SINULOSUS, CORIOLUS (Kl.), nov. comb.

sinulosa, Daedalea Kl., Linnaea, Vol. 8, p. 482, 1833.
microsinulosa, Daedalea Fr., Epicrisis, p. 495, 1838.

Collections at Kew from Australia are: "Endeavour River, Q., Persietz" and "Port Denison, Q., Shann".

sinuosa, Poria (Fr.) Cke. Cooke (*Hdbk.*, p. 156, 1892) recorded the species from New South Wales, but there are no specimens from Australia at Kew.

sordidus, Polyporus Berk. Two collections from Australia are filed under this cover at Kew. One, ex "Govt. Domain, Melbourne, Vic.", is fragmentary but probably *Coriolus paleaceus*; the second, ex "Queensland", is, as Bresadola had referred it on the sheet, a specimen of *Fomes strigatus*.

140. SPADICEUS, FOMES (Berk.) Cke., Grev., Vol. 14, p. 20, 1885.

spadiceus, Polyporus Berk., Ann. Nat. Hist., Vol. 18, p. 388, 1839.

The two collections at Kew agree with the type ex India. They are ex "Tropical Queensland, Bailey" and "Strickland River, New Guinea, Bauerlen, Nos. 50, 54". A third collection, filed under this cover, ex "Condamine River, Q., Hartmann, No. 513", Cooke referred to *P. substygius* Berk., Bresadola to *P. nilgheriensis* Mont. It is of *Fomes pectinatus*.

spathulatus, Favolus (Jungh.) Bres. A collection so referred, ex "Dunk Island, Q., W. Cottrell-Dormer, No. 41", is a branched specimen of *Polyporus grammocephalus*.

141. SPICULIFER, POLYPORUS Cke., Grev., Vol. 15, p. 20, 1886.

Two collections are at Kew, both labelled type: "North Gippsland, Vic., Webb" and "North Gippsland, Vic., H. Tisdall, No. 23". A third, ex "N.Z., Waitaki, No. 348", filed under this cover, is a weathered specimen of *Coriolus hirsutus*.

142. SPRUCEI, CORIOLUS (Berk.), nov. comb.

sprucei, Trametes Berk., Hook. Jour. Bot., Vol. 8, p. 236, 1856.

sprucei, Daedalea Berk., Trans. Linn. Soc., Ser. II, Vol. 1, p. 402, 1879.

Four Australian collections at Kew agree with the type, ex Panure, namely, "Cape York, Q.", "Endeavour River, Q., Persieh", "Rockhampton, Q., Mrs. Thozet, No. 136" and "Port Jackson, N.S.W., Dr. Wolls". The species is closely related to *C. lactineus*.

squamiger, Favolus Berk. = *Polyporus arcularius.*

sterinus, Polyporus Berk. & Curt. = *Polyporus liebmannii.*

stereoides, Polystictus Berk. & Curt. = *Polyporus liebmannii.*

stowardii, Trametes Lloyd. = *Trametes lilacino-gilva.*

strangeri, Polyporus Kalch., PROC. LINN. SOC. N.S.W., Vol. 7, p. 106, 1883. Type ex "Riverina, N.S.W.". No specimens are at Kew or British Museum of Natural History. The description suggests the species was based on a specimen of *P. hypomelanus* or *P. infernalis.*

striata, Lenzites Fr. = *Daedalea trabea.*

143. STRIGATUS, FOMES (Berk.) Cke., Grev., Vol. 14, p. 20, 1885.

strigata, Trametes Berk., Lond. Jour. Bot., Vol. 6, p. 502, 1847.

xerophyllaceus, Polyporus Currey, Trans. Linn. Soc., Ser. II, Vol. 1, p. 120, 1882.

lineato-scaber, Fomes Berk. & Br., Trans. Linn. Soc., Ser. II, Vol. 2, p. 59, 1883.

curreyi, Polyporus Berk. ex Cke., Grev., Vol. 15, p. 21, 1886.

The following collections from this region agree with the type ex Hautane, Ceylon, at Kew: "North Queensland, J. E. T. Wood, No. 357", type of *F. lineato-scaber* (in British Museum of Natural History), "Trinity Bay, Q., No. 527", "Clarence River, N.S.W., Wilcox" and "Yambi Bay, New Caledonia, Dr. Sarasin, No. 37".

strigoso-albus, Polyporus Lloyd = *Polyporus pelliculosus.*

strumosus, Polyporus Fr. Cooke (*Hdbk.*, p. 125, 1892) recorded the species from Victoria, but there are no specimens at Kew from this region.

stypticus, Polyporus Fr. A specimen so referred by Cooke, ex "Geographe Bay, W. Aus.", was filed by Miss Wakefield under *Polyporus australiensis.*

suaderis, Polyporus Lloyd = *Fomitopsis tasmanica.*

144. SUBCAPERATUS, CORIOLUS (Lloyd), nov. comb.

subcaperatus, Polystictus Lloyd, Myc. Notes, No. 64, p. 996, 1920.

Part of the type is at Kew, ex "Sydney, N.S.W., J. B. Cleland, No. 560".

subcongener, Daedalea Berk. = *Coriolus occidentalis.*

145. SUBFERRUGINEA, DAEDALEA (Berk.), nov. comb.

subferruginea, Lenzites Berk., Hook. Lond. Jour. Bot., Vol. 6, p. 134, 1854.

bifasciata, Lenzites Cke. & Mass., Grev., Vol. 21, p. 37, 1892, type ex Victoria, Mrs. Martin, No. 995.

adusta, Lenzites Lloyd, Myc. Notes, No. 65, p. 1072, 1921, type ex Australia, J. B. Cleland.

The type of *L. bifasciata* is the only Australian collection at Kew. It matches the type of *D. subferruginea* from Khasia Mts., India.

subsulcata, Daedalea Berk. & Br. Specimens ex "Goode Island, Torres Strait" = *Lenzites beckleri*; those from "Sunday Island, Kermadecs, W. R. B. Oliver" = *Lenzites aspera.* Both were filed under the cover of *D. subsulcata.*

subtenuis, Hexagona Berk. = *Hexagona polygramma.*

subvaporaria, Poria Cke. = *Poria versipora.*

146. SUBVINCTA, PORIA (Berk. & Br.) Cke., Grev., Vol. 14, p. 109, 1886.

subvinctus, Polyporus Berk. & Br., Jour. Linn. Soc., Vol. 14, p. 54, 1875.

The type ex "Tasmania" is the only collection at Kew.

subzonalis, Polyporus Cke. = *Coriolus blumei.*

147. SULPHUREUS, POLYPORUS Fr., Syst. Myc., Vol. 1, p. 357, 1821.

Three collections are at Kew from Australia: "Endeavour River, Q., Persietz, No. 26", "Brisbane, Q., F. M. Bailey, No. 647" and "Lake Cootharata, Q., F. M. Bailey".

148. SUPERPOSITUS, POLYPORUS Berk., Jour. Linn. Soc., Vol. 13, p. 161, 1873.

The type, from "New England, Vic., Australia", is matched by a second collection at Kew ex "Strickland River, New Guinea".

tabacina, Hexagona Lev. = *Inonotus setiporus.*

149. TABACINUS, INONOTUS (Mont.) Karst., Rev. Myc., Vol. 3, p. 19, 1881.

tabacinus, Polyporus Mont., Ann. Sci. Nat., Ser. III, Vol. 2, p. 349, 1835.
xerampelinus, Polyporus Kalch. ex Thuem., Grev., Vol. 4, p. 72, 1875, type ex Rockhampton, Q., A. Thozet.
glabro-tabacinus, Polystictus Lloyd, Myc. Notes, No. 67, p. 1152, 1922, type ex Hobart, Tasmania, L. Rodway, No. 1152.
rigidus, Polyporus Lloyd, Myc. Notes, No. 73, p. 1319, 1924, nomen nudum.
gilvo-rigidus, Polyporus Lloyd, Myc. Notes, No. 74, p. 1334, 1925, type ex N.Z., Dunedin, H. K. Dalrymple.

Additional collections at Kew from this region are: "National Park, Adelaide, S. Aus., W. N. Cheesman, No. 97" and "New Guinea, Rev. Chalmers", the latter being filed under *P. leprieurii* Mont.

tabulaeformis, Polyporus Berk. = *Coltricia schweinitzii.*

150. TARDA, PORIA (Berk.) Cke., Grev., Vol. 14, p. 109, 1886.

tardus, Polyporus Berk., Lond. Jour. Bot., Vol. 4, p. 56, 1845.

The type ex "Swan River, W. Aus., Drummond, No. 130" is the only collection at Kew from this region. One other specimen filed under this cover, ex "Melbourne, Vic., Reader" = *Poria medulla-panis.*

tasmaniae, Polyporus Berk. On the type sheet of *Poria membranicincta* is a specimen labelled *Polyporus tasmaniae* Berk. As Bresadola had noted on the sheet, both are synonyms of *Polyporus merulius.*

tasmanica, Daedalea Sacc. = *Trametes protea.*

151. TASMANICA, FOMITOPSIS (Berk.), nov. comb.

tasmanicus, Polyporus Berk., Fl. Tas., Vol. 2, p. 254, 1860.
cuneatus, Fomes Lloyd, Syn. gen. Fomes, p. 217, 1915, type ex N.Z., Colenso, No. 369.
suaderis, Polyporus Lloyd, Myc. Notes, No. 59, p. 859, 1919, type ex Tasmania, L. Rodway.

The type, ex "Tasmania, Archer", is matched by the types of the synonyms *F. cuneatus* and *P. suaderis.* These are the only collections at Kew.

tasmanicus, Polyporus Mass. = *Polyporus pes-caprae.*

152. TENUIS, HEXAGONA (Hook.) Fr., Epicrisis, p. 498, 1838.

tenuis, Boletus Hook., in Kuenth. Syn. Vol. 1, p. 10, 1822.
muelleri, Hexagona Berk., Jour. Linn. Soc., Vol. 13, p. 166, 1873.

Collections ex "Cape York, Q., Challenger Expedition" and "New England, Vic., Australia" (type of *H. muelleri*) are at Kew.

153. TENUIS, LENZITES (Berk.), nov. comb.

tenuis, Daedalea Berk., Lond. Jour. Bot., Vol. 1, p. 151, 1842.
aulacophylla, Daedalea Berk., Jour. Linn. Soc., Vol. 13, p. 166, 1873, type ex Australia, Schomburgk, No. 58.
schomburgkii, Daedalea Berk. ex Cke., in Muell. Fragm. Phyto. Aus., Suppl. to Vol. 11, p. 86, 1880, type ex Australia.

The following collections are at Kew, filed under *D. aulacophylla*: "Rockhampton, Q., Mrs. Thozet", "Cape York, Q.", "Goode Island, Powell", "Thursday Island, Bauerlen, No. 55", "Raoul Island, Kermadecs, H. M. Herald" and "Clarence River, N.S.W., Wilcox". They agree with the type ex Philippines.

tenuissimus, Merulius Berk. & Br., *Trans. Linn. Soc.*, Ser. II, Vol. 2, p. 62, 1883. The type ex "Brisbane, Q., Bailey, No. 173" now consists of some mycelium upon a fragment of rotting wood. It is unrecognizable.

154. TEPHROLEUCUS, POLYPORUS Fr., Syst. Myc., Vol. 1, p. 360, 1821.

Two collections are at Kew from this region: "Australia, Reader" was filed by Cooke under *P. epileucus*; "Endeavour River, Q., Pentzcke" was placed by Cooke under *P. galactinus.* A specimen ex "Tasmania, Mt. Wellington, Eaton" placed here by Cooke = *P. tephronotus.*

155. TEPHRONOTUS, POLYPORUS Berk., Fl. Tas., Vol. 2, p. 252, 1860.

angustus, Polyporus Berk., Fl. Tas., Vol. 2, p. 253, 1860.

Collections at Kew are the type ex "Tasmania, Archer", the type of *P. angustus*, ex "Tasmania, Archer", "Tasmania, Mt. Wellington, Eaton" filed under *P. tephroleucus*. One specimen ex "Clarence River, N.S.W., Wilcox" filed under *P. tephronotus* by Cooke = *Poria radula*; a second, ex "Pennant Hills, N.S.W., Challenger Expedition" = *Polyporus campylus*.

tepperi, Fomes Lloyd = *Fomes scaber*.

testudo, Polyporus Berk. & Br. = *Fomes durus*.

156. THELEPHOROIDES, POLYPORUS (Hook.) Fr., Epicrisis, p. 473, 1838.

thelephoroides, Boletus Hook., in Kuenth. Syn., Vol. 1, p. 10, 1822.
conchoides, Gloeoporus Mont., Hist. Cuba, p. 385, 1842.

The type, ex "Loxa, Peru, Humboldt, No. 222", is excellently preserved at Kew. It is matched by specimens of *Gloeoporus conchoides* later described by Montagne. Only one collection is at Kew from Australia, ex "Moruya, N.S.W., W. N. Cheesman", though there are several from New Zealand, where the species is abundant.

157. THWAITESII, HEXAGONA Berk., Am. Acad. Arts & Sci., Vol. 4, p. 122, 1860.

mirabilis, Hexagona Lloyd, Syn. gen. Hexagona, p. 37, 1910, type ex Samoa, C. G. Lloyd.

Specimens from Australia at Kew are "Port Denison, Q., Fitzalan, Shann", "Toowoomba, Q., Hartmann, No. 52", "Soane River, Behen", and "Bloomfield River, Bauer". The last was referred on the sheet by C. J. Humphrey to *H. capillacea* Pat.

tomentosus, Polyporus Fr. Recorded by Cooke (*Hdbk.*, p. 137, 1892) from Victoria and Queensland, but no specimens are at Kew from this region.

torrida, Lenzites Kalch. = *Lenzites beckleri*.

158. TRABEA, DAEDALEA (Pers.) Fr., Syst. Myc., Vol. 1, p. 335, 1821.

trabea, Lenzites Fr., Epicrisis, p. 406, 1838.
striata, Lenzites Fr., Epicrisis, p. 406, 1838.
protracta, Lenzites Fr., Vet. Akad. Forh., p. 52, 1851.
clelandii, Lenzites Lloyd, Myc. Notes, No. 61, p. 887, 1919.

Collections at Kew from this region are ex "Brisbane, Q., F. M. Bailey, No. 629", filed under *L. striata*; "Brisbane, Q., C. E. Broome", under cover of *T. rigida* Berk. & Mont.; "Angaston, S. Aus., W. N. Cheesman, No. 4" and "N.Z., Waitaki, No. 254", the latter being filed under *L. abietina*.

tricholoma, Polyporus Mont. One collection so referred by Cooke, ex "Endeavour River, Q., Persieh", is a small-pored form of *P. arcularius*.

trizonatus, Polyporus Cke. = *Coriolus zonatus*. The type, ex "Upper Yarra, Vic., Lucas", is a discoloured specimen of *C. zonatus*. On the type sheet Bresadola had noted "*Polyporus lenis* Lev., a form of *Polystictus occidentalis* Kl., old, naked". A second specimen of *C. zonatus* on the same sheet was noted by Bresadola as "Probably *P. velutinus*".

159. TUMULOSUS, POLYPORUS Cke. & Mass. ex Cke., Grev., Vol. 17, p. 55, 1889.

The type was from "Brisbane, Q., F. M. Bailey". Only a sclerotium is now at Kew.

160. UDUS, POLYPORUS Jungh., Ann. Sci. Nat., Ser. II, Vol. 16, p. 320, 1841.

fusco-maculatus, Polyporus Bres. & Pat., in Lloyd's Myc. Notes, No. 6, p. 49, 1901.

Under the cover of *P. rasipes* are two collections of *P. udus* at Kew from Australia, ex "Daintree River, Q., Pentzcke" and "Richmond River, N.S.W., Camara". *P. fusco-maculatus*, ex Samoa, was based on the same species.

umbrinella, Hexagona Fr. Cooke (*Hdbk.*, p. 165, 1892) recorded the species from Queensland, but no specimens from the region are at Kew.

161. UNDATA, PORIA (Pers.) Bres., Ann. Myc., Vol. 1, p. 78, 1903.

undatus, Polyporus Pers., Myc. Eur., Vol. 2, p. 90, 1825.
broomei, Polyporus Rabh. ex Berk. & Br., Trans. Linn. Soc., Ser. II, Vol. 1, p. 402, 1879, type ex Brisbane, Q., No. 398, in British Museum of Natural History.

Two collections at Kew from Australia are "Daintree River, Q., Pentzcke", filed under *Poria hyposclera*; and "Clarence River, N.S.W., Wilcox", which is under *Poria radula*.

ungulata, Trametes Berk. = *Fomitopsis ochroleuca*.

162. UNICOLOR, LENZITES (Fr.), nov. comb.

unicolor, Daedalea Fr., Syst. Myc., Vol. 1, p. 336, 1821.
pendula, Daedalea Berk., Fl. N.Z., Vol. 2, p. 180, 1855.

Cooke (Hdbk., p. 162, 1892) recorded the species from Queensland, but there are no specimens from Australia at Kew. *Daedalea pendula*, type ex "N.Z., Colenso", is a pendulous form.

163. VAPORARIA, PORIA (Fr.) Cke., Grev., Vol. 14, p. 111, 1886.

vaporarius, Polyporus Fr., Syst. Myc., Vol. 1, p. 382, 1821.

Collections from Australia which resemble European specimens are "Brisbane, Q., Bailey", "Swan River, W. Aus., Drummond", "Melbourne, Vic., No. 1014" (the last filed under *Fomes cryptarum*), and "Tasmania, Archer". Two collections so referred by Cooke, ex "Moe Swamp, Gippsland, Vic." and "Clarendon, Vic.", are of *Poria versipora*, as are all New Zealand specimens under this cover.

varia, Trametes Lloyd = *Fomitopsis ochroleuca.*

varius, Polyporus Fr. Cooke (*Hdbk.*, p. 116, 1892) recorded the species from Queensland, Western Australia and Tasmania. No specimens are at Kew from Australia.

vellereus, Polyporus Berk. Under this cover at Kew are two collections from Australia, ex "Endeavour River, Q., Persieh" and "Daintree River, Q., Pentzcke". Both are of *Coriolus pinsitus.*

164. VELUTINUS, CORIOLUS (Fr.) Quel., Ench., p. 175, 1886.

velutinus, Polyporus (Pers.) Fr., Syst. Myc., Vol. 1, p. 368, 1821.

One specimen of three from "South Australia", filed under *Polystictus versicolor*, is of this species.

165. VENUSTUS, POLYPORUS Berk., Lond. Jour. Bot., Vol. 4, p. 55, 1845.

Two collections are at Kew from Australia, the type ex "Swan River, W. Aus., Drummond, No. 135" and "Brisbane, Q., No. 101".

166. VERNICIFLUUS, POLYPORUS Berk., Fl. Tas., Vol. 2, p. 254, 1860.

rasipes, Polyporus Berk., Jour. Linn. Soc., Vol. 16, p. 49, 1878.

The type is ex "Tasmania, Archer". The type of *P. rasipes* ex "Admiralty Islands, Challenger Expedition" matches this. Other collections, filed under *P. rasipes*, are ex "Trinity Bay, Q., Sayer" and "New Guinea, Capt. Armit". The former Bresadola doubtfully referred to *P. brasiliensis.*

167. VERSATILIS, POLYPORUS Berk., Lond. Jour. Bot., Vol. 5, p. 150, 1846.

One collection is under this cover at Kew, ex "Nambour, Blackball Range, Q., W. N. Cheesman". Two others, filed under this cover, ex "Rockhampton, Q., Mrs. Thozet" and "Clarence River, N.S.W., Camara, No. 67", are probably specimens of *Irpex flavus.*

versicolor, Fomes P. Henn. = *Fomes rimosus.*

168. VERSICOLOR, CORIOLUS (Fr.) Quel., Ench., p. 175, 1886.

versicolor, Polyporus (L.) Fr., Syst. Myc., Vol. 1, p. 368, 1821.
aequus, Polystictus Lloyd, Myc. Notes, No. 62, p. 933, 1920, type ex Tasmania, L. Rodway.

The only specimens at Kew are "Mt. Lofty, Adelaide, S. Aus., W. N. Cheesman", "Tasmania" and the type of *P. aequus.* Others filed under this cover are of *C. zonatus.*

versicutus, Polyporus Berk. & Curt. Specimens so referred by Cooke, ex "Endeavour River, Q., Persietz" and "Daintree River, Q., Pentzcke, No. 143", are of *Coltricia acupunctata.* A third, ex "Endeavour River, Q., Persietz" = *Coriolus blumei.*

versiformis, Trametes Berk. & Br. An Australian collection ex "Rockhampton, Q., Mrs. Thozet, No. 131", so referred by Cooke = *Trametes protea.*

169. VERSIPORA, PORIA (Pers.) Rom. Svensk Bot. Tidskr., Vol. 20, p. 15, 1926.

versiporus, Polyporus Pers., Myc. Eur., Vol. 2, p. 105, 1825.
chlorina, Poria Mass., Kew Bull. Misc. Inf., p. 95, 1906, type ex Christmas Island.
subvaporaria, Poria Cke., in herb. Kew.

The following collections from Australia are at Kew: "Toowoomba, Q., Hartmann", filed under *Poria vulgaris*; "Guntawang, N.S.W., Hamilton, No. 4", under *Poria odontoporia*; "Moe Swamp, Gippsland, Vic." and "Clarendon, Vic.", under *Poria vaporaria.*

170. VESPACEA, HEXAGONA (Pers.) Fr., Epicrisis, p. 497, 1838.

vespaceus, Polyporus Pers., Gaud. Voy. Freyc., p. 169, 1827.
inconcinna, Daedalea Berk., Lond. Jour. Bot., Vol. 1, p. 151, 1842.
albida, Hexagona Berk., Jour. Linn. Soc., Vol. 16, p. 47, 1878.
intermedia, Daedalea Berk., Jour. Linn. Soc., Vol. 18, p. 385, 1881.
favoloides, Hexagona Cke., Grev., Vol. 14, p. 118, 1886.
cookei, Hexagona Sacc., Syll. Fung., Vol. 6, p. 363, 1888.

Collections at Kew from this region are: "Malamon Island, Challenger Expedition", type of *H. albida*; "New Guinea, Hollrung", filed under *H. albida*; "Strickland River, New Guinea, Bauerlen, No. 20", type of *H. favoloides* which Saccardo changed to *H. cookei* because the name was preoccupied; and "Australia", type of *Daedalea intermedia.*

vesparius, Polyporus Berk. = *Hexagona gunnii.*

171. VICTORIAE, PORIA (Berk.) Cke., Grev., Vol. 14, p. 111, 1886.

victoriae, Polyporus Berk. ex Cke., Grev., Vol. 10, p. 103, 1882.

Three collections are at Kew, the type ex "Victoria, F.v.M.", "Daintree River, Q., Pentzcke" and "Hawkesbury River, N.S.W., J. B. Cleland". Others under this cover from Australia are of *Poria calcicola.*

172. VICTORIENSIS, POLYPORUS Lloyd, Myc. Notes, No. 65, p. 1095, 1921.

Part of the type is at Kew, ex "South Australia, J. B. Cleland".

vinctus, Polyporus Berk. = *Poria radula.*

173. VINOSUS, POLYPORUS Berk., Ann. Mag. Nat. Hist., Ser. IX, Vol. 2, p. 195, 1852.

A collection ex "Strickland River, New Guinea, Capt. Armit" agrees with the type ex St. Domingo.

violacea, Poria (Fr.) Cke. A specimen so labelled by Cooke, ex "Victoria, No. 1029" = *Poria spissa.*

174. VIRGATUS, POLYPORUS Berk. & Curt., Jour. Linn. Soc., Vol. 10, p. 304, 1869.

novo-guineensis, Polyporus P. Henn., in Schum. & Hollr., Flora von Kais. Wilh. Land, p. 6, 1889.

The type of *P. novo-guineensis* matches specimens of *P. virgatus* at Kew from the type locality, Cuba.

175. VULGARIS, PORIA (Fr.) Gray, Nat. Arr. Brit. Pl., Vol. 1, p. 639, 1821. One specimen ex "Richmond River, N.S.W." is probably of this species, though I was not able to verify this by examination under the microscope. A collection ex "Toowoomba, Q., Hartmann" so referred by Cooke = *Poria versipora.*

176. WAKEFIELDIAE, PORIA Rodw. & Clel., Papers & Proc. Roy. Soc. Tas. for 1929, p. 85, 1929.

Part of the type is at Kew, ex "Sydney, N.S.W., J. B. Cleland, No. 20".

177. WEBERIANA, COLTRICIA (Bres. & P. Henn.), nov. comb.

weberianus, Polyporus Bres. & P. Henn., in litt.
weberianus, Fomes Sacc., Syll. Fung., Vol. 9, p. 174, 1891.

A specimen so named by Bresadola, ex "Brisbane, Q., C. E. Broome", is under the cover of *P. fruticum* at Kew. A second, ex "Trinity Bay, Q., Sayer, No. 40", is filed under *Fomes salicinus.*

178. WIGHTII, HEXAGONA (Kl.) Fr., Nov. Symb., p. 100, 1851.

wightii, Polyporus Kl., Linnaea, Vol. 7, p. 200, 1832.
wrightii, Hexagona (Kl.) Fr., Epicrisis, p. 496, 1838.

The following Australian collections are at Kew: "Port Denison, Q., Fitzalan, Shann", "Toowoomba, Q., Hartmann, No. 52", "Blomfield River, Bauer" and "Soane River, Behen".

wilsonianus, Polyporus Lloyd = *Polyporus campylus.*

179. XANTHOPUS, CORIOLUS (Fr.), nov. comb.

xanthopus, Polyporus Fr., Syst. Myc., Vol. 1, p. 350, 1821.
cupreo-nitens, Polyporus Kalch. ex Thuem., Myc. Univ., No. 1702, 1881.

Collections at Kew from this region are: "Goode Island, Q., W. Powell", "Cape York, Q., Challenger Expedition", "Brisbane, Q.", "Rockhampton, Q., Mrs. Thozet", "Dunk Island, Q., W. Cottrell-Dormer", "North Queensland", "Endeavour River, Q.,

Persietz", "Trinity Bay, Q., Karsten", "Upper Daintree River, Q., Harris, Pentzcke", "Port Denison, Q., Fitzalan", "Clarence River, N.S.W., Dr. Beckler", "Tweed River, N.S.W., Camara", "Mossman River, Barnard", "Melbourne, Vic." (type of *P. cupreonitens*), "New Guinea, Capt. Armit, No. 2", "Solomon Islands, Dr. Guppy", "Fly River, New Guinea, Everett's Expedition, Bauerlen".

xerampelinus, Polyporus Kalch. = *Inonotus tabacinus.*
xerophyllaceus, Polyporus Currey = *Fomes strigatus.*

180. XEROPHYLLUS, POLYPORUS Berk., Fl. N.Z., Vol. 2, p. 178, 1855.

Specimens at Kew which match the type, ex "N.Z., Hooker herb.", are "N.Z., Colenso, No. 532", filed under *P. rudis*; and "New Guinea, Strickland River, Bauerlen", which is under the cover of *P. russiceps.*

181. ZEALANDICUS, FOMES Cke., Grev., Vol. 14, p. 18, 1885.
zealandicus, Polyporus Cke., Grev., Vol. 8, p. 55, 1879.
hamatus, Fomes (Corner) Imaz., Jour. Jap. Bot., Vol. 16, p. 586, 1940.

The type, consisting of three specimens, "N.Z., Coromandel, Dr. Berggren, Nos. 309, 310" and an additional collection ex "Sunday Island, Kermadecs, W. R. B. Oliver", are at Kew.

zelandicus, Polyporus Cke. = *Polyporus berkeleyi.*

182. ZONALIS, POLYPORUS Berk., Ann. Nat. Hist., Vol. 10, p. 375, 1842.

Collections at Kew from this region are: "Near Trinity Bay, Q., W. A. Sayer", "Buderim Mts., Q., C. T. White", "Brisbane, Q." (the last is filed under *P. luteo-nitidus*), "Strickland River, New Guinea" and "New Guinea, Armit".

183. ZONATUS, CORIOLUS (Fr.) Quel., Ench., p. 175, 1886.
zonatus, Polyporus Fr., Syst. Myc., Vol. 1, p. 368, 1821.
bireflexus, Polyporus Berk. & Br. ex Cke., Grev., Vol. 10, p. 101, 1882.
rufo-rugosus, Polyporus Lloyd, Myc. Notes, No. 73, p. 1331, 1924.
orbiculata, Polyporus Col., in herb. Kew.
trizonatus, Polyporus Cke., Grev., Vol. 12, p. 17, 1883.

The following collections from this region are at Kew: "Brisbane, Q., No. 172" (type of *P. bireflexus*), "Illawarra, N.S.W., Camara" (under *P. elongatus*), "Nymitiballe, Bauerlen, No. 246" and "Upper Yarra, Vic., Lucas" (both under *P. trizonatus*), "Tasmania, L. Rodway" (type of *P. rufo-rugosus*), "N.Z., Colenso, No. 1052" (filed under *Polystictus versicolor* and in the herbarium labelled *P. orbiculata*), "N.Z., Colenso, Nos. 837, 838" and "N.Z., T. Kirk, No. 88" (filed under *P. versicolor*).

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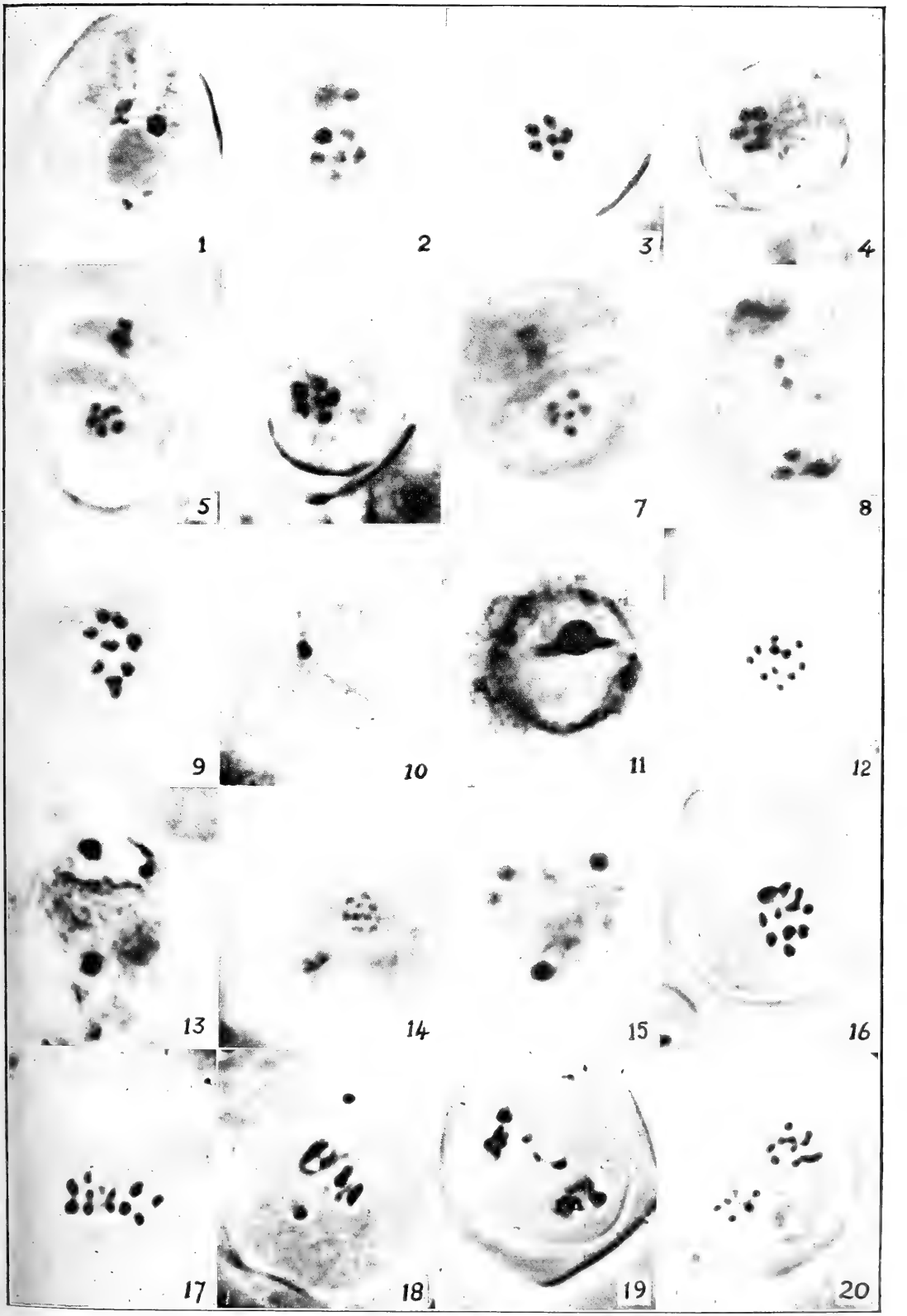

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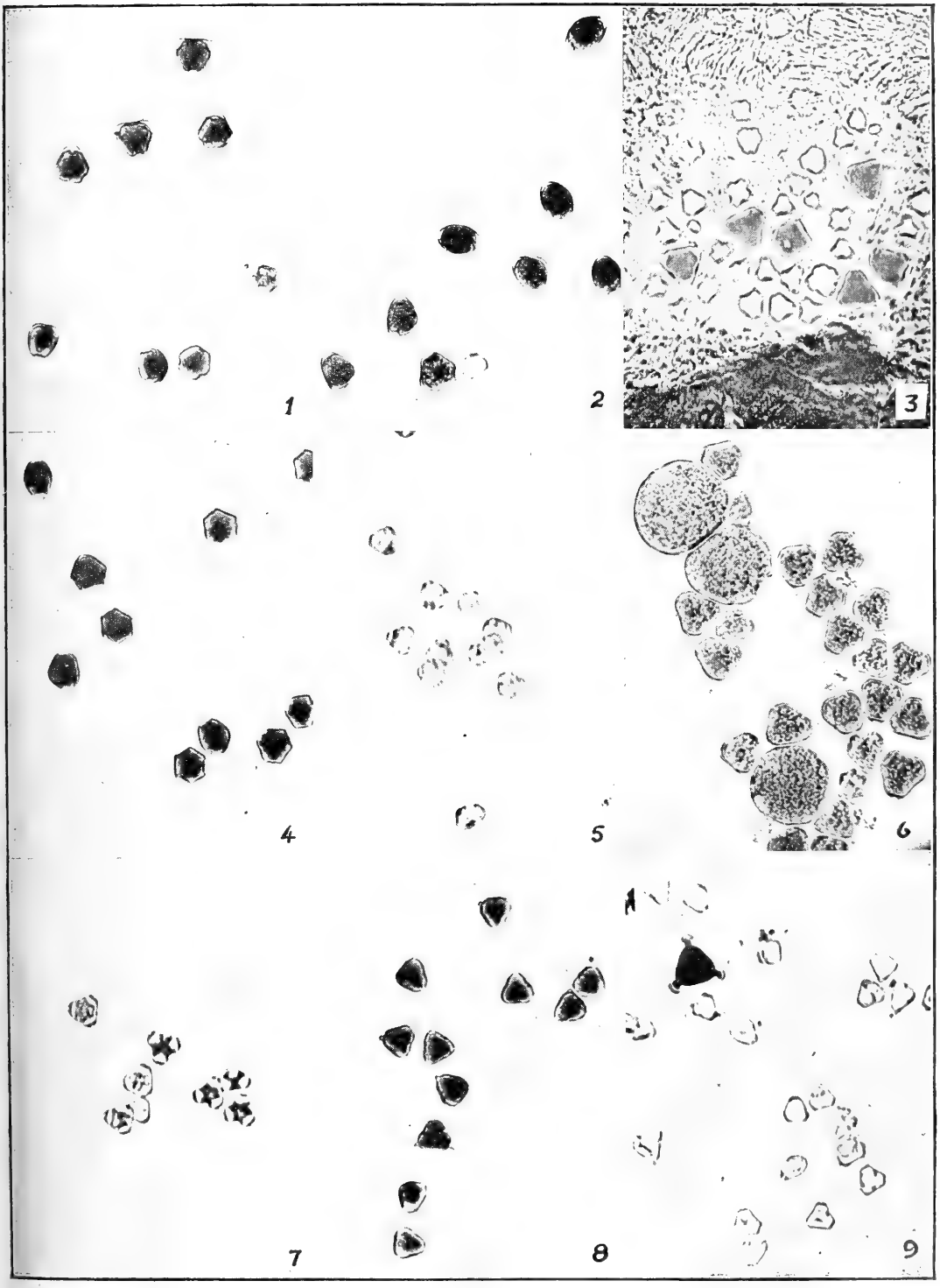

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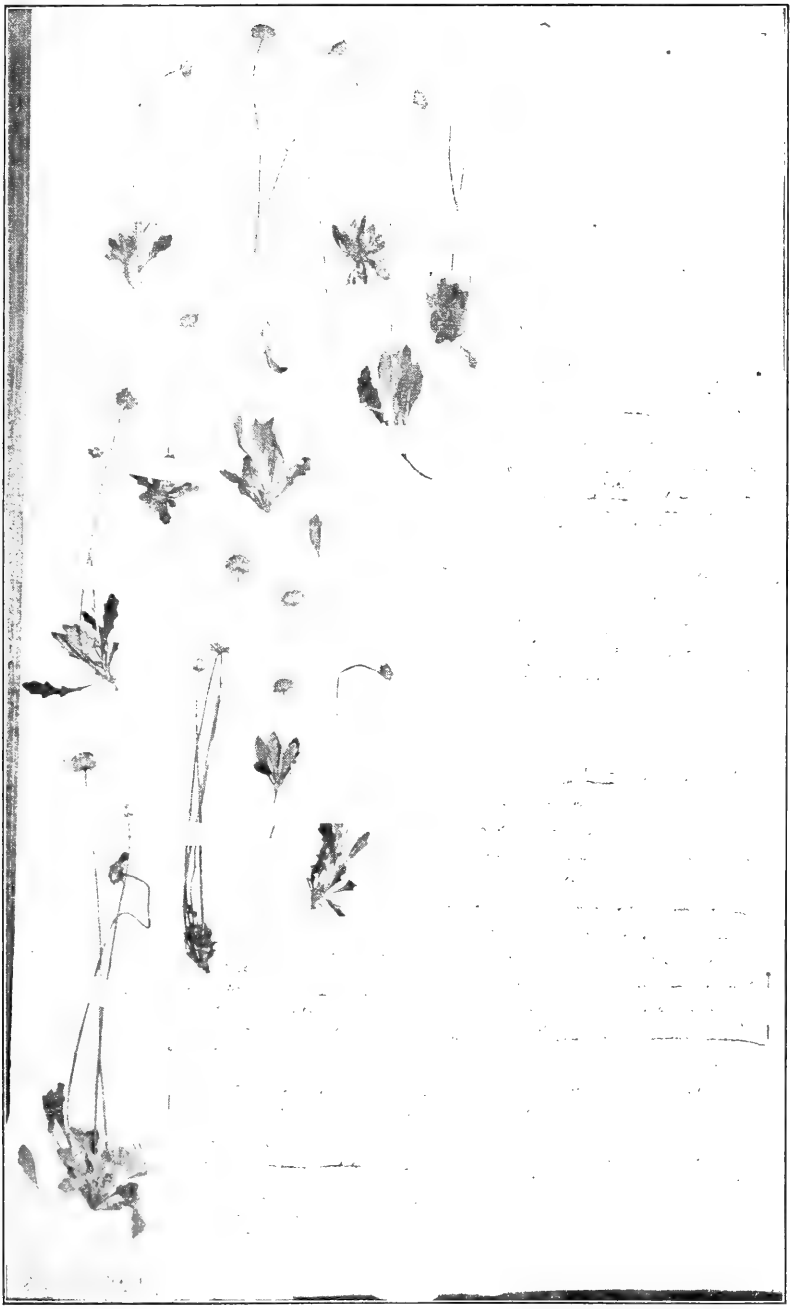

Australian Species of Lagenophora.

Immature Stages of *Aphodius howitti* Hope.

FURTHER NOTES ON EXPERIMENTAL CROSSING WITHIN THE AËDES SCUTELLARIS GROUP OF SPECIES (DIPTERA, CULICIDAE).

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[Read 27th September, 1950.]

Introduction.

In a previous paper (Woodhill, 1949) an account was given of experimental crossing between *Aëdes scutellaris scutellaris* Walker and *Aëdes scutellaris katherinensis* Woodhill, and in this it was shown that the cross was fertile using *katherinensis* males and *scutellaris* females, but that the reciprocal cross was sterile. In the present paper a description is given of back crosses with the original parents and also of crosses between other species of the *scutellaris* group. The distribution of the species used is given in the map accompanying the previous paper, and the breeding and crossing procedure is similar to that previously described.

Crossing Experiments.

Table 1.

Results of Crossing F_1 Hybrids (produced from Male Katherinensis × *Female* Scutellaris) *with Both Parents.*

Crosses, Numbers and Sexes.			Approximate Number of Eggs Produced.	Period of Egg Production.	Percentage Eggs Hatched.
Hybrid.	*scutellaris.*	*katherinensis.*			
137♂♂		107♀♀	1220	5/12/49–12/12/49	0
106♀♀		118♂♂	480	,, ,,	100
135♂♂	97♀♀		1150	21/12/49–27/12/49	100
97♀♀	135♂♂		650	,, ,,	100

It will be seen that the only sterile cross was that of hybrid males crossed with *katherinensis* females and that in each case where hybrid males were used the egg production was considerably greater than where males of the original parents were used. In all the above crosses copulation was frequent and samples of the females showed living spermatozoa in the spermathecae. Larvae resulting from the fertile eggs were bred through to the adult stage and appeared quite normal. No further information has been obtained as to a possible explanation of the sterility occurring in these crosses.

In all the crosses shown in Table 2 copulation was frequently observed and living spermatozoa were present in the spermathecae of the females, but nevertheless all the crosses were completely sterile.

Discussion.

In the back crossing experiments the only sterile cross occurred when *katherinensis* was used as the female parent, and it will be remembered that in the original crosses between *scutellaris* and *katherinensis* (Woodhill, 1949) the *katherinensis* females gave sterile eggs. There is obviously some factor associated with *katherinensis* females which renders them sterile unless crossed with males of their own subspecies. Two similar cases occurring in mosquito crosses are now known, i.e. Downs and Baker (1949), who crossed *Aëdes aegypti* with *Aëdes albopictus*, and Perry (1949), who crossed *Aëdes scutellaris scutellaris* (= *A. hebrideus*) with *Aëdes pernotatus*. Both these authors found that one cross was fertile, whereas the reciprocal cross gave sterile eggs, and

it would appear that this phenomenon is peculiar to closely related forms of mosquitoes. Partial sterility of this type is recorded in other groups of animals, but in these cases the sterility occurs in the F_1 adult hybrids, whereas in the experiments mentioned above no development of the embryo occurs in the F_1 eggs. With reference to Table 2, it is clear that *Aëdes pseudoscutellaris* Theobald and *Aëdes scutellaris scutellaris* Walker are completely isolated genetically, and this confirms the opinion of Farner and Bohart (1945), who accorded specific status to these forms on morphological and geographic evidence. As was to be expected, *Aëdes scutellaris katherinensis* Woodhill was completely sterile when crossed with *A. pseudoscutellaris*. Perry (see above) concludes as a

TABLE 2.

Results of Crossing A. pseudoscutellaris *Theobald with* A. scutellaris scutellaris *Walker and* A. scutellaris katherinensis *Woodhill.*

Crosses, Numbers and Sexes.			Approximate Number of Eggs Produced.	Period of Egg Production.	Percentage Eggs Hatched.
pseudo-scutellaris.	*scutellaris.*	*katherinensis.*			
158♂♂	105♀♀		2400	11/7/49–25/ 7/49	0
92♂♂	49♀♀		260	3/6/49–20/ 6/49	0
113♀♀	144♂♂		1090	11/7/49–25/ 7/49	0
63♀♀	99♂♂		120	3/6/49–20/ 6/49	0
164♂♂		170♀♀	4700	2/9/49–16/ 9/49	0
200♂♂		135♀♀	2700	30/9/49–14/10/49	0
99♀♀		63♂♂	790	2/9/49–16/ 9/49	0
93♀♀		125♂♂	1100	30/9/49–14/10/49	0

result of his work that *A. scutellaris scutellaris* and *A. pernotatus* should be regarded as distinct species owing to "their ability to hybridize only with extreme difficulty under laboratory conditions". It should, however, be pointed out that only small numbers of individuals, up to 16, were used in his experiments. The author found the same difficulty with small numbers of *scutellaris* and *katherinensis*, but when 60 to 100 individuals of both sexes were used, large numbers of hybrids were readily obtained. It could be argued, therefore, that the question as to whether *pernotatus* should be regarded as a subspecies of *scutellaris* or as a distinct species (on the grounds of genetical isolation) should be left open until more comprehensive crossing experiments are carried out. On the other hand, as Perry points out, the two forms differ markedly in their feeding habits, and no intermediate forms have been taken in the field in areas where the two forms occur together.

SUMMARY.

(1) Back crosses of F_1 hybrids (produced from male *katherinensis* × female *scutellaris*) were made with both parents. All the crosses were fertile with the exception of hybrid males crossed with *katherinensis* females. In this cross all eggs were completely sterile.

(2) *A. pseudoscutellaris* Theobald was crossed with *A. scutellaris scutellaris* Walker and with *A. scutellaris katherinensis* Woodhill, and in all cases the eggs were completely sterile.

(3) The bearing of these experiments on problems of speciation in the *Aëdes scutellaris* group of species is discussed.

ACKNOWLEDGEMENTS.

The author wishes to acknowledge with gratitude the assistance given by Miss M. Besly, Miss M. Morris and Mr. J. R. Henry, who acted as blood donors and submitted to considerable irritation on many occasions.

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THE HAIR TRACTS IN MARSUPIALS.

PART V. A CONTRIBUTION ON CAUSATION.

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[Read 27th September, 1950.]

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In Part IV (Proc. Linn. Soc., 1950, 75:89–95) of this work variations in expression of the centre of the radial field were considered. Convergent and divergent centres are now discussed in relationship to the development of definitive tract pattern and through them an approach is made to the problem of causation.

Statement of the Problem.

Authors are in general agreement on what constitutes the primitive hair arrangement in the mammalian skin. The primitive condition may be defined as "craniocaudal in the dorsal half, more or less, of head, neck and trunk, and for the rest, caudoventral in varying degree; on the limbs postaxially and distally" (Boardman, 1946*b*). None of the marsupials examined in the course of this work shows an unmodified primitive picture, though some of the phascogales and lower phalangers make a close approach to it. Regional alteration of major or minor extent in the direction of the hair shaft as found in the primitive condition gives rise to hair tracts such as have been described for many marsupials and other mammals.

No evidence has been forthcoming to justify doubt that tract pattern is species constant except for abnormalities of expression, and, once laid down, persists unchanged throughout life (*v. infra*). Constancy of pattern form implies that there is regularity in the direction of flow of hair within the tracts and that this regularity must be the outcome of a precise placing of the individual hair shaft in ontogeny. All attempts at explanation of regional differentiation in the direction taken by the growing hair have been and must continue to be a search for forces within the body which act to confer this orientation on the follicle. Any theory of causation must suggest explanation for the primitive condition and the derivation from it of the most complicated pattern, together with the varying degrees of pattern complexity which examination of this marsupial material has shown to exist between these extremes. The problem is essentially concerned with conditions in the skin at the time of the laying down of the follicle anlagen, which, from their earliest appearance exhibit a definitive orientation (Landauer, 1925*a*, has summarized the literature bearing on this point).

Species Constancy of Definitive Tract Pattern.

As early as 1837 Eschricht* expressed the belief that tract pattern was constant for the species, that different species may have different patterns, and that the pattern peculiar to a species was laid down in embryonic development and remained unaltered throughout life. Evidence of the soundness of these conclusions has steadily accumulated, particularly through the work of Schwalbe (1910, 1911) on the Anthropoidea, de Beaux (1917, 1918, 1924*a*, 1924*b*, 1927), on Anthropoidea, Carnivora and other groups, and of Niedoba (1917) on an extensive series of domestic animals. Further confirmation is provided by the large collection of marsupial pouch young examined in the course of the present work. Of a total of about one hundred and sixty known species of marsupials the hair tracts of fifty-three have been recorded, thirty-seven being described from series of two or more specimens. No grounds exist anywhere in this collection for doubting Eschricht's views on pattern constancy nor can any evidence be adduced that pattern changes with age over the range of size presented by the collection.

Species Constancy of Pattern Development.

A considerable literature exists relative to the ontogeny of the mammalian coat. To Voigt (1857) and Ludwig (1921) we owe details for man, Schwalbe (1910, 1911) for anthropoids other than man, Dry (1926) for the mouse *Mus musculus*, Fraser (1928) for the white rat, Colin (1943) for the guinea-pig, Toldt (1907)† for *Vulpes vulpes*, Gibbs (1938) for *Trichosurus vulpecula*, and Landauer (1925*a*) for *Didelphys virginiana*. Schönherr (1937) has provided a detailed account of hair succession in the region of a divergent whorl. It is not necessary to review this work in detail. Wherever development of the mammalian coat has been investigated the sequence of events leading to its formation has been found to be constant for the species and always involves the development of hair in small localized areas which gradually increase in extent until the primary coat is laid down. In the absence of negative findings the body of evidence available seems sufficiently substantial to accept the species constancy of pattern development as a generalization for the mammalia.

The Concept of Primary and Secondary Hair Configurations.

Voigt (1857) charted the hair tracts of man as a series of fields each of which has hairs radiating from a divergent centre. He considered that the slope of the hair and the direction in which it pointed were the outcome of regional differences between the rate of growth of the epidermis relative to the dermis. The divergent centres situated within the constituent fields of the pelage were held to be focal points of the growth forces concerned, and these centres he regarded as primary. All other hair configurations were classified as secondary in the sense that their occurrence at the margin of the fields indicated that they were brought into existence by forces operating within the fields.

Any approach to the question of causation must be preceded by an evaluation of the status of the peculiar configurations of hairs (centres, intervals, ridges and partings) associated with tract formation. What are the primary features of the pelt concerned with tract formation and what are the consequential effects? Voigt's classification arose from his theory that differential tensions in the skin layers were causal in tract formation. It is profitable to approach this question in the first instance independently of notions of causal factors. If a group of hair configurations is in fact dependent for expression on the presence of another group, then the latter will display attributes—mendelian inheritance and meristic repetition, for example—not found in the former. In what follows evidence is presented to demonstrate that divergent and convergent centres together constitute a primary group and that all other hair configurations are secondary.

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* Information from Voigt (1857).

† Information from Schwalbe (1911).

THE EVIDENCE FOR PRIMARY STATUS OF RADIAL FIELD CENTRES.

(*a*) *Genetics of Radial Fields.*

The genetics of radial fields in mammals, including man, has received considerable attention, but no work of this kind has been done for marsupials. The particular section of the literature relevant to the present purpose is that in which the alternative conditions of presence or absence of radial fields has been determined as due to gene substitution.

Craft and Warner (1934) report that in swine whorls which occur over the loin and rump are hereditary. Their data "support the hypothesis that the swirl is due to the interaction of two dominant complementary genes which may be present in either a homozygous or heterozygous combination to produce the swirl". Gates (1946) in resuming this work comments that a single factor with low penetrance would equally well explain the results.

The most complete analysis is provided by the work on the genetics of rosette pattern in the guinea-pig. The divergent centres (rosettes) characteristic of the rough coat are the outcome of a single gene substitution which, alone, produces reversal of hair direction on the hind toes; the extent of rosette formation is progressively increased by the presence of modifiers (Castle, 1925; Sewall Wright, 1935).

The cases given above could be added to considerably (see, for instance, Landauer, 1926, 1929).

No direct evidence from breeding experiments can be brought forward to support the contention that convergent centres are genetically determined in the same way as divergent centres. It is of interest that while geneticists working on the guinea-pig's coat have been preoccupied with divergent centres examination of a rough coat shows that both divergent and convergent centres may be involved in production of the roughness. That convergent centres are primary characters is suggested by the alternatives of presence or absence observed for some of them. Thus, within the genus *Perameles* all species examined, with the exception of *P. gunnii*, possess a convergence on the elbow. Further, in a series of *Perameles* sp. from Western Australia (Part III) containing three specimens, one member has the normally occurring elbow whorl absent. A case in the same category is provided in the occurrence of a convergent whorl on the cheek in one of the series of *Trichosurus vulpecula* (Part II, Fig. 19, Boardman, 1946*a*). The sporadic occurrence of the mid-dorsal convergent centre in man may also be cited (Wood Jones, 1927, 1934).

(*b*) *Meristic Repetition of Centres.*

Within the limits of the species alternative conditions are found for the focus of some radial fields; the centre may be single or double or, sometimes, multiple. The point may be illustrated by an extreme case, the mid-dorsal whorl of *Phascolarctos*, which, while most commonly single and median, is often bilaterally doubled and occasionally subdivided into as many as six separate centres collectively having the same general effect as the single median whorl. It is difficult to visualize these alternative conditions as other than an innate capacity of the field's centre to undergo duplication. Such a property could only be consonant with classification as a primary constituent of the pelt. Part IV of this series (Boardman, 1950*a*) deals at length with meristic repetition of the divergent centre in the Marsupialia, including a detailed account of the phenomenon in *Phascolarctos* cited above. The type of variation is shown to be one of common occurrence.

In the case of the convergent centre evidence of duplication as an alternative condition for the species is not so ample, being restricted to two cases—the chin convergence of *Phascolarctos* (Part II, Fig. 26) and the mid-ventral (umbilical) convergence in *Trichosurus vulpecula hypoleucus* (Part III, p. 194).

Supernumerary whorls, both divergent and convergent, have been recorded in marsupials. Reference to them is made in Part IV (Boardman, 1950*a*). The phenomenon is readily separable from that of duplication of the centres of radial fields discussed above, where additional whorls always lead to a condition that is symmetrical with respect

to the longitudinal median line of the dorsal or ventral surface and are demonstrably derived from single median structures. Supernumerary whorls, on the other hand, are sporadically distributed in the pelt and show no relationship to existing normally occurring centres.

THE SECONDARY STATUS OF INTERVALS.

Convergent and divergent intervals, usually referred to as "kreuze" in the German literature, constitute the principal hair configuration classified as secondary. The conditions for their formation are provided by currents from two divergent centres meeting and diverging towards two oppositely situated convergent centres or their equivalent. By equivalent is meant the extremities of the ears, limbs, and tail which act in the skin as though physiologically similar to the more precisely defined convergent centre. Examples of the method of formation are seen, for example, in *Dasyurinus geoffroii*, *Sarcophilus harrisii*, *Perameles gunnii*, and *Trichosurus vulpecula* (Part II, Figs. 5, 8, 13 and 20 respectively, Boardman, 1946*a*). The terms "convergent" and "divergent" as applied to the interval have no significance except as a convenience in description.

It is necessary to evaluate these intervals by the same criteria as have been used above to determine the status of the radial field. There is no genetic evidence that they are other than consequential effects of the presence of divergent and convergent centres. The facies of an interval is predictable by a consideration of the conditions in the surrounding fields. Intervals are not known to present alternative single or duplex expressions and the occurrence of an abnormal interval cropping up in the manner of a supernumerary whorl is unknown. The intervals satisfy the requirements of a feature of secondary status.

THE ORIENTATION OF THE FOLLICLE.

It is a well-established fact that the slope of the developing hair shaft with reference to the skin surface is apparent from the earliest stages of the hair anlagen (see Landauer, 1925*a*, for references). Additionally, it will be self-evident from a consideration of the regularity in direction of the hair streams composing a pattern of tracts that the individual hair will also emerge in a determined plane lying at right angles to the skin surface. In other words, the direction taken by the growing hair in any specified part of the body is a constant for the species.

Voigt (1857), working principally on human material, analysed the relationships of hairs in the divergent radial field and commented on their arrangement in lines radiating from the centre. Ludwig (1921), working on human material and a series of domestic animals, confirmed this finding and extended it by the statement that the lines always ran between a divergent and a convergent centre. That placing of hairs in rows is general in the Mammalia is implicit in the well-known work of De Meijere (1894) on hair arrangement. The truth of the generalization that the flow of hair is from divergent centre to convergent centre (or its equivalent) certainly holds for the Marsupialia, as evidenced by examination of the extensive collection on which this work rests. A search for exceptions in published accounts of the hair tracts of members of other orders of the Mammalia has yielded negative results.

The neglect of Ludwig's important generalization is probably attributable to the manner in which the arrangement of follicles in rows is distorted in some animals (the sheep, for example) by the simultaneous development of several generations of follicles with consequent crowding so that the lines are difficult if not impossible to see. In the phalanger, *Trichosurus vulpecula*, the primary hairs cover the whole body before the secondary and tertiary hairs break through (Gibbs, 1938). The primary coat is therefore presented in its simplest form. The species, moreover, has a moderately complicated tract pattern which, combined with the peculiarities of the hair succession, makes it particularly valuable for study in this context. The hair tracts of *Trichosurus vulpecula* are well known (Wood Jones, 1920; Boardman, 1946*a*, 1949). Gibbs (1938) has given an account of hair succession in the building up of the coat. The pattern of the tracts is determined by the disposition of a series of divergent centres in

association with a group of convergent centres or their equivalent. The divergent centres are situated on each side at the hind margin of the rhinarium, medially on the crown, at the medial canthus, on the chin, and in the axilla; the preauricular reversal presumably originates in a centre within the external ear, but its position could not be defined. Convergent centres occur on the mid-ventral line caudal of the interramal papilla, at about the middle of the abdomen (the umbilical convergence), at the cloacal hillock, and in the male associated with the base of the scrotum; a poorly defined convergent point is present just below and in front of the angle of the mouth. Occasionally a convergent centre is developed on the cheek (Part II, Fig. 19, Boardman, 1946*a*). The extremities of the limbs, tail and ears have, as explained above, the equivalence of convergent points in that hairs are directed towards them.

The account that follows is based on a series of pouch young not previously recorded. Only four specimens of the series are relevant and these are presented in order of extent of hair development. Th crown-rump length is given and the damp weight, the latter appearing to be a much more accurate basis for size comparison.

Specimen I. A male (crown-rump length 35 mm., weight 2·9 gm.), Canberra, Australian Capital Territory; coll. R. N. Wardle, May, 1943.

The specimen is at an early stage of coat development, not all of the body surface being yet clothed with the first generation hairs. Only a few vibrissae at the caudal margin of the mystacial zone have come through. Delimitation of the fields can be made accurately, as the hairs in general are arranged in rows which can be traced from divergent centre to convergent centre or equivalent. The region surrounding the central point of the occipital divergent radial field is still hairless, but an inner zone of shorter shafts, as shown by comparison with the next member of the series, indicates that the wave of growing hairs is progressing towards the centre. This phenomenon of initiation of hair growth on a circle surrounding the centre from which growth then proceeds both away from and towards the centre is apparently general in *Trichosurus*, as all divergent centres show it in some degree. The bilaterally paired whorls at the margin of the rhinarium, to the presence of which the rhinal reversal is due (Part II, Fig. 19, Boardman, 1946*a*) appear not to be active at this stage, so that their territory on the dorsal and lateral aspects of the snout is naked. The field associated with the canthal divergent centre which meets that of the rhinal centre in a divergent interval is formed anterior to the canthus as though the rhinal field were there. Apparently the skin is organized to conform with the definitive pattern before the development of the hairs.

The front of developing hairs from the occipital and axillary whorls which enters into the composition of the mid-dorsal (umbilical) convergence has not quite reached its central point. There are no indications that the centre is other than passive at this stage of coat formation. The same condition obtains for the interramal convergent point into whose formation enter hairs from all the divergent centres of the head, including the occipital whorl.

A mid-ventral longitudinal strip of skin apparently coincident with the site of the sternal gland and the medial and lateral surfaces of the ear are naked.

Specimen II. A headless female; Canberra, Australian Capital Territory; coll. W. Boardman, 25th November, 1943.

The second specimen is at about the same stage of hair development as the first and shows no differences from it.

Specimen III. A male (crown-rump length 40 mm., weight 3·2 gm.); Canberra, Australian Capital Territory; coll. W. Eldridge, 5th June, 1944.

This example, somewhat larger than the first, shows the development of hairs at a later stage. The mystacial vibrissae are well developed. The rhinal centres are

active, but a small zone on the rhinal side of the future divergent interval in front of the eye is still naked or at most the hairs are just breaking the skin surface. The growth wave from the dorsal and axillary whorls has reached the mid-ventral convergent point. Only the scrotum is without hair at the hinder end of the body. The medial naked patch on the ventral thorax presumed related to the sternal gland is still present and the ear both laterally and medially remains naked.

Specimen IV. A female (crown-rump length 45 mm., weight 10·7 gm.); Canberra, Australian Capital Territory; coll. W. Boardman, 16th October, 1944.

Hair development shows a considerable advance on the previous members of the series. The medial naked patch on the ventral thorax remains and the ear is hairless both laterally and medially.

The arrangement of hair around the interramal convergent centre of this specimen shows very clearly the manner in which hair streams from separate sources entering into the composition of the field surrounding such a centre retain, at this stage of development, their identity. The zone around this particular point receives hairs traceable back to divergent centres on the chin, medial canthus, margin of rhinarium, and occiput; each of these fields has its own peculiarities of hair length, hair density and so on.

The Divergent Centre Considered as a Growth Centre.

In 1934 Sewall Wright expressed the view that the rosettes of the rough guinea-pig's coat were "physiologically isolated growth centres" modifying the antero-posterior gradient. Sewall Wright does not appear to have developed his ideas beyond their formal statement. That this interpretation is correct for the guinea-pig is readily confirmed by examining embryos of rough forms at the earliest stage of hair development, where the positions of the rosette centres are marked by the precocious development of follicle clusters. Ludwig (1921), paying particular attention to the occipital whorl in man, has advanced the same idea of follicle activity spreading from a centre. In the marsupials, when the development of the pelt as shown, for example, in *Trichosurus* (see above and Gibbs, 1938) is considered, the conclusion is inescapable that the divergent centre, whatever its position in the skin, is a centre of activity from which a follicle growth wave is initiated. A crucial example is provided by a specimen of *Phascolarctos* in the lumbar region of which follicle activity has, judged by the differential density of the follicle population, originated in the sacral whorls, and on the distal hind-limb from the whorl on the ankle. The same phenomenon is readily observed in connection with the gular centres of the family Peramelidae.

The question arises: Can this demonstrated activity of the centre of the divergent field be regarded as general for mammals? The uniformity in structure of the skin and its appendages in the class suggests uniformity of underlying processes which, with the facts available, point to the generalization being tenable. Much of the literature dealing with coat development is not usable in this context, as the hair tracts of the forms considered are not known.

Coincidence of the initial centres of follicle growth with the position of divergent centres may be obscured. As indicated above in discussing *Trichosurus vulpecula*, the first development of follicles is on the circumference of a circle of considerable radius about the centre. Follicle differentiation then proceeds from this ring towards the centre and, beyond it, centrifugally. An extreme example of the phenomenon is seen in the development of the mid-back divergent centre of *Macropus major*. The condition as presented by well-furred pouch young is figured in Part II, Fig. 42, Boardman, 1946*a*. The centre is seen to lie over the lumbar region and from it there extends forwards to the occiput a feathering which terminates in a divergent interval. Examination of younger specimens shows that the centre of the system initially includes the feathering also. In the early stages of coat development on the dorsal trunk the hair is seen to radiate from the margin of a fusiform naked area extending along the mid-back from the occiput to the definitive centre.

Relationship of Divergent and Convergent Centres to the Process of Hair Growth.

Evidence is presented above to support the status of divergent and convergent centres as primary characteristics of the coat genetically controlled for position and incidence. A fundamental similarity between divergent and convergent centres is suggested by several considerations. Both kinds appear in an identical way as abnormalities (*v. supra*) and both may be at the focal point of whorled systems. Further, there are grounds for believing that occasionally a divergent system may be replaced at the same locus by a convergent system (*v. infra*). In the elaboration of the primary coat, however, these two structures are not of equal value. The divergent centre is apparently a focal point of activity (*v. supra*). Follicles first make their appearance at the site of divergent centres and from them the follicle growth wave extends centrifugally. It has been pointed out how the follicles are developed along lines and how each line is as it were drawn from a divergent to a convergent centre. There is no evidence in this marsupial material that the convergent centre is in any way concerned with the initiation of follicle growth as is the case with the divergent centre. Lines of successively developed follicles grow towards the convergent centre and finally enter into spatial relationships around it. The follicles around a convergent centre may be traceable through their developmental lines back to two or more divergent centres. It is readily observed and significant that for some time after a convergent centre has become defined by follicle growth the characteristics of the fields (hair density, hair character, etc.) associated with the several contributory divergent centres retain their identity (see the account of *Trichosurus vulpecula* above).

The tip of the tail, limb extremities, and tips of the ears are regarded as physiologically equivalent to convergent centres in that they enter into similar relationships with the divergent centres where the laying down of hair rows is concerned and appear not to be the site of initiation of hair growth unless, as in the case of the hind-limb of *Phascolarctos*, a divergent centre occurs distally.

The inactivity of the convergent centre with respect to hair production is probably due to its later appearance in the skin or later attainment of a threshold for some requirement necessary in the laying down of follicle anlagen.* Subsequent activity at the convergent centre is shown by such features of the mature pelt as regional difference in the length attained by the hairs. Thus many marsupials, particularly the macropods, show an early lengthening of the hairs of the cloacal hillock, which is the site of a convergent centre. The same phenomenon is observed in the mid-ventral trunk convergence of ungulates, and on man and other animals in the formation of the beard, which appears to be associated with the interramal convergence.

The Pelt Considered as a Mosaic of Polarized Hair Fields.

Establishment of the generalization that hairs develop in rows and that the rows, however tortuous their course might be, always run between divergent and convergent centres, justifies the viewpoint that the skin in its expression of hair tract pattern may be viewed as a mosaic of hair fields. Ideally the hair field will be spindle-shaped, with a divergent centre at one pole and a convergent centre at the other. All the hairs within a field will point towards the convergent centre. In general, both convergent and divergent centres will act as the poles of two or more of these fields. The hairs entering into the formation of convergent centres will not all be from the same source

* The system of divergent centres associated with a given pattern does not necessarily become active in follicle development for all its members simultaneously. In the account given above of the ontogeny of the coat in *Trichosurus vulpecula* it was noted that the medial canthal divergence emerges as a centre of follicle production before the more cranially situated rhinal centre, so that for some time the divergent interval between them is formed only on the side adjacent to the eye. If the postulate is sound that divergent and convergent centres are fundamentally similar in their physiological significance, then the existence of differences in timing of attainment of threshold levels as between divergent and convergent centres related through hair rows would be anticipated.

(divergent centres), and these differences, as shown by examination of the interramal convergence of *Trichosurus vulpecula* (*v. supra*), can often be seen as differences in the character and arrangement of the hairs.

Interchangeability of Divergent and Convergent Centres.

It has been suggested above that the divergent and convergent centres have the same fundamental function in relationship to the physiology of tract development. If there is this similarity between the two types of centre one might reasonably look for cases, either in ontogeny or phylogeny, where the one has become substituted for the other. No evidence is forthcoming from this marsupial material that alternative conditions of divergence or convergence may obtain at a given situation on the skin in the same species. From the phylogenetic aspect, however, a case from the Peramelidae seems relevant. In this family hair tracts of the groin may be determined by the presence of a single or double divergent radial system or of a medial convergent system. The two alternatives have been figured in Part I (Boardman, 1943*b*) for *Isoodon obesulus* (Fig. 9) and *Perameles myosura notina* (Fig. 10). Both alternatives occur within the genus *Perameles*. The probability is present, then, that substitution of one type of field for the other may take place.

Discussion.

Explanation has been the objective of nearly all workers on hair tracts. The theories* that have been advanced fall into two groups representing fundamentally different approaches to the subject.

In the first group the early primordium of the follicle is regarded as subject to tension and pressure through the influence of which the definitive slope and direction of the developing hair is determined. The literature of these mechanical theories has been reviewed by a number of authors, notably Schwalbe (1910, 1911), Landauer (1925*a*, 1925*b*) and Biedermann (1928). The most recent work advocating mechanical explanation is that of Landauer (1925*b*).

The second and smaller group of theories was inaugurated with Ludwig's paper (1921), using human material and some domestic animals. Colin (1943) followed with a study based on the embryogenesis of hair follicles in the guinea-pig. Both of these workers propounded theories to explain differential hair direction, and in each case the theory rests on a gradient-field concept. Ludwig advanced the idea that growth in area of the skin is "quantitatively pluricentral", each surface element increasing through reproduction of its own special cells. Further, these growing surface elements grow faster in some directions than others. From this postulated pluricentral expansion and differential growth rates Ludwig concludes that in the skin there exist growth lines running between points of the highest and lowest growth rate. These lines determine the pattern of follicles. This ingenious theory is primarily concerned with explanation of deviations from the arrangement of the hair in the primitive condition. The nature of the work makes a critical examination difficult in that histological or experimental evidence having bearing on it is at present not available. Colin (1943) accounted for hair direction by suggesting "that the more actively growing regions give off some kind of growth-promoting influence, possibly a substance or substances which diffuse to other regions. As a consequence, a physiological gradient with its high at the posterior end and its low somewhere on the head becomes established; similar gradients develop in the limbs and ears with highs at the distal ends. The resulting differential growth within the follicles directs the latter away from the more active regions . . . . The formed hairs accordingly point in general toward regions of active growth as measured at the time of formation of their primordia." Colin's hypothesis is open to a number of objections, both in itself and arising out of its application to explain the data derived from this study of marsupial hair tracts. The theory depends

* Hair tracts have been advanced by Kidd (1903, 1920) as a case of the inheritance of acquired characters. This viewpoint has been strongly supported by Wood Jones (1924, 1925, 1943). Consideration of the question is deferred to a later contribution.

on the reality of "differential growth of the opposite sides of the early primordium itself". Beyond an expression of opinion by the author arising from examination of his material, there seems to be no histological evidence presented in the paper that the slope of the early primordium is traceable to or correlated with differential growth. The theory would identify divergent centres and convergent centres as focal points of low and high metabolic activity respectively. The role of the divergent centres as the point from which the follicle growth waves take their origin and the passivity, at least in the early stages of coat formation, of the convergent centre, make it highly probable that the opposite is the case (*v. supra*). The idea of a diffusible substance being in any way connected with hair slope seems to be negatived by the absence of any evidence of graded effect from the centre of diffusion outwards. Also there is a complete absence (*v. supra*) of any kind of mingling or resultant of forces at tract margins as would almost certainly occur in the presence of such a substance diffusing from adjacent centres. Colin takes the view that the points of high growth rate of the developing body are coincident with convergent centres as they occur in the formed coat. The general growth in area of the skin is, of course, closely correlated with the changing contours of the body as growth proceeds. But the placing of divergent and convergent centres appears to have no relationship to probable regions of low and high growth rate for the body generally. The point is made clear by consideration of the variable position of the median dorsal divergent centre in the members of the macropod genus *Dendrolagus* (Rothschild and Dollman, 1936). This centre may be present at the level of the shoulders, the mid-back, or near the root of the tail. Superimposed upon the general growth contours of the skin there is apparently a second and separate system of centres of activity concerned with the elaboration of the hair covering with its extensive implications in skin organization.

The regional differential orientation of the hair that gives rise to tract formation presents a number of problems. It is necessary to seek explanation for:

1. The presence of divergent and convergent centres.
2. The slope of the hair, that is, the angle it makes with the skin surface.
3. The direction in which the sloping hair is pointed, that is, on a line joining a divergent to a convergent centre.
4. The centrifugal extension of the follicular growth wave from a centre and the arrangement of the developing follicles on lines joined to the centre.
5. The whorling of divergent and convergent centres.

Sufficient evidence has now accumulated (*v. supra*) to make tenable the view that divergent and convergent centres are centres of activity which, by their number and arrangement, determine the pattern of tracts. The follicle growth wave has been shown (in marsupials) to take its origin from or in the vicinity of a divergent centre. The subdivision of the growth wave to produce tract units is conditioned by the presence of the so-called convergent centres upon which a given segment of the growth wave converges. Further, evidence has been adduced that fundamentally the divergent and convergent centres are similar in nature. The manner in which the lines of follicles converge towards a point (centre) shows that while activity at the point is not such as to permit follicle initiation it is not quiescent, but is in some way concerned in the course taken by the follicles. As has been expressed earlier in this paper, it seems permissible to view any given pattern of tracts as a mosaic of separate fields each polarized by the presence of a divergent and convergent centre. The nature of the activity at the centres seems to be responsible for the initiation of the follicle growth wave.

Landauer (1925*b*) has emphasized the necessity for recognizing that the definitive orientation of the hair shaft involves two components—the angle that it makes with the skin surface, and the direction in which it is pointing. His theory of causation, however, advances a common cause (skin tension) as responsible for the two components. Within a given tract the slope of the hair tends to remain constant, but the direction in which it is pointing is subject to wide variation in relationship to the tract poles. Illustrative of the extent of fluctuation in direction, the course of hairs from the occipital

whorl in *Trichosurus* over the shoulder and then caudalwards on the ventral surface to the mid-belly convergence may be cited. There seems no reason why a common cause should be sought for the two components of hair direction. The tip of the developing hair is always directed away from the centre in association with which the follicle growth wave is initiated. This rule has no exceptions in the present marsupial material. There is, however, nothing to indicate that the primary slope as distinct from the direction of pointing is the outcome of activity at the centre. It is proposed here to advance the view that slope is an intrinsic quality of the follicle. It will be apparent that much more is involved than mere inclination of the hair shaft with reference to the surface of the skin. There are probably constant relationships of the sebaceous gland, the arrector pili muscle and the epithelial bed to the inclined follicle, though confirmation of this has not been included in the present research. Support for the viewpoint comes from two sources. Firstly, slope is correlated with features both in the structure of the hair itself and in its environment. Landauer (1925*a*) has gathered together the relevant literature. Thus Stoss reports correlation between skin thickness and angle of inclination of hair in the horse and cattle; Landauer, utilizing data from Frédéric, showed that the same was true for man, and later (Upham and Landauer, 1935) this was confirmed on more extensive material; Lehmann's data for sheep establish a correlation between thickness of hair and slope. Secondly, the experimentally demonstrated individuality of the feather with reference to orientation seems admissible as supporting evidence. Thus Lillie (1942) reports: "If a papilla dissected completely free from all attachments is rotated through 90° or 180° around its own axis and re-implanted, the feather which develops from it is similarly disorientated, in the latter case upside down with the dorsal surface next the skin." It should be borne in mind that feathers, like hairs, are arranged in tracts within which feathers develop in an orderly sequence from localized areas of proliferation (Holmes, 1935). There is, then, a probability that slope *per se* is an innate quality of the follicle correlated with certain physical features, particularly thickness of the skin and thickness of the hair shaft.

Evidence has been presented (*v. supra*) that the follicle growth wave is made up of lines of follicles which extend from a divergent centre towards a convergent centre. Two points here call for explanation—the fact that the hair shaft primarily grows away from the centre of initiation of follicle growth, and that the so-directed shaft lies on a line connecting the divergent centre with a convergent centre. No suggestion is forthcoming from this material as to how the growth centre from which the growth wave originates brings about this orientation of the follicle. The second point, the geometrical pattern of lines along which the follicles grow, finds a possible explanation by applying the principle of ultrastructural organization as put forward by Weiss in connection with his experimental work on the growth of nerve fibres. The advancing lines of follicles that constitute the growth wave, each line drawn along a definite route from a divergent centre to a convergent centre, produce an impression that there must be directive tracks of some kind in the skin. The phenomenon of follicle succession and line direction has been discussed above for *Trichosurus vulpecula*. Ludwig (1921) held the opinion that the growth lines existed at the microscopic level but was unable to detect them. Weiss (1934) writes:

"The formation of connecting fiber tracts is strikingly demonstrated when two spinal ganglia are cultivated in a thin plasma membrane at some distance from each other: A narrow streak of cells and nerve fibers grows then in a perfectly straight direction along the line connecting the two cultures. The assumption that the phenomenon is due to 'chemotactic' attraction could be decisively disproved. The real mechanism is as follows: The proliferative activity of the cultures entails dehydration of the surrounding medium. The dehydration causes contraction. Contraction proceeding from two centers establishes tensions which, in the space between the two centers, reach a maximum of intensity and are oriented in the direction of the connecting line. These oriented tensions evoke a correspondingly oriented pattern of ultrastructures in the medium, an ultrastructural 'bridge' leading from one center to the other. The

outgrowing nerve fibers simply follow the 'bridge'. On the basis of these results the fact is explained that in embryogenesis intracentral fiber tracts develop between centers of accelerated growth (Coghill) and that peripheral nerves are 'attracted' by growing organs (Detwiler)."

The existence of the system of centres of activity in the mammalian skin may be interpreted as an example *in vivo* of Weiss's tissue culture experiment, from which it might be concluded that the activity of the centres has produced between them a series of micellar lines related causally to the precise tracks followed by the advancing follicles.

The whorling of the hair often observed about the divergent and convergent centre finds no explanation in the present work. The phenomenon would seem to imply the existence of a separate problem. In *Trichosurus vulpecula* two centres usually strongly whorled in well-furred material, the occipital divergent centre and the mid-ventral (umbilical) convergent centre, appear not to be whorled at the time that the primary coat is laid down. This would suggest that the whorling is a later development, perhaps conditioned in its appearance by differential growth about the centre. The separateness of this phase of the hair tract problem is shown by Schwarzburg's (1927) work on the occipital whorl in man, where it was demonstrated that clockwise rotation is dominant to counter-clockwise rotation.

Summary.

1. The hair tracts problem is stated.

2. Evidence is submitted that tract pattern:

(*a*) is constant for the species,

(*b*) does not vary with age,

(*c*) is constant in its developmental stages for the species.

3. Divergent and convergent centres are shown to be primary configurations of the pelt in that their presence is dependent on the genotype. Divergent and convergent intervals are regarded as secondary characteristics the formation of which is conditioned by the grouping of centres.

4. The primary hairs are shown to be disposed in lines running between divergent and convergent centres.

5. The divergent centre is identified as a growth centre from which the growth wave of follicles takes origin.

6. Evidence is tendered that divergent and convergent centres, while not equally concerned in the development of the pelt, are fundamentally similar in nature.

7. There are grounds for believing that the divergent and convergent centres may occasionally be interchangeable.

8. The skin, with reference to its hairy covering, is considered to be a mosaic of fields each unit of which is polarized by a divergent and convergent centre.

9. Current theories of causation involving gradient-field concepts are critically examined.

10. Two components are recognized in the definitive orientation of the hair shaft—the angle made by it with the skin surface (its slope) and the direction in which it is pointing. Slope is considered to be an intrinsic quality of the follicle.

11. The geometrical pattern of follicle lines running between divergent and convergent centres is considered as possibly due to activity at the centres having produced an ultrastructural organization of micellar (ultramicronic) lines along which the follicles grow.

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THE HAIR TRACTS IN MARSUPIALS.

PART VI. EVOLUTION AND GENETICS OF TRACT PATTERN.

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[Read 27th September, 1950.]

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Evidence has been submitted (Part V) that any given tract pattern may be regarded as a mosaic of fields the number and form of which is determined by the skin's complement of divergent and convergent centres. The centres are shown to be primary characteristics of the skin in the sense that their presence or absence is conditioned by the genotype. It follows that the phylogeny of pattern is most likely to be expressible in terms of change in the number and distribution of these centres. Support for this view is provided by a consideration of tract sequence in the polyprotodont family Dasyuridae and the diprotodont subfamily Phalangerinae. The material representing other families is not sufficiently ample for formal presentation in this context.

The Primitive Condition Considered in Relationship to Growth Centres.

In the primitive condition (Boardman, 1946*b*), the simplest of tract patterns, divergent (growth) centres are situated at the extremity of the snout and on the chin so that growth of hair is initiated at the cranial end of the body and spreads as a growth wave caudally. The course of the lines of follicles is determined by the influence of the caudal end of the body and the distal extremities of the ears and limbs which are regarded as physiological equivalents of convergent centres. The relationship of the follicle growth wave to divergent and convergent centres has been discussed in Part V. Universally in marsupials the cloacal hillock is the site of a convergence with, in consequence, a convergent interval between it and the root of the tail. A litter of seven *Antechinus flavipes* (M.4793) in which the primary coat is not completely laid down illustrates these points. The dorsal half of the body is haired from the cranial end almost to the level of the hind limbs and it is apparent from the direction taken by the lines of follicles and the gradient in hair size from the edge of the advancing wave cranially that hair growth was initiated at the extremity of the snout. The fore-limb is haired laterally but not the hind-limb. The gular region is haired and there is weak hair growth on the ventral thorax. Origin of the growth wave on the ventral aspect is similarly traceable back to the divergent centre on the chin. Confirmatory evidence from the Marsupialia is provided by juveniles of *Myrmecobius fasciatus* (Part III, Boardman, 1949), which in its tracts differs very little from the primitive condition.

Stages in Tract Development and their Evolutionary Significance.

The comparative approach to pattern made possible by the wide selection of marsupials available for this study early showed that pattern could be classified using as criteria for separation the number and situation of the centres of radial fields. Differences between one pattern and another are essentially differences involving divergent and convergent centres. This is best shown on the present material by analysing

pattern form in the polyprotodont family Dasyuridae and the diprotodont subfamily Phalangerinae. It is seen that pattern within these two groups falls readily into stages of ascending order of complexity from the most primitive to the most highly developed genera. The authority of Bensley (1903) is cited for what are primitive and what are highly developed genera in the groups discussed.

The facility with which the tracts can be classified as indicated leaves no doubt that pattern evolution has been through successive additions of divergent and convergent centres on a primitive ground-plan. While primitive genera and highly developed genera can be nominated with reasonable certainty the relationship of genera between the two extremes is less clear so that no attempt has been made to elaborate a phylogenetic arrangement. The pattern grades are arranged below in an arbitrary linear series. It is not at present known what constitutes a unit of increment in this evolutionary sense for divergent and convergent centres so that the presentation does not necessarily portray in detail the actual sequence of events leading to the formation of any particular pattern.

Family Dasyuridae.

Of the five pattern grades observable in the family the first and second belong to the Phascogalinae, the third, fourth and fifth to the Dasyurinae.

Subfamily Phascogalinae.

Stage 1.—The simplest pattern amongst the polyprotodonts is that of the Sminthopsis-group (*Sminthopsis*, *Antechinus* and *Planigale*) of this subfamily. The coat presents a close approach to the hypothetical primitive condition. Except in the scrotal region of the males (*v. infra*) there are no added divergent or convergent centres so that the hairs generally flow from the nasal region caudally or caudally and ventrally. The cloacal hillock is the site of a convergence common to all marsupials.

Stage 2.—The second stage is represented by the Dasycercus-group (*Dasycercus*, *Dasyuroides* and *Phascogale*). It is derivable from the Sminthopsis-group by the addition of the elbow and interramal convergences. The interramal convergence is not always fully expressed (see *Dasycercus cristicauda*, Boardman 1946*a*, Figs. 1 and 2).

Subfamily Dasyurinae.

Stage 1.—The first stage, represented in this material only by the single genus *Sarcophilus*, differs from the Smithopsis-group of the Phascogalinae by the addition of the mid-ventral (umbilical) convergence.

Stage 2.—A re-examination of the specimens of *Dasyurus quoll* leads to the conclusion that the somewhat vague disturbances in the gular hair are caused by a bilateral pair of whorls associated with a distorted interramal convergent point. If this be so the stage would be derivable from the Dasycercus-group of the Phascogalinae.

Stage 3.—Stage 3 follows Stage 2 by the addition of an umbilical convergence. It includes the Dasyurinus-group (*Dasyurinus* and *Dasyurops*). The derivation of Stage 3 from Stage 2 implies homology of the blurred gular whorls of *Dasyurus* and the axillary whorls of *Dasyurinus* and *Dasyurops*. This homology is in accord with the facts of variation in whorl pattern (*v. infra*).

Subfamily Phalangerinae.

Four stages in tract elaboration may be defined between the primitive *Acrobates* and the highly developed *Trichosurus*.

Stage 1.—In *Acrobates pygmaeus* the simple condition shown in the Sminthopsis-group of the Phascogalinae is repeated. The primitive nature of the tracts is not altered by the presence of the flying membrane.

Stage 2.—The second stage is represented by *Cercartetus*, in which appear an interramal convergent point and a preauricular stream (presumably from a divergence associated with the external ear). These two features together give rise to a divergent interval on the face.

Stage 3.—The third stage is reached in *Petaurus* and *Schoinobates* by the addition of a convergent point in the vicinity of the lateral canthus (in *Petaurus papuanus* this point has not been recorded).

Stage 4.—*Pseudocheirus* differs from Stage 3 by the addition of an umbilical convergent point. The conspicuous convergent whorl between the eye and the ear is regarded as homologous with the point more usually situated nearer to the eye.

Stage 5.—In *Dactylopsila* a dorsal whorl is found superimposed on the conditions characteristic of Stage 4.

Stage 6.—*Trichosurus* represents a considerable advance on the preceding stage in that both axillary and medial canthal divergent centres are present. The centre at the lateral canthus is not developed.

The polyprotodont and diprotodont stocks of the Marsupialia represented above by the Dasyuridae and Phalangerinae respectively are distinct lines of descent that appear to have separated very early in the history of the group. In polyprotodonts tract patterns have, except for the medial canthal divergence of the Peramelidae, evolved beyond the primitive state only on the ventral aspect of the body. In diprotodonts, on the other hand, dorsal and ventral surfaces have been affected. Comparing the ventral surface of polyprotodonts and diprotodonts with reference to tract evolution it is readily seen that the end result is in both cases the same. That is to say, for each divergent or convergent centre in the Polyprotodontia there is an equivalent structure occupying a similar situation in the Diprotodontia. This fact is illustrated by reference to the analysis of tract pattern in the Dasyuridae and Phalangerinae. Inguinal radiating fields, not represented in either dasyures or phalangers are, when they occur, the same in both polyprotodonts and diprotodonts. An apparent exception to this generalization is provided by the ventral thoracic convergence in *Thylacinus* which is not found elsewhere in marsupials. It might be anticipated that one or both of the two principal marsupial stocks would, in their more highly developed forms, produce centres not represented in the other. Elsewhere than in this particular centre *Thylacinus* conforms to the generalization. A consideration of the tracts of the dorsal surface as presented by the constituent families of the Diprotodontia leads to a similar conclusion, namely, that the most highly developed group, the Macropodinae, includes all features present in other groups.

A given centre may show considerable variation in position. Consider the paired divergent whorls, most commonly situated in the axilla, which are present in all Macropodidae examined except *Dendrolagus*. The whorl may be displaced laterally onto the upper arm (*Macropus major*) and the forearm (*Thylogale* sp.), or cranially onto the upper thorax (*Bettongia penicillata*) and even as high as the root of the neck (*Potorous tridactylus apicalis*). The occurrence of this series of positions about the more frequent axillary locus for the whorl, the relationship of the genera cited, and the absence of other whorled systems with which it might be confused, make tenable the assumption that in each case it is the same whorl that is being considered. The material presents no evidence of overlap of the territory that might be occupied by adjacent centres.

Comparison of the hair tracts of the Polyprotodontia and Diprotodontia, bearing in mind the restriction on the variation in position of any particular centre, raises the point that there must exist for the marsupial skin a maximal complement of divergent and convergent centres. The evaluation of this maximal figure (to be discussed in a subsequent contribution) can be satisfactorily made only through comparative studies which enable what might be called initial centres to be distinguished from derived centres produced by subdivision of the initial units. The subdivision (meristic repetition) of centres is discussed at length in Part IV.

With reference to tract pattern, then, the situation in the marsupial skin is such that evolution proceeds by successive increments of centres towards the attainment of a saturation density, each centre, divergent or convergent, having its own territory of occurrence. A similar end-result is attained irrespective of whether development is along the distinct lines presented by the Polyprotodontia or the Diprotodontia.

Genetics of Hair Tracts.

The genic mechanism may have evolved in two ways. There may have been successive mutations leading to the production of centres in particular areas. With this interpretation the relatively precise zonation of centres and the relationships of divergent and convergent types, affecting similarly polyprotodont and diprotodont lines, is difficult to explain except in terms of orthogenetic trends. On the other hand, it may be postulated that a mutation or mutations occurred in the ancestral stock permitting the potential development of centres up to the saturation density (*v. supra*) for the marsupial skin and that the extent to which they appear is conditioned by the effects of a system of modifying genes. This viewpoint would explain the similar steps by which pattern has evolved in polyprotodonts and diprotodonts and the attainment in both of these major groups of a similar end-point for pattern form. While no evidence from marsupial breeding is available, support for the second alternative is given by the work of Sewall Wright (1934, 1935) on the genetics of rosette pattern in guinea-pigs which is interpreted by him as implying the existence of such a genic background to the pattern grades that may be defined for these rodents. Variation of rosette pattern in guinea-pigs is similar to pattern variation in the Marsupialia as a whole.

Sex-Dimorphism with Reference to Pattern.

Only one aspect of pattern following sex is found in the Marsupialia and that is what has been referred to in the descriptive portion of this work as the prescrotal reversal or prescrotal reversed triangle. The condition has been figured for *Dasycercus cristicauda* (Part II, Fig. 3, Boardman, 1946*a*). It consists of the development of a divergent centre at the base of the scrotal stalk with a convergent centre in the middle line a short distance in front. The distinctness of the triangular field of reversed hairs is attested by the fact that often the field remains naked or very sparsely haired after the development of dense hair in the surrounding areas. In species having prescrotal reversal of this type the female shows no variation in the primitive caudo-ventral trend of the inguinal region except in *Tarsipes spenserae* where a similar but indistinctly developed area of reversal is found in a female also.

Prescrotal reversal is probably universal in the Phascogalinae but there is at present no evidence that the peculiarity is found in the Dasyurinae. It occurs in *Myrmecobius fasciatus*. As mentioned above, it is recorded for *Tarsipes spenserae* and is also found in some of the less highly developed members of the Phalangerinae (*Acrobates pygmaeus* and *Cercartetus nanus*). It is doubtful if the reversal as found in the Phascogalinae occurs in the higher phalangers or in the macropods, but the material is insufficient to provide data on which to formulate a rule.

The simplest explanation is that the dimorphism is a secondary sexual character. The fact that the female of *Tarsipes spenserae* has the configuration weakly developed is against interpretation as a case of sex-limited inheritance.

The Gular Whorls of the Peramelidae—a Phenogenetic Explanation.

The family Peramelidae is characterized by the presence of a whorled system on the throat. What might be called the typical condition is that of a bilateral pair of whorls, mirror images of each other and conforming to the marsupial directional rule for the ventral surface, that is, the left member is always clockwise (Part IV, Boardman, 1950*a*). The paired condition has been figured for *Perameles nasuta* (Boardman, 1943*a*). Variation consists in the suppression of one whorl independently of the other which maintains unchanged both its lateral position and direction of flow. This unilateral suppression is indiscriminately on the right or left side within the species and is independent of sex (see *Echymipera cockerelli*, Part I, Fig. 11, Boardman, 1943*b*; *Isoodon obesulus obesulus*, Part II, Fig. 9, Boardman, 1946*a*). The chances of one or other of the three conditions of the gular whorled system (paired whorls, a single right whorl, or a single left whorl) occurring are approximately equal if the family is considered as a whole. Of a total of nineteen specimens showing gular whorls, seven (three males and four females) have the double system, six (one male and five females) have a single whorl clockwise and on the left, six (again one male and five females) have a single

whorl counter-clockwise and on the right. Three of the ten examples with a double-whorled system have one member of the pair more strongly developed than the other. Specimen R.13042, a female of the *Perameles gunnii* series, shows this point very well in having the right member very small and practically overwhelmed so far as effect on hair disturbance is concerned by the whorl on the left side.

The male of the series of *Macrotis minor minor* (Part II, Boardman, 1946*a*) has no gular whorls at all. The remainder of the series consists of two females having each a single whorl. At first sight this seems to be an extreme case of the tendency to suppress whorls, but the fact that the male is devoid of elbow and inguinal whorls as well suggests that a more profound genic disturbance is involved which is not in this context comparable with the phenomenon of unilateral suppression.

Right-left asymmetry, the essential peculiarity of the variability of these gular whorls, has a parallel in certain teeth in fossil skulls. Simpson (1944) has recorded of the functionless P^2 in the Oligocene viverrid *Hoplophoneus* that it "may vary from a well-developed state to complete absence, not only within one race but also within one individual (i.e., the left side may differ from the right side)". A further case is provided by the degenerating P^3 of the Paleocene Multituberculate *Ptilodus montanus*. Simpson does not deal specifically with the probable genetic background of these tooth vagaries but it would appear from the general viewpoint expressed that he visualizes the accumulation of small degenerative mutations leading gradually, in the absence of selection-pressure, to the suppression of the teeth and during suppression bringing about disequilibrium in the factors determining the range of variability.

Goldschmidt (1946) has commented at length on the examples of tooth variation given by Simpson. Goldschmidt has also submitted an alternative phenogenetic explanation which rests on known genetic facts. Briefly, this explanation takes cognisance of the peculiarities of some of the homoeotic mutants in *Drosophila*. Amongst these mutants are genes of low penetrance, highly variable expression, and with a high degree of right-left asymmetry. The right-left asymmetry has been shown by statistical analysis of tetraptera, tetraltera and podoptera to be the outcome of the absence of right-left correlation, each wing reacting independently in the mutant. Goldschmidt demonstrates that, by altering such features as time of determination and threshold levels "one single mutant with a type of action as discussed, causing any rudimentation, can produce all the conditions of variability, etc., described by Simpson". Goldschmidt has developed his thesis at length in several works (see, for example, 1938 and 1940).

The genetics of the peramelid gular whorls may be viewed from a similar angle—a mutation having arisen to alter the developmental timing characteristic of the whorl pair relative to each other thus introducing right-left asymmetry. The situation is, then, comparable to the cases of Simpson's relative to degenerating teeth and to the homoeotic mutants (though no homoeosis is involved in such a situation).

In discussing the high variability of degenerating teeth Simpson stresses that "It is so commonly true that degenerating structures are highly variable that this may be advanced as an empirical evolutionary generalization". There is no evidence that this high variability with right-left asymmetry is associated with degeneration in the case of peramelid gular whorls. The variability of the whorls probably differs from the expression of variability in Simpson's skulls in that only one member of the whorl pair is involved. Whether the whorl is absent or at any stage of development short of complete expression the remaining whorl is invariably fully developed and produces hair reversal on the throat little if at all less than the pair together would produce. The question of degeneration does not seem to arise with reference to these whorls.

Summary.

1. The primitive condition of hair tracts is expressed in terms of growth centres.

2. Successive levels of tract complexity within the Dasyuridae (Polyprotodontia) and Phalangerinae (Diprotodontia) are presented as evidence that tract evolution has proceeded by the continued addition of divergent and convergent centres on a primitive ground-plan.

3. Evolution of the gene complex with reference to tract pattern is considered. The phylogeny of tract pattern suggests that there is operative a principal gene or genes the expression of which is dependent on a system of modifiers.

4. The known cases of sex-dimorphism of tracts are recorded. It is concluded that the phenomenon is interpretable as a secondary sexual character.

5. The vagaries of phenotypic expression of the gular whorls in the Peramelidae are stated. The right-left asymmetry is attributed to a mutation having altered developmental timing of the whorl pair relative to each other. It is concluded that the high variability shown by the whorls is not associated with degeneration.

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THE HAIR TRACTS IN MARSUPIALS.

PART VII. A SYSTEM OF NOMENCLATURE.

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(Two Text-figures.)

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Divergent and convergent centres the distribution and relationships of which condition hair tract formation (Part V) are not disposed haphazardly in the skin but are found only in particular situations. Each centre has a territory of occurrence and the territories of neighbouring centres seem not to overlap (Part VI, Boardman, 1950*c*). There is apparently a definable maximal concentration of centres which is an intrinsic property of the group. Comparison of polyprotodont and diprotodont tract evolution and consideration of the probable genetic mechanism (Part VI, Boardman, 1950*c*) leaves little doubt that centres of the same type occupying the same situation are homologous structures.

With these generalizations it becomes possible to formulate provisionally a system of nomenclature using symbols applicable to the Marsupialia and almost certainly (*v. infra*) to the Mammalia as a whole. In the proposed terminology divergent and convergent centres are recorded relative to the dorsal and ventral surfaces. The centres, divergent and convergent respectively, are numbered from the cranial end caudally except as mentioned below. In drawing up such a nomenclature several points must be taken into consideration. Evidence has been presented (Part IV, Boardman, 1950*a*) that some bilateral pairs of centres are derived from a single medial structure. The derivation of bilateral pairs from medial units may be general. Thus, the divergent centre on the forearm in *Thylogale* has been shown to be equivalent to the axillary centres of macropods generally (Part VI, Boardman, 1950*c*) and must be presumed equivalent to the single medial whorl as exhibited by *Vombatus hirsutus* (Parts II and IV, Boardman, 1946*a*, 1950*a*). However, in cases such as the divergence at the medial canthus, the elbow convergence and the genal convergence where the specimens give no evidence that affinity is with a known medial point, the centre is referred to by abbreviation and is not included in the numerical system used for other centres. The material suggests but does not provide further grounds for believing that duplication may have occurred longitudinally and that subsequent bilateral duplication has taken place in one only of the daughter centres. Longitudinal duplication is a well-established phenomenon as a meristic variant and simultaneous longitudinal and transverse duplication is known to occur (Part IV, Boardman, 1950*a*). This viewpoint would explain the triangular arrangement of divergent centres in the groin of *Perameles gunnii* (Part II, Figs. 12 and 13, Boardman, 1946*a*), and of the interramal convergence and the convergent centre occasionally seen in the vicinity of the angle of the mouth, as in *Trichosurus vulpecula* (Part II, Fig. 19, Boardman, 1946*a*). The nearness of the centres in the cases cited is suggestive of relationship through duplication. The unique convergence on the mid-ventral thorax of *Thylacinus* (Part I, Fig. 8, Boardman, 1943) is omitted from consideration at this stage pending further information relevant to its status; it may be related to the elbow convergence in the same manner as has been suggested above for the convergence at the angle of the mouth and the interramal convergence.

The following abbreviations are used: a = ankle, C = convergent, D = divergent, d = dorsal, e = elbow, g = genal, h = heel, k = canthal, l = lateral, m = medial, p = ear (pinna).

Divergent Centres of Head, Neck and Trunk.

Dorsal Surface.—There appear to be four divergent centres in the dorsal series additional to those associated with the eye and ear (*v. infra*). The first (Dd1) is placed at the end of the snout and is present in all marsupials. The centre of this system is usually diffuse and apparently may be single or bilaterally doubled. When doubled and whorled, as in *Trichosurus vulpecula* and *Onychogalea fraenata* (Part II, Figs. 19 and 38, Boardman, 1946*a*), the result is the well-known rhinal reversal. The second (Dd2), restricted to diprotodonts, occupies the region from the crown back to about the level of the inferior angle of the scapula. The third (Dd3) is fairly constant in position on the mid-back; it is represented in the collection only by *Macropus major* (Part II, Fig. 42, Boardman, 1946*a*). The fourth (Dd4) occurs over the sacrum back to the root of the tail; *Phascolarctos* has this centre doubled over the sacrum; in *Dendrolagus dorianus* it is situated at the root of the tail (Miklouho-Maclay, 1885).

Ventral Surface.—Three divergent centres occur on the ventral surface. The first (Dv1), of universal occurrence in marsupials, is situated on the chin. The second (Dv2) is most commonly found in a bilaterally paired condition associated with the axilla, but may be located on the gular region, ventral thorax in front of the caudal limits of the axilla, or on the fore-limb (Part VI, Boardman, 1950*c*). Dv3, in the inguinal region, may be single and median as in *Isoodon obesulus* (Part I, Fig. 9, Boardman, 1943), bilaterally doubled as in *Thylacinus* (Boardman, 1945, Fig. 1) or arranged as three centres at the angles of a triangle as in *Perameles gunnii* (Part II, Figs. 12 and 13, Boardman, 1946*a*). In *Macropus major* the lateral members of the Dv3 pair have again subdivided to give a transversely placed pair on each side of the middle line (Part II, Fig. 43, Boardman, 1946*a*).

Convergent Centres of the Head, Neck and Trunk.

Dorsal Surface.—Other than that associated with the lateral canthus (*v. infra*) only one convergent centre (Cd1) is found on the dorsal surface. It occurs on the crown in the Macropodinae (see, for example, *Macropus major*, Part II, Fig. 42, Boardman, 1946*a*) and occasionally in *Phascolarctos*.

Ventral Surface.—Four sites of convergence are found on the ventral aspect of the body. The cranialmost (Cv1) is that at or in the vicinity of the interramal papilla. Most marsupials possess it. The second (Cv2) is the familiar umbilical convergence, though its coincidence with the umbilical scar is exceptional rather than otherwise. Cv2 is bilaterally doubled in *Thylacinus* (Part I, Fig. 6) and *Thylogale* (Part I, Fig. 24, Boardman, 1943). The third (Cv3) occurs in front of the cloacal hillock and may be present within the pouch area or near the root of the scrotum. The caudalmost member of the series, Cv4, is the convergence at the cloacal hillock; it is universal in marsupials. All of these convergent centres are shown by *Trichosurus vulpecula* (Part II, Fig. 20, Boardman, 1946*a*).

A genal convergence (Cg) is present in some marsupials.

Centres Associated with the Eye, Ear and Limbs.

Except for the divergent centre on the upper arm of *Macropus major* and on the forearm of *Thylogale* (Part VI, Boardman, 1950*c*) none of the centres found associated with the eye, ear and limbs can be demonstrated as derived from medial loci of the head, neck or trunk, although, as mentioned above, it is possible that this is so. Consequently, no attempt is made at this stage to give them a place in the numbered series defined above and they are here referred to by non-committal abbreviation.

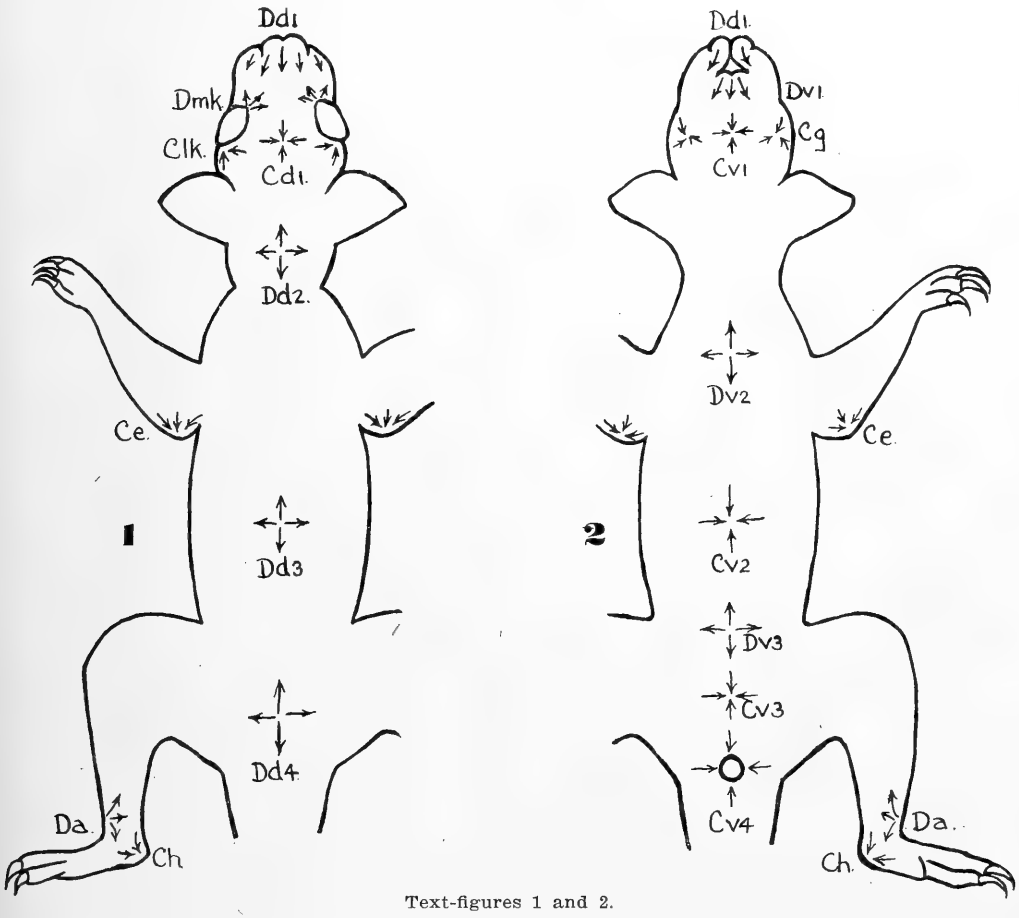
The eye.—Very often in marsupials a divergent centre (Dmk) is associated with the medial canthus (for example, *Onychogalea fraenata*, Part II, Figs. 37 and 38, Boardman, 1946*a*). A further centre, apparently always convergent (Clk), is similarly associated with the lateral canthus. Sometimes the centre at the lateral canthus may be at some distance from it as in *Pseudocheirus* (Part II, Fig. 17, Boardman, 1946*a*).

The ear.—The frequent presence of a preauricular reversal would suggest that a divergent centre may be situated in the cavity of the external ear but its focal point is difficult to define in this marsupial material. It is provisionally designated Dp.

The fore-limb.—The elbow convergence (Ce) is often present in marsupials; it is always single.

The hind-limb.—Except in the Phascogalinae, the heel seems always to be the site of a convergence (Ch) which produces reversal to a greater or lesser extent on the plantar surface. It is very strongly developed in peramelids.

Divergent systems on the hind-limb occur rarely. *Phascolarctos* (Part II, Fig. 27, Boardman, 1946*a*) possesses a distinct whorl on the ankle (Da), and what is probably a duplicated version of this has been recorded for two macropods (*Thylogale* sp. and *Onychogalea fraenata*).



Text-figures 1 and 2.

Schematic representation of the relationship of divergent and convergent centres in the marsupial skin (Fig. 1, dorsal aspect; Fig. 2, ventral aspect). See text for explanation of symbols.

Applicability of the System of Nomenclature to the Mammalia as a Whole.

The symbolized terminology submitted above is based on the collection of marsupial young, a systematic account of the hair tracts of which has been recorded in Parts I-III (Boardman, 1943, 1946*a*, 1949) of this work. The collection is representative of the several groups and includes nearly a third of known marsupials. A summary of the hair tracts of these species arranged according to the terminology is presented in Table 1.

TABLE 1.

Résumé of Marsupial Tract Pattern Arranged in Accordance with the Proposed Nomenclature. Centres of Universal Occurrence in the Order are Omitted. (For explanation of symbols, see text.)

Name.	Dd2.	Dd3.	Dd4.	Dv2.	Dv3.	Cd1.	Cv1.	Cv2.	Cv3.	Dmk.	Clk.	Cg.	Ce.	Ch.	Da.	Reference.
PHASCOGALINAE—																
Sminthopsis crassicaudata c. ..	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1949*, p. 192
Sminthopsis crassicaudata macrura	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1943, p. 97
Sminthopsis crassicaudata centralis	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1946*a*, p. 181
Antechinus flavipes flavipes ..	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1943, p. 96
Antechinus maculatus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1943, p. 96
Planigale ingrami	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1943, p. 97
Dasycercus cristicauda	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	1946*a*, p. 179
Dasyuroides byrnei	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	1946*a*, p. 181
Phascogale sp.	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	1949, p. 192
DASYURINAE—																
Sarcophilus harrisii	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	1943, p. 98; 1946*a*, p. 182
Dasyurus quoll	–	–	–	+	–	–	+	–	–	–	–	–	+	+	–	1943, p. 97
Dasyurinus geoffroii g.	–	–	–	+	–	–	+	+	–	–	–	–	+	+	–	1946*a*, p. 181
Dasyurinus geoffroii fortis ..	–	–	–	+	–	–	+	+	–	–	–	–	+	+	–	1946*a*, p. 181
Dasyurops maculatus	–	–	–	+	–	–	+	+	–	–	–	–	+	+	–	1946*a*, p. 182
THYLACININAE—																
Thylacinus cynocephalus	–	–	–	–	+	–	+	+	–	–	–	–	+	+	–	1943, p. 98†
MYRMECOBIIDAE—																
Myrmecobius fasciatus	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	1949, p. 192
PERAMELIDAE—																
Isoodon obesulus obesulus	–	–	–	+	+	–	–	–	–	+	–	–	+	+	–	1946*a*, p. 182
Isoodon obesulus fusciventer ..	–	–	–	+	+	–	+	–	–	+	–	–	+	+	–	1943, p. 100
Isoodon macrourus	–	–	–	+	+	–	–	–	–	+	–	–	+	+	–	1943, p. 101
Isoodon torosus	–	–	–	+	+	–	–	–	–	+	–	–	+	+	–	1943, p. 101
Perameles nasuta	–	–	–	+	+	–	+	–	–	+	–	–	+	+	–	1943, p. 102
Perameles gunnii	–	–	–	+	+	–	+	–	–	+	–	–	–	+	–	1946*a*, p. 183
Perameles myosura myosura ..	–	–	–	+	–	–	+	–	–	+	–	–	+	+	–	1943, p. 101
Perameles myosura notina ..	–	–	–	+	–	–	+	–	–	+	–	–	+	+	–	1943, p. 101
Perameles sp.	–	–	–	+	+	–	+	–	–	+	–	–	+	+	–	1949, p. 194
Macrotis minor minor	–	–	–	+	–	–	+	–	–	+	–	–	+	+	–	1946*a*, p. 184
Echymipera cockerelli	–	–	–	+	+	–	+	–	–	+	–	–	–	+	–	1943, p. 102
TARSIPEDINAE—																
Tarsipes spenserae	–	–	–	+	–	–	+	–	–	+	–	–	–	+	–	1943, p. 102; 1949, p. 194
PHALANGERINAE—																
Acrobates pygmaeus	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	1943, p. 103; 1946*a*, p. 185
Cercartetus concinnus	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	1949, p. 194
Cercartetus nanus nanus	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	1946*a*, p. 185
Cercartetus nanus unicolor ..	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	1946*a*, p. 185
Petaurus breviceps	–	–	–	–	–	–	+	–	–	+	+	–	–	+	–	1946*a*, p. 186
Petaurus australis	–	–	–	–	–	–	+	–	–	+	+	–	–	+	–	1946*a*, p. 186
Petaurus norfolcensis	–	–	–	–	–	–	+	–	–	+	+	–	–	+	–	1946*a*, p. 186
Petaurus papuanus	–	–	–	–	–	–	+	–	–	+	–	–	–	+	–	1946*a*, p. 187
Schoinobates volans	–	–	–	–	–	–	+	–	–	+	+	–	–	+	–	1943, p. 104; 1946*a*, p. 188
Pseudocheirus laniginosus ..	–	–	–	–	–	–	+	+	–	+	+	–	–	+	–	1946*a*, p. 187
Pseudocheirus convolutor c. ..	–	–	–	–	–	–	+	+	–	+	+	–	–	+	–	1946*a*, p. 187
Dactylopsila picata	+	–	–	–	–	–	+	+	–	+	+	–	–	+	–	1943, p. 104
Trichosurus vulpecula v.	+	–	–	+	–	–	+	+	–	+	–	–	–	+	–	1946*a*, p. 188
Trichosurus vulpecula hypoleucus	+	–	–	+	–	–	+	+	–	+	–	–	–	+	–	1946*a*, p. 190; 1949, p. 194
Trichosurus fuliginosus	+	–	–	+	–	–	+	+	–	+	–	–	–	+	–	1946*a*, p. 190
PHASCOLARCTIDAE—																
Phascolarctos cinereus	+	–	+	+	–	–	+	+	–	+	–	–	–	+	+	1943, p. 106; 1946*a*, p. 192
VOMBATIDAE—																
Vombatus ursinus u.	–	–	–	+	–	–	–	–	–	+	–	–	–	+	–	1946*a*, p. 192
Vombatus ursinus tasmaniensis ..	–	–	–	+	–	–	+	–	–	+	–	–	–	+	–	1946*a*, p. 194
Vombatus hirsutus h.	–	–	–	+	–	–	–	–	–	+	–	–	–	+	–	1946*a*, p. 195
POTOROINAE—																
Bettongia penicillata	–	–	–	+	–	–	+	+	+	+	–	–	+	+	–	1943, p. 106
Potorous tridactylus apicalis ..	–	–	–	+	–	–	+	+	+	+	–	–	–	+	–	1946*a*, p. 195
Bettongia lesueur graii	–	–	–	+	–	–	+	+	+	+	–	–	+	+	–	1949, p. 195
Caloprymnus campestris	–	–	–	+	–	–	+	–	–	+	–	–	–	+	–	1946*a*, p. 196

TABLE 1.—*Continued.*

Résumé of Marsupial Tract Pattern Arranged in Accordance with the Proposed Nomenclature. Centres of Universal Occurrence in the Order are Omitted. (For explanation of symbols, see text.)

Name.	Dd2.	Dd3.	Dd4.	Dv2.	Dv3.	Cd1.	Cv1.	Cv2.	Cv3.	Dmk.	Clk.	Cg.	Ce.	Ch.	Da.	Reference.
MACROPODINAE—																
Dendrolagus inustus	+	−	−	−	−	+	+	+	+	+	−	−	−	+	−	1943, p. 107
Onychogalea fraenata	+	−	−	+	+	+	+	+	+	+	−	+	−	+	+	1946*a*, p. 197
Thylogale sp.	+	−	−	+	+	+	+	+	+	+	−	−	−	+	+	1943, p. 108
Wallabia agilis	+	−	−	+	+	+	+	+	+	+	+	+	−	+	−	1946*a*, p. 199
Wallabia bicolor	+	−	−	+	+	+	+	+	−	+	−	+	−	+	−	1943, p. 111; 1946*a*, p. 199
Wallabia dorsalis	+	−	−	+	+	+	+	+	−	+	−	+	−	+	−	1946*a*, p. 200
Wallabia sp.	+	−	−	+	+	+	+	+	−	+	−	+	−	+	−	1946*a*, p. 200
Setonix brachyurus	+	−	−	+	+	+	+	+	−	+	−	+	−	+	−	1949, p. 195
Osphranter robustus	+	−	−	+	+	+	+	+	−	+	−	−	−	+	−	1943, p. 112; 1946*a*, p. 200
Macropus major	+	+	−	+	+	+	+	+	+	+	−	−	−	+	−	1946*a*, p. 201

* Dates refer to papers by W. Boardman, see list of references. † See also Boardman (1945).

The scattered literature on hair tracts of mammals other than marsupials is, in the aggregate, extensive and appears to be sufficient to serve as a basis for consideration of the question of general applicability of the nomenclatural system that has been proposed.

For groups other than Primates the extensive work of Kidd (1900, 1902, 1903*a*, 1903*b*, 1904, 1920) is the principal source although the usefulness of the descriptions is restricted since only adult material was examined. To this should be added the papers of De Beaux (1924*a*, 1924*b*, 1927, 1931) on fissiped carnivores, *Potamochoerus* and *Bradypus*, of Wood Jones (1940) on *Pedetes*, of Niedoba (1917) on domestic animals (rabbit, guinea-pig, sheep, goat, pig, cat, dog, cow, buffalo, horse, ass, mule), and of Boardman (1946*b*) on monotremes.

The Primates have attracted more attention than other eutherian groups. In addition to the classical accounts of the hair tracts of man accurate information is available on a wide range of genera. Schwalbe (1910, 1911) has written on *Galago*, *Lemur*, *Propithecus*, *Indris*, *Tarsius*, *Macacus*, *Semnopithecus*, *Nasalis*, *Simia*, *Gorilla* and *Anthropopithecus*. De Beaux (1917, 1918) has written on *Hylobates*, *Guereza*, *Cercopithecus*, *Maimon*, *Choiropithecus*, *Papio*, *Hamadryas*, *Hapale*, and *Galago*. Brandt (1940) has discussed hair arrangement in *Chiromys*, *Loris*, *Tarsius*, *Cebus*, *Pithecia*, *Macacus*, *Cercopithecus*, *Hylobates*, *Anthropopithecus*, and *Gorilla*, and Osman Hill (1947) in *Arctocebus*.

A critical examination of this literature, particularly of that concerning the Primates, leads to the conclusion that the principle of classification of tract pattern by reference to divergent and convergent centres is applicable generally in mammals. Further, the scheme drawn up for marsupials would serve equally well for other mammalian orders as far as their hair tracts are at present known. There appears to be throughout the class a unity of plan for the distribution of divergent and convergent centres. It would seem that the spatial relationships of this system of centres (Part V) is within limits, constant from order to order and therefore is to be regarded as a class attribute. As Kidd (1904) has written, "No mammalian *Order* has been found possessing characteristic whorls, featherings and crests".

SUMMARY.

1. A symbolized nomenclature is proposed of divergent and convergent centres as they are found in the marsupial skin.

2. The principle of classification is held to be applicable to the Mammalia generally.

3. It is suggested that the spatial relationships of the system of centres (divergent and convergent) is, within definable limits of variation, constant for the Mammalia as a whole.

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A NOTE ON NON-RECIPROCAL FERTILITY IN MATINGS BETWEEN SUBSPECIES OF MOSQUITOES.

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[Read 27th September, 1950.]

The non-reciprocal fertility of matings between *Aëdes scutellaris scutellaris* and *A. scutellaris katherinensis* reported by Woodhill (1949*a*, 1949*b*, 1950) is of very considerable interest in relation to the problem of speciation in mosquitoes. Matings between the two subspecies prove to be fully effective and give fertile hybrids which show characteristic Mendelian segregation in the F_2 when the subspecies *scutellaris* is used as the female parent, but when *katherinensis* is used as female parent the mating is ineffective and the eggs laid are completely inviable. The occurrence of cryptic and sibling species in *Anopheles* and other genera has been well established (Mayr, 1942), but in such cass sterility barriers and sexual isolation between the forms is complete. In the present case, Woodhill has pointed out that the two forms would presumably interbreed should they occur in the same territory, and in the absence of evidence to the contrary they must be ranked as geographical subspecies. Comparable cases of non-reciprocal isolation between "races" of other species of *Aëdes* have been described by Perry (1949) and by Downs and Baker (1949), so that the case is not exceptional. It may even be of frequent occurrence in the Culicidae.

The origin and nature of non-reciprocal fertility is also of genetical interest because it may involve an interaction between the nucleus and the cytoplasm. The existence of undefined cytoplasmic incompatibility as an isolating mechanism between species is probably of frequent occurrence, but, where it involves complete and absolute isolation, it is not susceptible to analysis. It is important that the present case should be actively investigated by any methods which may become available.

Woodhill's data demonstrate that in the sterile mating *A. s. katherinensis* × *A. s. scutellaris** effective copulation and insemination occur, live spermatozoa being found in the spermathecae of the mated females. Two possibilities must be considered.

Firstly, the *scutellaris* sperm may fail to enter the *katherinensis* eggs, either because of the serological conditions in the host females or for mechanical or other reasons. There is abundant evidence that insemination, or even the mere act of copulation, may serve as a stimulation to ovulation in insects, and the high egg production obtained from the mating is of little significance. However, the low egg production of females of *Aëdes albopictus* when mated with *A. aegypti*, as reported by Downs and Baker (*l.c.*) may indicate that copulation alone provides insufficient stimulus. It must be emphasized that unless partial development of the hybrid embryos or actual fusion of gamete nuclei can be demonstrated, it will be difficult to exclude this first possibility, but it would seem to be improbable in view of the backcross data obtained by Woodhill.

The second possibility is that the sperm enter the eggs but that either they fail to effect fertilization or the hybrid embryos die at a very early stage of development. This possibility has implications of far-reaching significance, involving an incompatibility interaction between the cytoplasm of the *katherinensis* egg and the *scutellaris* nuclear genom in the haploid sperm or the hybrid embryo. An explanation, tenable in mammals, that the hybrid embryo is serologically incompatible with the body fluids of the mother, is untenable in the Culicidae, where the entry of the sperm into the egg occurs immediately prior to oviposition and where the actual nuclear fusion may occur

* In conformity with the usual genetical convention, throughout this paper the female parent is written to the left and the male parent to the right of the × sign in denoting a mating.

after this act, as it does, for example, in *Anopheles* (Nicholson, 1921). In the reciprocal mating *scutellaris* × *katherinensis* the cytoplasm of the *scutellaris* egg must be fully compatible with the *katherinensis* genom both in the haploid sperm and the hybrid embryo.

The following formal scheme, although at present little more than speculation, could explain such behaviour and leads to predictions capable of experimental verification or disproof. It is dependent on the assumption that the insect sperm contributes little or no cytoplasm to the zygote. There is evidence from the inheritance of CO_2-sensitivity in *Drosophila* (L'Heritier, 1948) that the sperm of Diptera may contribute a little cytoplasm to the zygote, but this would be small in comparison with the quantity of cytoplasm contributed by the egg and would not be likely to invalidate the hypothesis.

If the cytoplasm of *katherinensis* contains a factor or factors incompatible with the nuclear genom of *scutellaris*, the nuclear-cytoplasmic constitution of *katherinensis* may be written as KK_k, where K represents one haploid *katherinensis* genom, and $_k$ represents the cytoplasmic factor. Similarly, *scutellaris* may be written SS_s, where $_s$ may represent a different cytoplasmic factor, characteristic of *scutellaris* cytoplasm, but not incompatible with the *katherinensis* genom, or merely the absence of $_k$. It is not at present suggested that $_k$ is necessarily particulate in nature.

The mating $SS_s \times KK_k$ would involve S_s eggs and K sperms and would give the hybrid combination SK_s, which would be viable, but the mating $KK_k \times SS_s$, involving K_k eggs and S sperm, would fail. Further, it would be expected that, on backcrossing the fertile hybrid SK_s to both parents reciprocally the following matings would be successful and would give progeny of the constitutions indicated.

Mating.	Gametes Involved. ♀	♂	Constitution of Backcross Progeny.*
SKs × SSs	$\frac{SKs}{2}$	S	$\frac{SK}{2}$Ss
SSs × SKs	Ss	$\frac{SK}{2}$	$\frac{SK}{2}$Ss
SKs × KKk	$\frac{SKs}{2}$	K	$\frac{SK}{2}$Ks

On the other hand, the mating $KK_k \times SK_s$, which would involve K_k eggs and $\frac{SK}{2}$ sperms, might give either partial or complete sterility, according to the number of genes in the S genom involved in the incompatibility interaction. In the unlikely event that only one such "S" gene were involved, 50% of compatible sperm would be produced, but with a number, n, of "S" genes, inherited independently, the expected proportion of compatible sperm would be only $1/2^n$. With numerous "S" genes randomly distributed on the chromosomes and inherited in three linkage groups (the chromosome complement of *Aëdes* consists of three pairs of chromosomes), sperm from F_1 males lacking all portions of the S genom, and consequently pure for the K genom, would be rare, if they occurred at all, and the backcross to KK_k females would be completely unsuccessful. It may be noted that meiosis in F_1 males has been found to be normal, with a mean chiasma frequency above 2 per bivalent and not obviously lower than that in the parental subspecies.

So far prediction is in agreement with the experimental evidence, on the assumption that many "S" genes are involved. It is desirable that the hypothesis should be

* The formula $\frac{SK}{2}$ is used to designate the product of reduction division in the hybrid to indicate that as the result of crossing over it will contain a haploid genom made up in part of units of the S genom and in part of the K genom. The actual result in any particular gamete will depend on the positions of chiasmata and the accidents of chromosome assortment.

tested further, and this could be done by a series of backcrosses of the types

$$KK_k♀♀ \times (SK_s♀♀ \times KK_k♂♂)♂♂$$
$$KK_k♀♀ \times [(SK_s♀♀ \times KK_k♂♂)♀♀ \times KK_k♂♂]♂♂$$
etc.

It would be expected that the S genom would be gradually broken down, at a rate dependent on the number of "S" genes involved and on their chromosomal distribution, leading to partially fertile matings in the later backcross generations.

If this thesis of incompatibility between nuclear genes and cytoplasmic factors can be established, it would be theoretically possible to analyse the nature of the cytoplasmic factors involved and, so far as the author is aware, no comparable cases of interspecific or inter-racial cytoplasmic sterility barriers have been analysed in plants or animals, with the possible exception of the mating groups and the "killer" (kappa) substance in *Paramoecium* (Sonneborn, 1947). In the present case it should be possible to determine the nature of the cytoplasmic factors, whether particulate and plasmagenic or diffuse and perhaps hormonal, and also to determine the degree of dependence of the $_k$ factor on the KK nucleus. Such an analysis would seem to require the use of a third subspecies, compatible with *katherinensis* when the latter is used as female, and compatible also with *scutellaris*. Since many subspecies of *Aëdes scutellaris* are already known (Woodhill, 1949*a*), and probably many more remain to be discovered, these requirements should not be unattainable. Crosses of *katherinensis* with this third species, and back crosses to the third species, always using the latter as the male, would, when outcrossed to *scutellaris*, indicate whether the $_k$ cytoplasmic factor was dependent on the KK nucleus or independent and permanent.

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RESPIRATION AND CELL DIVISION IN DEVELOPING OYSTER EGGS.

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(Five Text-figures.)

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Introduction.

Since Hertwig made the first observation of fertilization in the Naples sea urchins in 1875, work on invertebrate eggs has played an important part in the history of cytology and cell physiology. Warburg in 1908 initiated the study of the relations which exist between the metabolism of developing eggs and the morphological changes which they undergo; since this time many workers have contributed to the study of these relations, which today are still far from elucidated.

The advantages of invertebrate, more particularly marine invertebrate, eggs are not far to seek: they are readily removed from the parent in the form of cells and washed free of other ovarian contaminants; they are usually in physiologically uniform state and, being separated in a fluid to which they would normally be voided, are remarkably stable *in vitro*; their availability in large numbers, physiologically uniform, is an advantage not possessed by tissue cultures, and the rapid, continued synchronized division in developing eggs makes them unique as material for physiological studies on cell division.

Most of this type of work has been done on the small, relatively alecithal eggs of sea urchins. Since they keep poorly under laboratory conditions, their study implies the existence of a well equipped marine laboratory in reasonable proximity to extensive sea urchin beds, facilities which are not locally available.

On the other hand, the rock oyster (*Ostrea commercialis*) which is cultivated in the Sydney area, will remain viable for up to six weeks out of water and is readily maintained under laboratory conditions in small amounts of aerated sea water. Its eggs, which are small, although made rather opaque by the presence of large numbers of small yolk granules (Cleland, 1947), are available for a considerable period in a fertilizable condition, have a satisfactory respiratory rate, and develop very rapidly.

The present work, which, with the exception of the colchicine experiments, was performed in the 1948 season, was undertaken in order to define the gross aspects of the respiratory metabolism of unfertilized and developing oyster eggs. In succeeding papers the metabolism will be analysed in greater detail and such knowledge applied to physiological studies of the mitotic process.

Apart from the work of Ballentine (1940) on *O. virginica*, there have been no previous observations made on respiration in developing oyster eggs; Lucke and Ricca (1941) have made a study of the permeability of the eggs of *O. virginica*.

Materials and Methods.

The source and maintenance of the adults and the method of preparing crude egg suspensions are the same as described in a previous paper (Cleland, 1947).

Crude egg suspensions were strained and freed of cytolysed eggs, vesicular tissue contaminants, etc., by three cycles of suspension in 50 volumes of sea water and centrifugation at 35 × g for 60–75 seconds. The number of viable eggs separated from one oyster varied between 5 and 30 × 10^6.

Sperm was separated from male oysters in about 5 ml. of sea water and, after straining, the resulting suspension centrifuged at 125 × g for 3 minutes to remove spermatocytes, spermatids and non-motile sperm. Such sperm suspensions will retain their fertilizing capacity for up to four days when stored in a refrigerator at about 3°C.

Fertilization was performed by suspending the washed eggs in 50–100 volumes of sea water, adding about 1/200 volume sperm suspension and leaving for 15 minutes. After this time eggs were washed once, as above, to remove sperm, suspended in about 10 volumes of sea water and placed either into Warburg vessels for immediate use or into 150 ml. Erlenmeyer flasks shaken on the Warburg bath. In all the experiments quoted in this paper the fertilization and development percentage was in excess of 90% unless otherwise stated.

Oxygen consumption was determined by the direct method of Warburg in vessels of 15–20 ml. capacity. R.Q. was determined by the direct method of Warburg and by the first method of Dickens and Simer. All procedures are described in Dixon (1943) and Umbreit *et al.* (1945). In the tables and figures the respiratory rate quoted for any given time is the rate found for the previous time period; e.g., a value quoted for 3 hours after fertilization refers to the rate over the period $2\frac{1}{2}$–3 hours.

Egg numbers were determined on aliquots made to a final volume of 5 ml. This was placed in an optically plane-bottomed dish of 4 cm. diameter, the suspension agitated and, after settling, the number of eggs in twenty-four microscope fields counted. The mean number per field was multiplied by the factor relating the surface area of the microscope field to that of the dish. The coefficient of variation of the mean number of eggs per field was 3·9%, which was a close approach to the theoretical value derived from the Poisson distribution.

The volume of the eggs used was determined on aliquots by the centrifuge method of Shapiro (1935). Because of the absence of jelly layer the eggs pack more quickly and compactly than sea urchin eggs; a centrifugal field of 700 × g acting for 5 minutes gave a valid estimate of volume.

Total nitrogen was determined by combustion of aliquots in H_2SO_4 and H_2O_2, steam distillation of the digest in the Parnas Wagner apparatus, and Nesslerization of the distillate.

After the completion of an experiment on developing eggs, development was followed in aliquots diluted 1/200 for a further 18 hours. In all cases at least 50% of the eggs were alive and had developed into typical trochophores. The low percentage development is associated with, and was presumed due, to bacterial growth in the cultures.

Determinations of the stage of development in the closed Warburg flasks were made by reference to a parallel shaken Erlenmeyer flask culture. This flask was stoppered and had provision for CO_2 absorption.

All experiments were conducted in a Warburg bath maintained at 26°C. with a shaking rate of 90 cycles/min. and an amplitude of 6 cm.

Results.

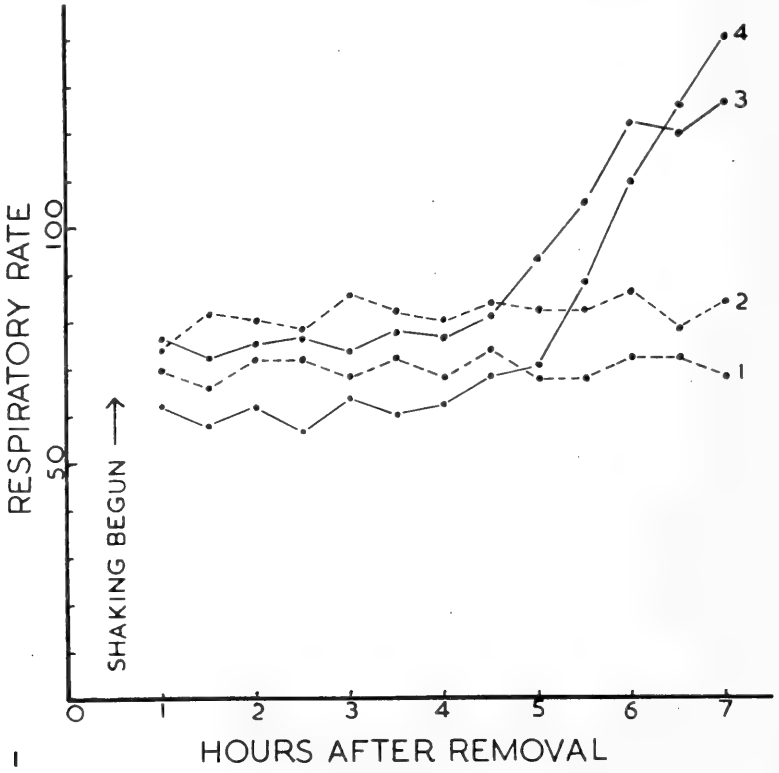
In twenty-nine determinations the number of eggs in 1 ml. of packed eggs was found to vary from 13–17 million, with an average of 14·5. One ml. of eggs contained an average of 28 mg. of total nitrogen with a range of 24–32 (25 determinations). One ml. of eggs had a dry weight of 240 mg. with a range of 230–260 (six determinations).

Effect of Continued Shaking.

It has been known for a long time that the rate and amplitude of shaking is an important factor in studies of invertebrate egg respiration by the Warburg method (e.g. Whitaker, 1933). In the present instrument the amplitude was fixed and the rate could not be reduced below 90 cycles per minute. The influence of time of shaking was investigated by removing aliquots from a large, gently aerated, stationary culture of fertilized eggs at hourly intervals, recovering the eggs by centrifugation, and transferring them to a Warburg vessel, which was then shaken and read until the end of the experiment. The results, which are shown in Table 1, indicate that eggs may safely be shaken for periods of seven hours without altering their respiratory rate. Loss of eggs by cytolysis, as determined by successive estimates of volume, was almost invariably found in both stationary and shaken cultures, up to a maximum of 10% of the initial volume of eggs.

Respiration of Unfertilized Eggs.

The majority of unfertilized egg batches could be shaken constantly in Warburg flasks for 7–8 hours without change in respiratory rate (Text-fig. 1, curves 1 and 2). In about 20% of cases eggs shaken for 5–6 hours showed a considerable increase in respiratory rate as shown in Text-figure 1 (curves 3 and 4). Examination of such eggs revealed signs of cytolysis, the eggs appearing perfectly spherical and more light-



Text-figure 1.

Respiratory rate of unfertilized eggs during prolonged shaking. Curves (1) and (2) were typical of the majority of batches studied, curves (3) and (4) from batches showing evidence of cytolysis at the end of the experiment. The respiratory rate is expressed as μl. $O_2/5 \times 10^6$ eggs/$\frac{1}{2}$ hour.

TABLE 1.

Effect of Continued Shaking on the Respiratory Rate of Developing Eggs.

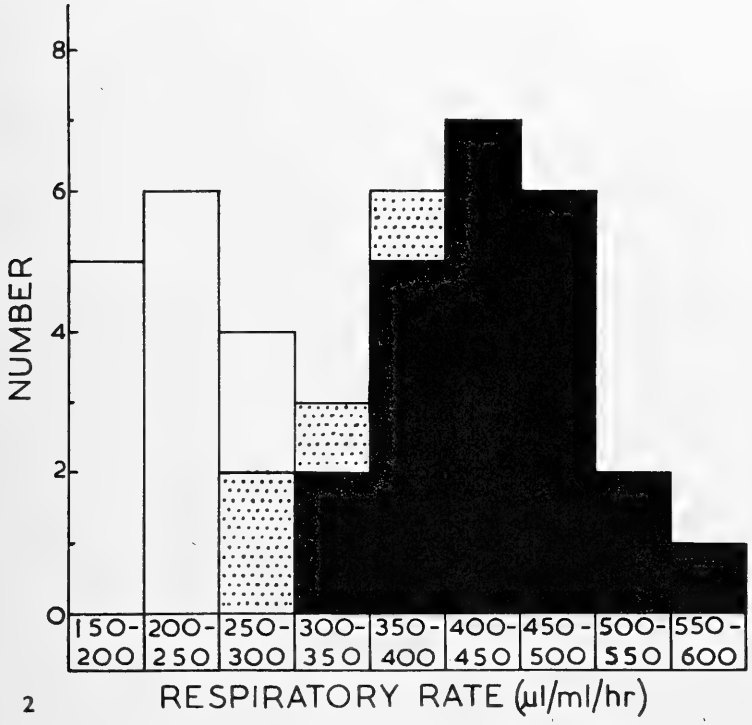
Flask No.	Hours After Fertilization.										
	2	2½	3	3½	4	4½	5	5½	6	6½	7
1	30	35	45	52	52	63	68	65	85	95	103
2	—	—	43	50	50	59	63	62	78	89	94
3	—	—	—	—	49	55	63	60	75	86	95
4	—	—	—	—	—	—	66	63	83	90	100
5	—	—	—	—	—	—	—	—	78	85	95
				a				*b*			

Respiratory rate is expressed as μl. O_2 per flask per half hour. The slight variations between flasks are probably due to varying losses of eggs during the centrifugation and resuspension of the samples. The times of somatoblast division and hatching are shown at *a* and *b*.

absorbing or having an appearance corresponding to abnormality I in egg suspensions described in a previous paper (Cleland, 1947). It is not known whether this increased respiratory rate occurred in stationary cultures also.

The respiratory rate of 40 batches of unfertilized eggs, some of them from individual oysters, some from pooled eggs, is shown in Text-figure 2, where observations of the state of the germinal vesicle are also recorded.

In a previous paper (Cleland, 1947) it was stated that the germinal vesicle of the oyster does not break down until fertilization occurs. More intensive study of the material has shown that the eggs fall into three classes in their behaviour to fertilization: (*a*) those which show no germinal vesicle breakdown and no activation even though sperm penetration has occurred; (*b*) those which show no breakdown or incomplete breakdown in sea water but which show activation on fertilization; (*c*) those which pass



Text-figure 2.

Frequency histogram of the respiratory rate of unfertilized eggs. The black areas are from batches showing more than 75% germinal vesicle breakdown, the dotted areas from batches with 10-75% breakdown, and the white areas from batches showing less than 10% breakdown.

to the first maturation metaphase in sea water and remain at this stage in the absence of fertilization. Eggs taken at the height of the breeding season almost invariably belong to the latter class; the second class was only rarely found.

The respiratory rate of class (*c*) eggs has tended in later seasons to be lower than that found characteristic for eggs in the 1948 season.

Effect of Fertilization on Respiratory Rate.

The effect of fertilization on the respiratory rate was investigated on six batches of eggs (each from one oyster only), fertilization being effected by tipping in 0·1 ml. of sperm suspension from the sidearm of the Warburg flask. The respiration of this quantity of sperm was negligible.

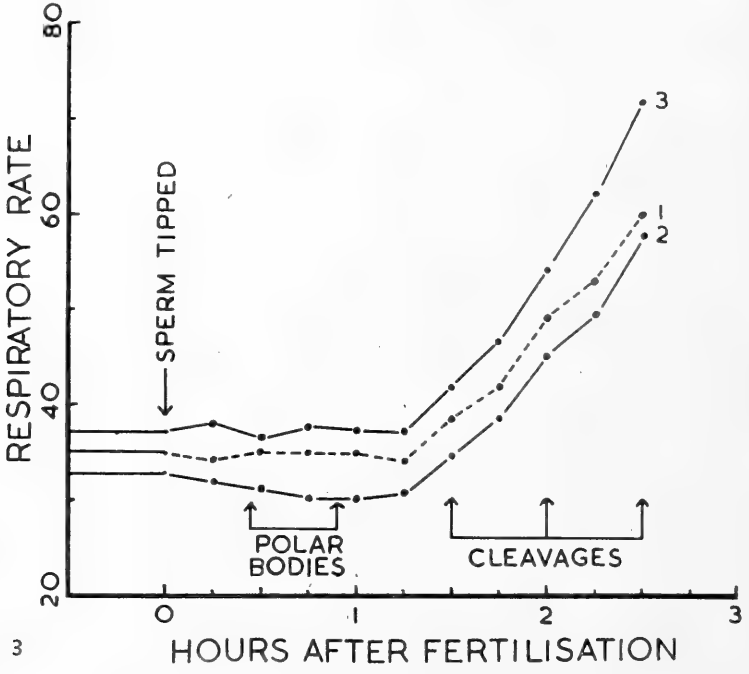
In Text-figure 3 the mean and two of the experimental curves are shown. In all curves no increase in respiratory rate was found until the onset of the first cleavage

division. After this the curve may show a smooth rise, as in curve 3, or show differences in respiratory rate with different stages of the division cycle, as in curve 2 or curve 1, which is the mean curve of the six experiments.

The data indicate that the maximal respiratory rate is found in the prophase, metaphase and possibly anaphase, while the minimal rate is found in telophase and the resting stage. The differences between different experiments were evidently due to differences in the degree of synchronization of division; in the poorly synchronized eggs the curve was smooth, while in the well-synchronized it showed the mitotic correlation. Synchronization as good as that found in *Arbacia* eggs (Fry, 1936) has never been found in the present material.

Respiratory Rate during Development.

The respiratory rate of 20 batches of eggs was followed for periods of up to eight hours after fertilization. At the peak of the season, when synchronization is good and more than 95% of the eggs are fertilized curves typical of those shown in Text-figure 4



Text-figure 3.
Respiratory rate (expressed as μl. $O_2/5 \times 10^6$ eggs/¼ hour) following fertilization.

were found. The first two plateaus may be somewhat masked, as in Text-figure 5, when the middle of the critical periods falls near a point of reading.

The early development of *O. commercialis*, which does not appear to differ significantly from the development of *O. gigas* (Fujita, 1929) or *O. virginica* (Brooks, 1880) may be divided into the following stages: (*a*) stage of meiotic divisions lasting for about 60 minutes after fertilization; (*b*) early cleavage stage up to 3–4 hours after fertilization; (*c*) stage of disappearance of the macromere, due to division of the "somatoblast" (Fujita) and occurring at about three and a half hours after fertilization; this is associated with depressed cleavage rate; (*d*) later cleavage stage terminating at 4–5 hours after fertilization; (*e*) the first signs of hatching are seen in a non-translational rotatory movement of the larvae which becomes progressively more rapid and appears to be associated with a depressed mitotic activity; (*f*) stage of increasing swimming intensity which is associated with increasing rate of translational movement

and occurs between hatching and about six and a half hours after fertilization; (*g*) stage of maximum swimming intensity is reached about seven hours after fertilization. At this stage the larvae in stationary cultures arrange themselves in vertical columns as described by Roughley (1933).

It will be noted in Text-figure 4 that three plateaus in the respiratory rate are present: one at somatoblast division, one at hatching and one when maximal swimming rate is reached. The first two are evidently due to depression of the mitotic rate and the third to attainment of maximal ciliary activity, the latter probably accounting for most of the rise after hatching.

Effect of Blocking Cell Division on Respiratory Rate.

In order to see what relation the rise in respiration had to cell division, experiments were performed in which colchicine was added from the side arm at different times after fertilization. The results of such an experiment (one of three) are shown in Text-figure 5. Similar results were obtained with a number of other mitotic inhibitors and will be considered in a later paper of this series. Almost identical results were obtained over a hundredfold colchicine concentration range (M/100–M/10,000), suggesting that penetration of the alkaloid or primary inhibition of respiratory enzymes was not the explanation of the results. Below the minimum biologically effective concentration (approximately 10^{-4}M) colchicine was without effect on respiration.

Effect of Suspending Medium.

Table 2 records one of the three experiments performed showing the influence of suspending medium and pH on the respiration of developing eggs. Glycine has been

TABLE 2.

Effect of Suspending Medium and pH on Respiration of Developing Eggs.

Medium.	Initial pH.	Hours After Fertilization.										Final pH.
		1½	2	2½	3	3½	4	4½	5	5½	6	
Sea water	7·85	70	82	108	144	148	179	178	225	280	275	7·4
Sea water + $\frac{M}{100}$ KH_2PO_4	5·8	65	74	100	130	130	150	150	184	220	230	5·7
Sea water + $\frac{M}{50}$ glycine	7·5	70	84	117	150	160	190	190	250	315	325	7·6
Sea water + $\frac{M}{100}$ phosphate buffer ..	6·3	67	75	106	135	137	165	165	207	245	250	6·3
Sea water + HCl ..	6·0	65	78	104	135	138	162	164	195	235	240	6·0
					a			*b*				

The respiratory rate is expressed as µl. O_2 per half hour per 5×10^6 eggs. *a* and *b* show the times of somatoblast division and hatching. No difference in the amount of egg breakdown in the various media could be detected.

recommended as a sea water buffer by Robbie (1948). The increased respiratory rate with glycine, especially after hatching, was presumed due to utilization of the glycine; glycine added from the side arm to larvae in sea water causes a rise in respiratory rate to the level of larvae which have developed in glycine sea water. The more marked depression of respiratory rate after hatching at low pH was associated with, and presumed due to, depressed ciliary activity. Similar results were obtained with unfertilized eggs.

Respiratory Quotient during Development.

Since development proceeds normally at a pH where CO_2 retention can be satisfactorily corrected for by the method of Johnson (Umbreit *et al.*, 1945) periodic respiratory quotient determinations on one egg sample are possible by the direct

method of Warburg. The validity of the KCO_2 correction for pH was established by estimates of the increase in the CO_2 which could be liberated by H_2SO_4. Continuous R.Q. determinations were made on fifteen batches (from individual females) of developing eggs suspended in M/100 KH_2PO_4 and sea water (pH 5·8) and, in about half of these, values typical of those shown in Nos. 1–3 of Table 3 were obtained where little or no change during development was found. In the other half (Nos. 4–6) the R.Q. appeared to rise, especially after hatching. No pH change was detectable in any of these experiments. Since this rise occurred in cases where the amount of eggs per flask was higher it was suspected that some metabolic product was causing a depression of oxygen uptake in the flask without KOH. Since this was abolished by replacing the gas atmosphere by fresh air it appeared that the relatively high CO_2 tension in the gas space was responsible.

It will be seen from Table 3 that some variation in respiratory quotient is found in different egg batches and that little or no change occurs on fertilization.

TABLE 3.
Respiratory Quotient during Development.

Expt. No.	Unfertilized.	Hours After Fertilization.									
		1½	2	2½	3	3½	4	4½	5	5½	6
1	0·82	0·84	0·82	0·82	0·83	0·81	0·86	0·83	0·85	0·84	—
2	0·80	0·78	0·79	0·78	0·78	0·76	0·81	0·77	0·82	0·83	0·82
3	0·78	0·81	0·78	0·83	0·85	0·81	0·84	0·80	—	—	—
4	0·78	0·79	0·79	0·79	0·77	0·81	0·82	0·81	0·84	0·89	—
5	0·84	0·84	0·86	0·91	0·87	0·94	0·84	0·90	0·96	0·94	0·97
6	0·76	0·78	0·80	0·82	0·84	0·83	0·86	0·88	0·94	0·99	1·03

In eight experiments parallel determinations of R.Q were made with the direct method of Warburg and the first method of Dickens and Simer. These determinations, some of which are shown in Table 4, indicated that the results of the two methods were in fair agreement. The R.Q. of eggs in sea water was the same as that of eggs in KH_2PO_4 buffered sea water in determinations by the Dickens and Simer method.

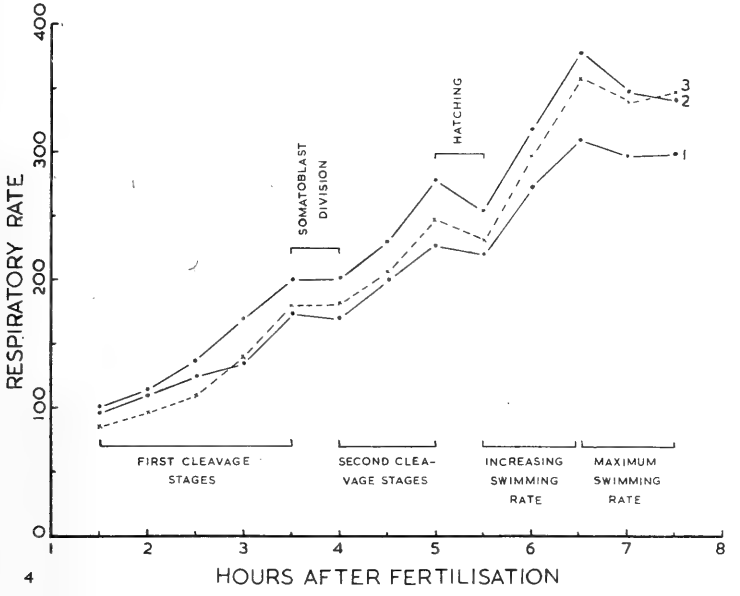
TABLE 4.
Comparison of the Respiratory Quotient by the Direct Warburg and Dickens and Simer Methods.

Experiment Number.	Method.	Hours After Fertilization.			
		1½–3	3–4	4–5	5–6
1	Direct Warburg	0·79	0·80	0·82	0·84
	Dickens and Simer	0·78	0·78	0·80	0·81
2	Direct Warburg	0·79	0·78	0·82	0·83
	Dickens and Simer	0·82	0·80	0·84	0·85
3	Direct Warburg	0·80	0·82	0·82	0·85
	Dickens and Simer	0·80	0·80	0·79	0·82

DISCUSSION.

In Cartesian diver determinations of respiratory rate Holter and Zeuthen (1944), Zeuthen (1944) and Borei (1948) have found that there is a decline of respiration of unfertilized eggs of *Ciona*, *Rana*, *Psammechinus* and *Asterias*, with time after removal from the ovary, a constant rate being reached 2–3 hours after removal. Such a

declining respiration appears never to have been observed in Warburg experiments and was not found in the present case even when the first reading was made twenty minutes after removal of eggs from the ovary.



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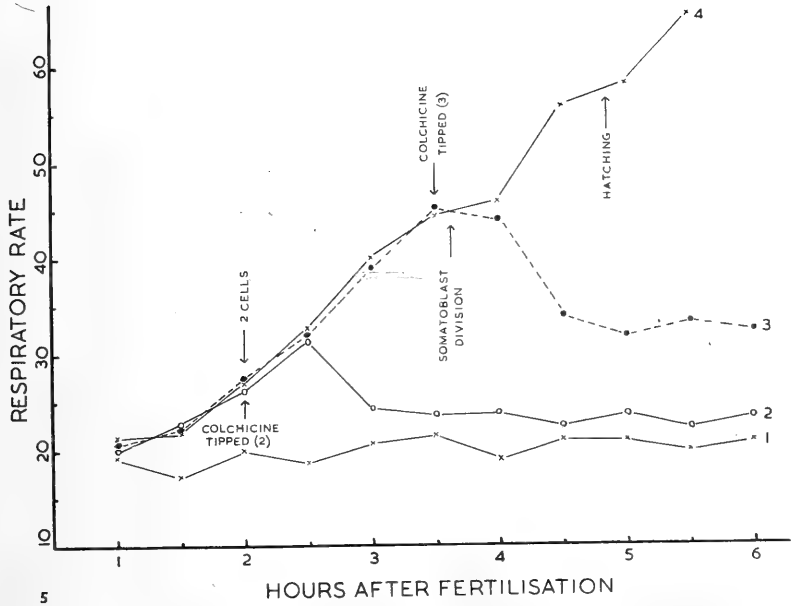

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Text-figures 4 and 5.

Fig. 4.—Respiratory rate (expressed as μl. $O_2/5 \times 10^6$ eggs/$\frac{1}{2}$ hour) and the recognizable morphological stages during development.

Fig. 5.—Effect of the addition of colchicine on the respiratory rate of developing eggs. Curve 1 shows the respiratory rate of colchicine-treated unfertilized eggs, and curve 4 is the control developing eggs. In curves 2 and 3 colchicine was tipped from the sidearm at the points indicated; the development ceased in these eggs at the following metaphase. Colchicine concentration 10^{-3}M; respiratory rate is expressed as μl. O_2/flask/$\frac{1}{2}$ hour.

The rather sudden marked increase in respiratory rate which is capable of occurring in unfertilized eggs (Text-figure 1) suggests that the respiratory mechanism is not normally functioning maximally but is presumably limited by such factors as rate of substrate mobilization (cf. Ballentine, 1940).

The effect of fertilization of an egg on its respiratory rate has been determined in a large number of animals. These observations have been collected in Table 5. An attempt was made to make this table comprehensive in respect of genera that have been studied, but not in respect of authors studying the same form. References not quoted in the present paper may be found in Ballentine (1940).

The only previous attempt to explain the difference in respiratory change at fertilization in different animal species appears to have been that of Whitaker (1933). He suggested that there was a normal range of respiratory rate which was necessary for development in any egg; unfertilized eggs with rates lower than this showed an

TABLE 5.

Changes in Respiratory Rate on Fertilization in Eggs of Different Animals.

Nuclear Type.	Genus.	Ratio F./UF.	Author.	Nuclear Type.	Genus.	Ratio F./UF.	Author.
1	*Nereis.*	1·4	Whitaker (1931).	3	*Rana.*	*ca.* 1	Brachet (1934*a*).
	,,	1·25	Barron (1932).		,,	*ca.* 1	Zeuthen (1944).
	Mactra.	1·8	Ballentine (1940).		*Bufo.*	1	Stefanelli (1938).
2	*Ciona.*	1·7	Tyler and Humason (1937).		*Fundulus.*	16·7	Boyd (1928).
					,,	1 (?)	Philips (1940).
	,,	*ca.* 1	Holter and Zeuthen (1944).	4	*Dendraster.*	2·6	Tyler and Humason (1937).
	Cumingia.	0·45	Whitaker (1931).		*Fucus.*	1·85	Whitaker (1931).
	Ostrea.	1·4	Ballentine (1940).		*Strongylocentrotus.*	3·7	Tyler and Humason (1937).
	,,	1·0	Cleland (1950).				
	Chaetopterus.	0·81	Ballentine (1940).		*Psammechinus.*	1·9	Laser and Rothschild (1939).
	,,	0·54	Whitaker (1931).				
	*Asterias.**	0·98	Tang (1931).		,,	6	Shearer (1922).
	,,	*ca.* 1	Borei (1948).		,,	3·6	Borei (1948).
	Sabellaria.	1·12	Faure Fremiet (1922).		*Paracentrotus.*	6	Warburg (1915).
	Urechis	1·15	Tyler and Humason (1937).		,,	4·7	Brock *et al.* (1938).
					Arbacia.	5	Tang (1931).
					,,	2·6	Shapiro (1936).
					,,	4·5	Ballentine (1940).

*Proceeds to complete maturation in the absence of fertilization.

References not quoted in this paper may be found in Ballentine (1940).

increase, while eggs with higher rates showed a decline on fertilization. He noted a number of exceptions to this rule, and the criticism of Gerard and Rubenstein (1934) have cast some doubt on the whole hypothesis. Indeed calculation of the respiratory rate per unit volume of eggs in the animals shown in Table 5 indicates that the fertilized egg values vary over a sevenfold range. Four types may be distinguished in the nuclear state of eggs on spawning (Just, 1938; Wilson, 1925): (*a*) eggs in which the germinal vesicle remains intact on spawning and breaks down only after fertilization; (*b*) eggs which proceed to the first maturation metaphase after spawning; (*c*) eggs which proceed to the second maturation metaphase after spawning; (*d*) eggs with haploid resting nucleus on spawning.

The data recorded in Table 5 have been arranged in these four groups. The assigning of animals to any group has been difficult, requiring a reference to the embryological literature, where available or by analogy with related forms.

The following points emerge from this comparison: (*a*) in the fourth group, comprising mostly the sea urchins, a rise in respiratory rate of at least twofold occurs on

fertilization; (*b*) the third and second groups are characterized by either no change or a depression; the first group shows a moderate or marked rise.

The apparent exceptions to this generalization are: (1) *Ciona*, where Tyler and Humason find a considerable increase on fertilization; this rise was not found in *Ciona* eggs by Holter and Zeuthen. (2) *Ostrea*, where Ballentine found a rise at some unspecified time after fertilization, while the present work has failed to find any rise associated with fertilization per se. (3) *Fundulus*, where Boyd found a marked increase which disappeared when the first cleavage division occurred. This phenomenon could not be confirmed by Philips (1940) and the rise on fertilization is therefore questionable.

It will be seen that these exceptions are all capable of other explanation and cannot be considered as critical evidence against the generalization. There is thus a fair correlation between no rise in respiratory rate on fertilization and a kinetic state of the nucleus and a moderate or marked rise with a non-kinetic nucleus. This suggests that respiration is increased in eggs either by previous entry into the meiotic divisions or by sperm penetration.

That this postulated rise in respiratory rate on entry into the meiotic divisions is not due solely to the increased respiratory rate found in cells during the division cycle is indicated by those cases in the second class where the rate shows a considerable decrease on fertilization. This suggests that the germinal vesicle breakdown itself is the main cause of the rise in the second class, the higher rate being subject to further modification by fertilization in forms such as *Chaetopterus* and *Cumingia*.

The rise in respiratory rate on germinal vesicle breakdown may well be due primarily to physical changes in the cytoplasm and is perhaps analogous to the rise found in some of the batches of unfertilized eggs shown in Text-figure 1. It is not difficult to imagine how liberation of the very aqueous germinal vesicle contents (Cleland, 1947; Harris, 1939) could cause such physical change and, indeed, a considerable difference in cytoplasmic viscosity has been found (centrifuge method) between eggs with intact and broken germinal vesicles in the present material.

Generalizations such as this, derived from a comparative study of respiratory change on fertilization, cannot be regarded as any more than suggestive. There are, however, two more direct lines of evidence suggesting its validity. In the present material there is a difference in respiratory rate between unfertilized eggs with and without germinal vesicle breakdown (Text-figure 2). At first sight these two groups seem to overlap somewhat in respiratory rate. However, when the percentage of germinal vesicle breakdown is considered it will be seen that the overlapping could be quite satisfactorily explained on that basis, the probable mean respiratory rate for eggs showing more than 90% germinal vesicle breakdown, being about 425 μl./ml./hr. and that of eggs showing less than 10% breakdown being about 200 μl./ml./hr. A considerable number of attempts were made to follow the respiratory rate of eggs during the germinal vesicle breakdown. No increases could be demonstrated, but by the time readings could be begun breakdown was already well advanced and the experiments are thus not conclusive. In a previous experiment (Cleland, 1947) the respiratory rate of unfertilized eggs was found to be considerably lower than that found in the present series. This difference may be due to the fact that the germinal vesicles did not break down until after fertilization in the 1947 experiment.

Borei (1948) has been able to follow the respiration of Asterias eggs during germinal vesicle breakdown and the meiotic divisions. He was able to show that there was a marked increase in respiratory rate when these processes occurred and that the activation persisted after the meiotic divisions were completed.

Studies on the respiratory rate during mitosis are of considerable importance in assessing the metabolic cost of the various stages. The available evidence has been considered by Zeuthen (1944, 1947). The present work confirms that of Zeuthen, but owing to the incomplete synchronization, accurate quantitative estimates of the respiratory rate at various stages were not possible. It would appear, however, that the main energy requirement occurs during prophase when chromosome synthesis is

occurring.* The absence of any demonstrable change in the maturation divisions where no chromosome synthesis occurs is in agreement with this.

Changes in respiratory rate and development with pH have been studied by a number of authors. In general there is a depression of respiration with increasing hydrogen ion concentration (e.g., Warburg, 1910; Root, 1930) and the present material is no exception. The effect of pH on development is different in different forms, even among genera of sea urchins. For example, Warburg (1910) found that *Strongylocentrotus* eggs were unable to develop at pH 6, while *Arbacia* eggs can develop over a wide pH range (Smith and Clowes, 1924). In the present form a slight delay in development of ten minutes in four and one-half hours is found at pH 5·8.

In a series of papers (1933–1938, reviewed by Tyler, 1942) Tyler has presented strong evidence that the amount of energy (as measured by respiration) required to perform a given amount of morphogenetic work is remarkably constant under a variety of experimental conditions. He was unable satisfactorily to "dissociate" (Needham, 1936, 1942) the total respiration from the final morphogenetic result. It would appear that a partial dissociation has been accomplished in the present material by low pH; up to the hatching stage the same amount of morphogenetic work is accomplished with the consumption of significantly less oxygen.

A number of authors have attempted to study the causes and significance of the rise in respiration during development by experimental interference with normal development. Lindahl (1936), Lindahl and Ohman (1938) found that when normal development of sea urchin eggs was modified by lithium treatment, which itself had no effect on initial respiratory rate, the normal rise in respiration was inhibited. Brachet (1938) found that *Chaetopterus* eggs, when activated by KCl to a differentiation without cleavage in which the nucleus undergoes anomalous division cycles and the cytoplasm differentiates to give a unicellular trochophore-like body, show a slower rise in respiration than in normally developing eggs. Tyler and Horowitz (1938) have reported a similar situation in *Urechis* eggs, and Andresen *et al.* (1944) have demonstrated the very anomalous "development" of *Ciona syncytia* is associated with considerable rise in respiratory rate. Horowitz (1940) demonstrated that when gastrulation of sea urchin eggs is prevented by thiocyanate, the respiration of the "dauerblastulae" does not rise above that typical of the normal blastula. Andresen *et al.* (1944) have expressed the opinion that the reason for the rise of respiratory rate during development lies in the chemical potentialities of the unfertilized egg and that once the egg is activated there follows a more or less predetermined series of chemical events which are largely independent of associated morphological changes.

The experiments reported in this paper do not support this opinion; when cleavage is prevented by colchicine there is no increase in respiratory rate and the normal rise in respiration is evidently a function of both the increase in cell number and the continued sequence of cell divisions, during which the respiration is raised (cf. Text-fig. 3).

The facts emerging from the colchicine experiment are as follows: (i) Unfertilized eggs show a slight decrease (10%) in respiratory rate after treatment with colchicine. This decrease is greater than that found in unfertilizable eggs (other experiments), suggesting that at least part of the depression is due to interference with the meiotic spindle. (ii) The application of colchicine at the two-cell stage causes a greater depression of respiratory rate than that found in unfertilized eggs. (iii) The application at later stages leads to an even greater inhibition, but the constant level is higher than that found in the earlier stages of development. (iv) In all cases except unfertilized eggs there is a time lag between application of colchicine and full respiratory depression. The absence of a concentration effect implies that this is not due to delay in penetration of the eggs by the alkaloid.

* The recent experiments of Bullough (*Nature*, 165 : 493, 1950) are in agreement with this. Also *Exp. Cell. Res.*, 1 : 410.

The interpretation placed on these observations is as follows. Colchicine, by interference with the spindle, abolishes part of the activity of the cell and, this activity being abolished, that fraction of the total respiration concerned in producing the energy required for it is also abolished. The lag in effect is due to the phase specificity of the colchicine and to the absence of complete synchronization of the divisions both in single eggs and in different eggs of the batch. The colchicine stable residual respiration, which increases with development, is regarded as a maintenance respiration, i.e. the respiration concerned in the production of the energy normally used in the maintenance of the cells as living systems. The observations indicate that this fraction of the total respiration increases with increase in cell number (cf. Robbie, 1948), although not proportionately to the increase in the activity metabolism associated with the increase in the number of dividing cells.

This interpretation is supported by the study of the respiratory rate during development. When the mitotic rate is physiologically depressed, as it is at the somatoblast division and hatching stages, the respiratory rate either remains constant or shows a decrease, the former presumably when synchronization of development and the choice of observation times is not good, the latter when it is more perfect.

The above concept of maintenance metabolism has no relation to that proposed by Fisher and Stern (1942), Fisher and Henry (1944), Ormsbee and Fisher (1944), Moog (1944). In the latter concept the maintenance metabolism is regarded as qualitatively different from the activity metabolism, while in the former the distinction is purely quantitative.

The observation that CO_2 causes an inhibition of respiration in oyster eggs is in qualitative agreement with the work of Root (1930) on *Arbacia* eggs. Invertebrate eggs evidently differ in this respect from mammalian tissues, which are often stimulated or stabilized by the presence of CO_2 or bicarbonate (Laser, 1935, 1942).

Needham (1930) has adduced considerable evidence, mostly from respiratory quotient studies, to support a hypothesis of a succession of energy sources during development (carbohydrate → protein → fat). Later (Needham, 1942) this was modified to exclude the cleavage stages and the succession considered to begin at gastrulation. During the cleavage stages the R.Q. differs widely in different animals: 1·0–0·8 for *Urechis* (Horowitz, 1940); 0·6–0·7 for the frog (Brachet, 1934); 0·75–0·85 for *Paracentrotus* (Ohman, 1940). Assuming that these determinations are valid, there thus appears to be no substrate preferentially oxidized during the cleavage stages.

In the developing oyster egg the R.Q. suggests combustion of either protein or a mixture of carbohydrate and fat. Analyses of ammonia production of eggs indicated that the combustion of protein was insufficient to account for more than a part of the normal respiration; thus the second alternative seems the more likely.

Summary.

1. Methods of handling the eggs of the oyster are described.

2. Some batches (about 20%) of unfertilized eggs may show sudden marked increases in respiratory rate about four hours after removal from the ovary; this is usually correlated with morphological signs of cytolysis.

3. A considerable difference in respiratory rate was found between unfertilized eggs in which the germinal vesicle remains intact and those in which it breaks down. It is suggested that a causal relation exists between these two factors.

4. No increase in respiratory rate occurs on fertilization until the onset of the first cleavage division.

5. Slight periodicity in the respiratory rate during the early cleavages suggests that the respiratory rate is higher in the pre-anaphase stages of division.

6. The respiratory rate rises considerably during development and shows three plateaus: at somatoblast division, at hatching and at attainment of maximum swimming intensity. The first two plateaus are associated with depression of the mitotic rate.

7. Eggs are capable of unretarded normal cleavage at pH 5·9. At this pH significantly less oxygen is consumed in reaching the hatching stage than in sea water at a pH of 7·5.

8. Colchicine abolishes the rise in respiratory rate during development. A respiratory depression is found after colchicine treatment, and it appears after a lag period. The depression is slight in unfertilized eggs but becomes greater with increasing development of the eggs. A lower constant level is reached which is higher with increasing development of the egg. These facts are interpreted in terms of activity and maintenance fractions of the total respiratory metabolism.

9. The respiratory quotient has a value of about 0·80. This is apparently due to combustion of a mixture of carbohydrate and lipide. No change in R.Q. takes place during the first six hours of development.

10. The reported respiratory change on fertilization in a range of animals is examined and a hypothesis suggested which accounts for most of the facts.

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THE INTERMEDIARY METABOLISM OF UNFERTILIZED OYSTER EGGS.

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(Three Text-figures.)

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Introduction.

In a previous paper (Cleland, 1950*a*) a study was made of the respiratory rate and respiratory quotient of oyster eggs during early development.

If work such as this is to be elaborated and related to a lower biochemical level, some knowledge of the enzyme constitution of the egg, the pathways of its metabolism and the method of biological energy production is essential.

Since the final aim of the present work is to study the mitotic process, the above knowledge is required before any results obtained with the present material can be related to other biological material of which metabolic properties are known. This knowledge is also required as a background for the study of the effect of inhibitors on the mitotic process.

The aim of the present paper is, then, to outline the intermediary metabolism of the oyster egg. Since only an outline is necessary at this stage, no detailed study of the properties of the enzymes involved has been attempted. Wherever possible whole eggs have been studied or, when these were inapplicable, unfractionated homogenates.

The work was performed in the 1948–50 seasons.

Materials and Methods.

The preparation of egg suspensions has already been described (Cleland, 1950*a*). In the present case no attention was paid to the fertilizability of the eggs, but most reactions have been studied on both fertilizable and unfertilizable batches.

Standard manometric methods (Umbreit *et al.*, 1945) were employed, the flasks having 15–20 ml. capacity. Some experiments were performed with flasks of 5–6 ml. capacity. Differences of 5% in respiratory rate are considered significant. The temperature of incubation has been 26°C. throughout.

Cozymase was prepared by the method of Williamson and Green (1939); ATP by the method of Le Page (1945); adenylic acid by the alkaline hydrolysis of ATP (Lohman, 1932) with subsequent separation in the Ba soluble alcohol precipitable fraction of the hydrolysate; cytochrome C by the method of Kielin and Hartree (1937); acetoacetic acid by the method of Schaffer (1921); crystalline sodium pyruvate from freshly vacuum distilled pyruvic acid by the method of Robertson (1942). All other chemicals were from commercial sources (usually B.D.H.).

All substrates and inhibitors were used as neutral solutions (glass electrode) of the sodium salt.

Unless otherwise stated, preparations for analysis were deproteinized with trichloracetic acid in a final concentration of 10%.

Lactic acid was determined by the method of Barker and Summerson (1941); pyruvic acid by the method of Friedman and Haugen (1941); phosphorus by the method of Fiske and Subbarow (1925); amino acids in tungstic acid filtrates by the method of Folin (1922) with glycine as standard; tyrosine by the method of Folin and Ciocalteu (1929).

Glycogen was separated by the method of Lindberg (1945), hydrolysed for 30 minutes with 5N H_2SO_4, and the reducing sugar determined by the method of Folin and Malmros (1929). The latter method was also used for the determination of the reducing substance in tungstic acid filtrates.

The barium salt fractionation and analysis was performed according to Le Page and Umbreit (1945).

Nitrogen was determined by direct Nesslerization of H_2SO_4–$HClO_4$ digests. Protein N was taken as that precipitable by 10% trichloracetic acid, polypeptide N as the N precipitable by tungstic acid treatment of trichloracetic filtrates, and non-protein N that remaining in the filtrate after tungstic acid precipitation.

All colour determinations were made with a Spekker photoelectric absorptiometer.

The cyanide studies were made according to the instructions of Robbie (1946) for balanced KOH/KCN centre well mixtures.

The nitrogen and nitrogen CO_2 mixtures were from commercial cylinders used without further purification. The oxygen uptakes were usually nil and always less than 2% of the control in air.

The preparation of homogenates is described later.

Analyses of Eggs.

Glycogen, monosaccharide, lactic acid and amino acids are present, as shown in Table 1, where the distribution of nitrogen is also shown. Rough analytical values for fat and phospholipide have already been given (Cleland, 1947). The fairly high amino acid content (4% of dry weight) is still considerably less than the amounts found in Urechis eggs (Horowitz, 1939) or Strongylocentrotus eggs (Orstrom, 1942). Fairly high amino acid contents are found in rapidly growing normal mammalian material (Christensen and Streicher, 1948; Christensen *et al.*, 1948). Although elevated in hyperplastic epidermis, the amount is much reduced when neoplastic change supervenes (Roberts and Tishoff, 1949). The large proportion of the total nitrogen in granules is expected from the high P granule content of the eggs (Cleland, 1947).

The glycogen and total carbohydrate content of the eggs is considerably less than that found in sea urchins (Hutchens *et al.*, 1942; Orstrom and Lindberg, 1940).

A number of the phosphorylated intermediates found in mammalian material are also present in oyster eggs, as shown in Table 2.

The figures for inorganic phosphate are very much higher than those quoted by Chambers and White (1949) for Arbacia eggs, but are of the same order as the figures

of Crane (1947) for Arbacia. The seven-minute hydrolysable P values are somewhat lower than those found in Arbacia by the above authors.

Of the total seven-minute hydrolysable P approximately 65% was present in the Ba insoluble fraction and is thus considered to be from ATP and ADP (hexose diphosphate

TABLE 1.
Presence of Substrates and Distribution of Nitrogen.

Substance.	Number of Analyses.	Content, Mg./ml. Eggs.	
Glycogen	9	3·1 –6·2	
Reducing substance	5	1·0 –2·3	
Lactic acid	18	0·02–0·05	
Pyruvic acid	15	0 –0·01	
Free amino acids	6	7–11	
„ tyrosine	9	1– 1·5	
Granular* N	4	40–55	% Total N
Protein N	3	74–80	% Total N
Polypeptide N	3	6–15	% Total N
Non-protein N	3	6–10	% Total N

* Precipitable by a force of 20,000 xg. acting for 30 minutes.

contributes less than 5% of the total). A further 15% can be accounted for as glucose-1-phosphate in the Ba soluble fraction. The remainder was not accounted for.

EXPERIMENTS ON WHOLE EGGS.

(*a*) *Effect of Inhibitors.*

The effect of a number of inhibitors on the respiration of whole eggs is shown in Table 3.

TABLE 2.
Presence of Phosphorylated Compounds.

Fraction.	Material.	Number of Analyses.	Content or Method of Identification.
Inorganic P	Whole eggs.	7	250–530 μg./ml. eggs.
7 min. hydrolysable P	„	5	100–320 μg./ml. eggs.
ATP and ADP	Ba-insoluble fraction.	5	P_7, pentose, N, 2650 Å absorption.
Hexose diphosphate		5	Fructose.
Phosphoglyceric acid		2	Resistant P.
Fructose 6 phosphate	Ba-soluble, alcohol precipitable fraction.	5	Fructose.
Cozymase		4	Absorption at 3400 Å.
Adenylic acid		4	Ratio absorption at 2650 and 3400 Å.

Spectrophotometric studies were made with a Beckman spectrophotometer.

These inhibitor studies suggest: (*a*) that most of the oxygen consumption is mediated by a cytochrome oxidase system (cyanide and azide and possibly fluoride inhibition); (*b*) that respiration is accompanied by phosphorylation and that the phosphorylation partly controls the respiratory rate (dinitrophenol effect cf. Loomis and Lipmann, 1946); (*c*) that enzymes whose activity depends on –SH groups, either oxidative or glycolytic or both, are concerned in the respiration (iodoacetic acid and phenylmercuric nitrate effects).

(*b*) *Effect of Methylene Blue and Possible Substrates.*

Methylene blue at concentration M/300 was found to stimulate the respiration some 200%–400%, a fact usually interpreted to mean that the cytochrome-cytochrome oxidase step is limiting. Hydroquinone at a concentration of M/10 gave, after correction for auto-oxidation by the method of Schneider and Potter (1943) stimulations varying from 0–30% (pH 7·0), while the addition of succinate, malate, malonate, glucose or pyruvate (M/20) had no demonstrable effect on respiration.

TABLE 3.
Effect of Inhibitors on Respiration.

Inhibitor.	Concentration (M.).						pH.
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	
Cyanide	—	—	7	15	65	95	7·5
Azide	50	64	85	100	—	—	6·5
Fluoride	21	94	100	—	—	—	,,
Iodoacetate	—	33	64	92	100	—	,,
Phenylmercuric nitrate ..	—	—	20*	90	100	—	7·5
Dinitrophenol†	—	—	120	140	175	100	,,

The figures quoted are percentages of the control respiration, the absolute value of which varied from 100–300 µl. in the experimental period of 60–120 mins.

* Variations of 10–25% were encountered.

† Both the position and height of the maximum stimulation have varied considerably in different egg batches (cf. Table 7).

In order to determine whether the absence of stimulation by the latter substances might be due to their non-penetration, experiments of the following type were performed.

To an approximately 50% suspension of eggs in sea-water, pyruvate was added and the suspension shaken for 45 minutes. Samples of the whole suspension and the cell-free supernatant were treated with trichloracetic acid and analysed for pyruvate. The non-solvent volume was determined by the osmotic method (cf. Brooks and Brooks, 1940).

The results of a typical experiment are shown in Table 4. No pyruvate was used by the eggs during the period of the experiment.

TABLE 4
Penetration of Pyruvate into Eggs.

Volume of suspension (ml.) ..	3·4	Calculated pyruvate in supernatant	
,, ,, eggs ,, ..	1·35	on an hypothesis of:	
Pyruvate in suspension (µg./ml.)	240	non-penetration	397 µg./ml.
,, ,, supernatant ,,	388	complete ,,	300 ,,
Non-solvent volume per cent. ..	42		

The data in Table 4 indicate that, within the limits of experimental error, no penetration of pyruvate occurs. Since it appeared likely that the eggs would be impermeable to most substrates, all studies of intermediary metabolism were conducted on homogenates.

(*c*) *Glycolysis.*

The experiments shown in Text-fig. 1 and Table 5 indicate (*a*) that considerable quantities of CO_2 are evolved by eggs suspended in bicarbonate–CO_2 buffers, and that the CO_2 arises from bicarbonate, (*b*) that the amounts of lactic and pyruvic acids accumulating are far less than expected from the manometric acid production, (*c*) that although similar quantities of lactic and pyruvic acid accumulate under aerobic conditions no manometric acid production is demonstrable.

TABLE 5.
Acid Production under Aerobic and Anaerobic Conditions.

Experiment Number.	Gas Phase.	Initial Bicarb. (μl.)	CO_2 Evolved. (μl.)	Bicarb. Recovered. (μl.)	Total. (μl.)	Change in Lactic. (μg.)	Change in Pyruvic. (μg.)
1	N_2/CO_2	310	48	265	313	+15	+10
	O_2/CO_2	315	—	307	—	+12	+2
2	N_2/CO_2	316	59	250	309	+10	+12
	O_2/CO_2	320	—	323	—	+12	−2
3	N_2/CO_2	360	35·5	315	350·5	+7	+8
	O_2/CO_2	340	—	334	—	+7	+3

All figures are expressed as μl. CO_2. Approx. 0·3 ml. egg/flask. Experiment duration 60 mins. Gas phase 5% CO_2 in N_2 or O_2.
Egg breakdown was slight and identical in amount under aerobic and anaerobic conditions.

Glycolytic inhibitors cause a depression of acid production, as shown in Table 6. It will be seen that the inhibition by iodoacetate and phenylmercuric nitrate increases with time, a phenomenon probably due to the relative impermeability of the eggs to these substances, while the fluoride inhibition is constant. Since fluoride seems less likely to affect other acid-producing mechanisms (e.g., ATP splitting) than the other two inhibitors, and is later shown to inhibit glycolysis in extracts, a conclusion that at most only 25% of the acid production is due to glycolysis seems warranted.

TABLE 6.
Effect of Glycolytic Inhibitors on Acid Production.

Time Period.	NaF. (10^{-2} M.)	Iodoacetate. (10^{-2} M.)	Phenyl Mercuric Nitrate. (10^{-3} M.)
0–45′	75	93	83
45–90′	77	72	52

The figures quoted are percentages of the control which evolved 33 μl. CO_2 in the 90 minutes. The flasks were of 5 ml. capacity and contained 0·15 ml. eggs, 0·02 M. bicarbonate (final conc.) and 5% CO_2 in N_2 in the gas phase.

Large amounts (about 800 μl. CO_2/ml. eggs) of acid are released on cytolysis of oyster eggs by saponin or taurocholate under both aerobic and anaerobic conditions. This acid production is proportional to the amount of eggs taken after retention corrections have been applied; this is difficult to reconcile with even a one-step enzyme system let alone a complex system like the glycolytic system. The cytolytic acid production is very insensitive to inhibitors (iodoacetate, phenylmercuric nitrate, fluoride, phloridzin) and to the addition of glycogen, and it was therefore concluded that it was non-metabolic. The most likely explanation is that the egg's interior is normally maintained at a more acid pH than the surrounding environment.

Injury of eggs under anaerobic conditions, with subsequent increased permeability and a slow neutralization of intracellular acid groups, is probably responsible for a portion of the acid production of whole eggs; eggs recovered from these anaerobic experiments frequently fail to fertilize, while those from the aerobic experiments are still capable of fertilization.

A further fraction of the acid production can be ascribed to splitting of ATP (cf. Table 7) and phosphomonoesterase and lipase activity (cf. Table 16).

No acid production could be demonstrated on fertilization of oyster eggs.

If the conclusions derived from the inhibitor experiments are correct, the glycolytic acid production is equivalent to approximately 15 μl. of CO_2/hr./0·3 ml. eggs, or to 60 μg. of lactic acid and/or pyruvic acid. This latter value is considerably less than that found in actual analyses (Table 5). This implies either that the conclusion is invalid or that some mechanism of anaerobic removal of pyruvate or lactate exists. That the latter alternative is correct is indicated by the homogenate experiments on pyruvate utilization (Table 19), where it is shown that the rate of anaerobic removal is sufficient to account for the non-accumulation of these substances.

In a previous study (Cleland, 1950*a*) it was inferred from the respiratory quotient values that carbohydrate and lipide were being oxidized. If aerobic and anaerobic glycolytic rates are equal, as appears likely from the analyses, the inferred glycolytic rates are sufficient to account for the carbohydrate moiety of the respiration of these eggs (intact germinal vesicles), provided the pyruvate is completely oxidized. That the latter occurs is indicated by the homogenate experiments (Table 21).

(*d*) *High Energy Phosphate Formation.*

High energy phosphate bonds, which are regarded as the ultimate source of usable biological energy, are formed in mammalian tissues both in breakdown of carbohydrate to lactic acid and in the oxidation of pyruvate through the tricarboxylic acid cycle (Lipmann, 1941; Potter, 1944; Cross *et al.*, 1948).

The production of high energy phosphate bonds is strongly inhibited by dinitrophenol (Loomis and Lipmann, 1946; Cross *et al.*, 1948), the stimulating effect of this compound on respiration being ascribed to its obviating the need for inorganic phosphate in the aerobic process (Loomis and Lipmann, 1946), or to its ability to cause or promote breakdown of some labile phosphate ester and thus to make available more inorganic phosphate (Tepley, 1949).

In the present material the presence of ATP and/or ADP and adenylic acid has been shown (Table 2). The latter compound causes a stimulation of glycolysis (Table 15), which implies that high energy phosphate bonds arise in this reaction. The stimulating effect of dinitrophenol on respiration has been shown in Table 3.

The effect of varying experimental conditions on the high energy phosphate content of whole eggs is shown in Table 7, where one of the five experiments performed is described. It will be seen that a graded decrease in the content occurs when the

TABLE 7.
High Energy Phosphate Changes under Various Conditions.

Inhibitor.	Conc.	Gas Phase.	μl. O_2.	P_0. (μg.)	P_7. (μg.)	P_{0+7}. (μg.)
—	—	Air.	128	87	99	186
—	—	Reduced O_2.	69	108	84	192
—	—	N_2.	3	113	75	188
Cyanide	10^{-3} M.	Air.	11	115	73	188
Dinitrophenol	10^{-3} M.	,,	346	123	69	191
,, ...	10^{-4} M.	,,	485	120	69	189
,, ...	10^{-5} M.	,,	194	96	90	186

Each flask contained approximately 0·3 ml. of eggs with broken germinal vesicles. Experiment duration 90 mins.

P_0 refers to inorganic phosphate and P_7 to 7 min. hydrolysable P.

respiration is decreased either by reduction of oxygen tension or by the action of cyanide. This indicates that the bonds are formed during oxidative metabolism, the decrease presumably being due to utilization and/or autolysis by apyrase action. It will furthermore be seen that dinitrophenol in appropriate concentration causes a greater decrease than anaerobic conditions, although causing a marked increase in

oxygen consumption; this is possibly due to interference with high energy phosphate formation not only in respiration but also in glycolysis.

By analysis of barium salt fractions it was shown that most of the decrease occurred in the Ba precipitable fraction. This implies that the greatest loss occurs in the ATP and ADP fractions.

Barth and Jaeger (1947) have studied the effect of anaerobic conditions on the high energy phosphate content of developing frogs' eggs. A much slower decrease was found than in the present material. This is presumably due to the fact that the glycolytic system is quite active in the frog's egg and is capable of providing sufficient energy for the early cleavages to occur. In the oyster egg anaerobic conditions cause arrest of development.

Robbie (1947) has described in developing eggs three types of response to anaerobic conditions: (*a*) those which are capable of maintenance and development, e.g. Fundulus; (*b*) those capable of maintenance only, e.g. Arbacia; (*c*) those incapable of either maintenance or development under anaerobic conditions, e.g. Echinarachnius (Robbie, 1948). These differences are possibly due to variations in the energy supply available from the glycolytic system.

Experiments on Homogenates.

(*a*) *General.*

Homogenates were prepared in an ice-jacketed ground-glass apparatus of the Potter type. Depending on the type of reaction under study either distilled water or a phosphate saline mixture (0·2M KCl, 0·1M $MgCl_2$, 0·01M–phosphate buffer pH 7·6) was used as an homogenizing medium.

Table 8.
Effect of Additions on Homogenate Respiration.

Addition.	Concentration. (M.)	Respiration. Percentage Control.
Cytochrome C	2×10^{-5}	195
Methylene blue	$3{\cdot}3 \times 10^{-3}$	145
Succinate	10^{-2}	185
Malonate	10^{-1}	65
Cyanide	10^{-3}	8·5
Iodoacetate	10^{-2}	57
Arsenite	10^{-2}	82
Phenyl mercuric nitrate	10^{-3}	50

Each flask contains 1 ml. 50% homogenate (phosphate saline) in a final volume of 2 ml. Experiment duration, 90 mins. Control respiration, 114 µl.

The respiration of homogenates was relatively independent of time of homogenation. In all cases the time selected was that required to cause complete cytolysis of 95% of the eggs (with the present apparatus about two minutes). Packed eggs were usually homogenized in an equal volume of homogenizing fluid.

While the pH optimum of homogenate respiration is approximately 8·0 it is difficult to buffer at this pH. For this reason phosphate buffers (final concentration M/25) of pH 7·5 were used in the homogenate experiments. Cytochrome, when added, was used at a final concentration of 2×10^{-5}M.

Typical homogenate respiration curves are shown in Text-figures 2 and 3. The decline in the respiratory rate of uncomplemented homogenates is evidently due to at least two factors: depletion of substrate in those cases where linear respiration occurs in the presence of added substrate, and changes in the enzyme system itself where this linearity is not found.

The metabolic properties of whole eggs and homogenates are compared in Table 23.

The effects of a number of additions on homogenate respiration are shown in Table 8.

The stimulation found with cytochrome C is expected from previous deductions on whole eggs, the stimulation found with methylene blue is less than that found with whole eggs, and, in contrast to its lack of effect on whole eggs, which it evidently did not penetrate, succinate gives a marked stimulation of homogenate respiration.

In contrast to its marked effect on the respiration of whole eggs, dinitrophenol has only a small effect on homogenate respiration (cf. Table 22). This effect is reduced still further when the phosphate concentration is increased.

Malonate and arsenite powerfully inhibit certain specific enzymes of the tricarboxylic acid cycle in vertebrate tissues (Krebs, 1943). The results quoted above imply either that none or only part of the homogenate respiration proceeds through the cycle or that the enzymes of the oyster cycle have different properties from the classical material.

The addition of phosphate is without effect on the respiration of homogenates. This is possibly due to the fact that sufficient is already present; Cross *et al.* (1949) have found that the vastly more active cyclophorase preparation from kidney requires only 1 μM/ml. for maximal activity.

(*b*) *Effect of Carbohydrates.*

The effect of some carbohydrates on homogenate respiration is shown in Table 9. It will be seen that significant stimulation of respiration is found with glycogen and fructose. When ATP is also added, glucose as well as fructose gives increases in the respiratory rate (cf. Text-figure 2). ATP alone has little or no effect.

TABLE 9.
Effect of Carbohydrates on Homogenate Respiration.

Substance.	Concentration.	Number of Experiments.	Percentage Control.
Glycogen	0·6%	4	115–130
Glucose	$\frac{M}{100}$	4	92–103
„ +ATP	„	1	130
Fructose	„	4	107–120
„ +ATP	„	1	150
Arabinose	„	4	100–105
Galactose	„	4	102–106
Xylose	„	4	98–104

Control respiration, 90–220 μl. Duration, 1–2 hrs. Cytochrome added in two experiments. ATP, when added, 1 mg./flask.

(*c*) *Effect of Lipides.*

The effect of some lipides and lipide derivatives on homogenate respiration is shown in Table 10. It will be seen that some of the lower fatty acids give significant increases in some preparations and that lecithin gives a considerably greater and more consistent stimulation. A considerable portion of the lipide store of the eggs is phospholipide (Cleland, 1947). Hydrolytic products of fats and phospholipides, glycerol and glycerophosphate, stimulate respiration, but higher fatty acids are consistently inhibitory. This inhibition has been described in rat liver preparations, but in these the addition of ATP enables utilization of the fatty acid to occur (Lehninger, 1945). This is not the case in egg homogenates. The non-oxidation of higher fatty acids was not affected by the addition of tricarboxylic acid cycle intermediates (cf. Lehninger, 1945; Graffin and Green, 1948).

(*d*) *Effect of Amino Acids.*

The following amino acids (M/100 final concentration) failed to cause a significant increase or decrease in homogenate respiration (3 expts.): glycine, dl-alanine l-leucine,

l-tyrosine. Dl-aspartate gave stimulations of 5–20% (5 expts.) and l-glutamate caused stimulations of 50–100% (12 expts.). The stimulation by glutamate was increased about 20% by addition of cozymase and the oxidation of glutamate was accompanied by ammonia production.

TABLE 10.

Effect of Lipides and Lipide Derivatives on Homogenate Respiration.

Substance.	Concentration. (M.)	Number of Experiments.	Percentage Control.
Acetate	10^{-2}	6	90–98
Propionate	,,	4	96–103
Butyrate	,,	4	105–144
Valerate	,,	4	95–130
Caproate	,,	4	103–133
Caprylate	,,	4	67–78
,,	10^{-3}	2	92–97
,, +ATP	,,	2	90–100
Palmitate	,,	2	91–95
,, +ATP	,,	2	92–97
Lecithin	0·5%	4	115–160
Glycerol	10^{-2}	3	120–128
Glycerophosphate	,,	2	120–130
Acetoacetate	,,	4	92–103

The control respiration varied from 90–170 µl. in different experiments of duration 1–2 hrs. Cytochrome added in some experiments; ATP when added 2 mg./flask.

(*e*) *Effect of Tricarboxylic Acid Intermediates.*

The effect of a number of tricarboxylic cycle intermediates on the respiration of homogenates is shown in Table 11. It will be seen that all caused a marked stimulation of homogenate respiration whether the homogenate was made in distilled water or phosphate saline and in the presence or absence of added carrier (methylene blue or cytochrome). Typical curves are shown in Text-figure 2.

TABLE 11.

Effect of Tricarboxylic Acid Cycle Intermediates on Homogenate Respiration.

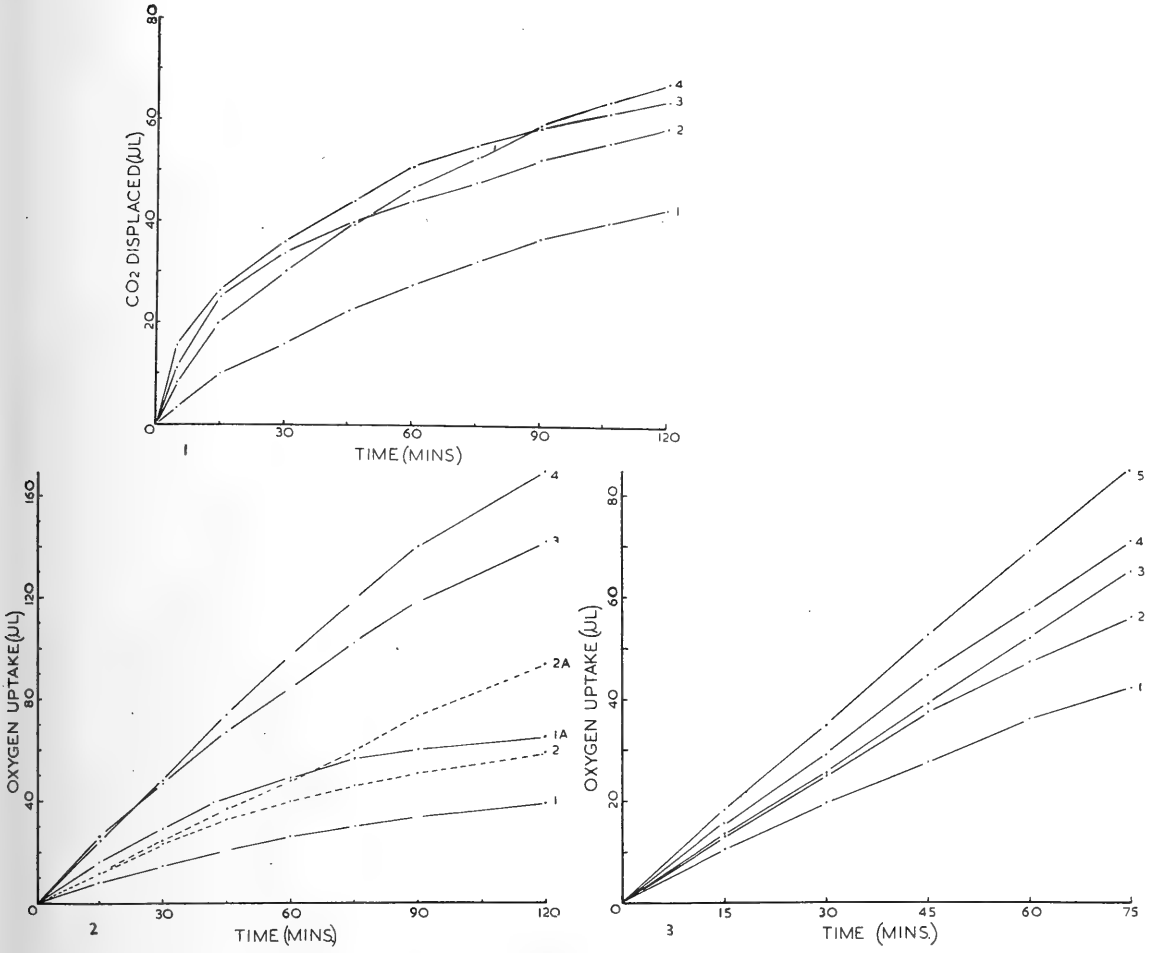
Substrate $(\frac{M}{100})$	Number of Experiments.	Percentage Control.
Citrate	8	130–160
α keto glutarate	5	135–175
Succinate	40	150–350
Fumarate	7	140–190
Malate	20	150–200
Pyruvate	12	130–185
Lactate	6	122–140

Some experiments were performed without added oxygen carrier, some with methylene blue and some with cytochrome. The final homogenate concentration has varied between 12% and 25%.

SPECIFIC ENZYMES IN HOMOGENATES.

(*a*) *Cytochrome Oxidase.*

The presence of cytochrome oxidase in the egg homogenate is indicated by the typical experiment shown in Table 12.



Text-figures 1-3.

Fig. 1. Typical anaerobic acid production curves for whole eggs. The eggs were suspended in sea-water to which was added 0·1 volume of M/4 bicarbonate. The flasks were gassed with 5% CO_2 in N_2 for 5-10 minutes, and equilibrated for 10 minutes in the bath before the first reading was taken. The volume of packed eggs per flask was as follows: 0·15 ml. in (1), 0·38 ml. in (2), 0·29 ml. in (3); 0·32 ml. in (4).

Fig. 2. Typical respiration curves for homogenates, and the effect of substrate additions.

Curve 1: Flask of 5 ml. capacity containing 0·5 ml. of 25% homogenate, 0·1 ml. cytochrome and 0·2 ml. phosphate buffer in a final volume of 1 ml. Curve 1A: Same with M/100 pyruvate added. Curve 2: Different homogenate but flask contents same as in (1). Curve 2A: Same with fructose (M/100) and ATP (1 mg.) added. Curve 3: Flask of 15 ml. capacity containing 1 ml. of 50% homogenate and 0·4 ml. of phosphate buffer in a final volume of 2 ml. Curve 4: Different homogenate but flask contents similar to (3) except for the addition of 0·2 ml. of cytochrome. Phosphate buffer: M/5, pH 7·5. Cytochrome: 2×10^{-4}M. It will be seen that addition of substrate may prevent the decline in respiratory rate of uncomplemented homogenate (2 and 2A) or have little effect on the decline (1 and 1A). This suggests that two factors are concerned in the decline: exhaustion of substrate and injury to the enzyme system. The endogenous respiration of the homogenates in curves (3) and (4) is more nearly linear owing to the smaller dilution of the homogenate. The addition of cytochrome did not consistently affect the linearity.

[Legend continued on page 306.]

The enzyme is saturated with a cytochrome concentration of approximately 4×10^{-5}M. Bands indistinguishable from those of cytochrome C may be seen in egg suspensions with a comparison microspectroscope.

TABLE 12.
Cytochrome Oxidase in Egg Homogenates.

	System.	µl. O_2.
1	Homogenate	14
2	(1) +hydroquinone	88
3	(1)+cytochrome	22
4	(2)+cytochrome	217
5	(4)+cyanide	60
6	(4)−homogenate	46

Each flask contained 1 ml. of 10% distilled water homogenate, 0·5 ml. $\frac{M}{5}$ phosphate buffer (pH 7·5) in a final volume of 3 ml. Hydroquinone final concentration $2\cdot5\times10^{-2}$ M, cytochrome 4×10^{-5} M, cyanide 10^{-3} M. Experiment duration, 60 minutes.

(*b*) *Succinoxidase.*

In Table 13 the results of a typical experiment indicating the presence of succinoxidase are shown.

The complete system, (4) in Table 13, is saturated by approximately $1\cdot5\times10^{-5}$M cytochrome, and is slightly stimulated by the presence of 5×10^{-4}M Ca^{++} and Al^{+++}, as noted by Schneider and Potter (1943) for the mammalian enzyme. The pH optimum was approximately 8·2.

TABLE 13.
Succinoxidase in Egg Homogenates.

	System.	µl. O_2.
1	Homogenate	19
2	(1)+cytochrome	26
3	(1)+succinate	31
4	(3)+cytochrome	75
5	(4)+cyanide	3
6	(3)+methylene blue	48
7	(5)+methylene blue	39

Each flask contained 1 ml. of 10% distilled water homogenate, 0·5 ml. $\frac{M}{5}$ phosphate buffer (pH 7·5) in a final volume of 3 ml. Succinate final concentration $2\cdot5\times10^{-2}$ M, cytochrome 2×10^{-5} M, cyanide 10^{-3} M, methylene blue $3\cdot3\times10^{-3}$ M. Experiment duration, 1 hr.

The effect of some inhibitors on the succinoxidase system is shown in Table 14. The effects of these inhibitors are in general similar to those described by Humphrey (1947) for adductor muscle. As in the latter, the relatively small inhibition by malonate

Fig. 3. Effect of tricarboxylic acid cycle intermediates on homogenate respiration. Each flask, of 5 ml. capacity, contained 0·5 ml. of 40% homogenate, 0·2 ml. phosphate buffer (M/5, pH 7·5), and 0·1 ml. cytochrome (2×10^{-4}M) in a final volume of 0·9 ml. Curve 1 is the control, curve 2 has pyruvate, curve 3 α-ketoglutarate, curve 4 malate, and curve 5 succinate, added in final concentration M/100. Apart from the control the greatest deviation from linearity is found in the pyruvate curve. This is consistent with the operation of the tricarboxylic acid cycle, since pyruvate is not oxidized as such, but only after a labile condensation reaction, while the other substrates are oxidized directly.

is noteworthy. Indeed the effect with changing succinate concentration does not give much support to the idea that malonate is acting competitively with succinate in the present material.

TABLE 14.
Effect of Inhibitors on Succinoxidase.

Inhibitor.	Concentration. (M.)	Succinate. (M.)	O_2 Uptake Percentage Control.
Malonate	10^{-1}	5×10^{-2}	35
,, ...	,,	10^{-2}	36
,, ...	,,	2×10^{-3}	30
,, ...	10^{-2}	10^{-2}	65
,, ...	10^{-3}	10^{-2}	90
Iodoacetate	10^{-2}	$2\cdot5\times10^{-2}$	59
,, ...	10^{-3}	,,	88
Phenyl mercuric nitrate ..	10^{-3}	,,	3
,, ,, ..	10^{-4}	,,	65
,, ,, ..	10^{-5}	,,	110
Arsenite	10^{-2}	10^{-2}	54
,, ...	10^{-3}	,,	85

Flask contents similar to those indicated in Table 13. Data are from several experiments of duration 1–2 hrs. The inhibitions are calculated from the ratio of (System (4)+inhibitor minus system (2)+inhibitor) and (system (4) minus system (2)), the code numbers being those shown in Table 13.

(*c*) *Malic Oxidase.*

The oyster egg homogenate contains a system capable of oxidizing malic acid, which is similar to that described in mammalian tissues by Potter (1944). The results of a typical experiment are shown in Table 15.

TABLE 15.
Malic Oxidase in Egg Homogenates.

	System.	μl. O_2.
1	Homogenate	10
2	(1)+malate	21
3	(1)+glutamate	18
4	(1)+cytochrome	16
5	(4)+malate	37·5
6	(4)+glutamate	29·5
7	(6)+malate	107
8	(7)+cozymase	122

Each flask contains 1 ml. of 12·5% distilled water homogenate 0·5 ml. $\frac{M}{5}$ phosphate buffer (pH 7·5) in a final volume of 3 ml. Malate final concentration 2×10^{-2} M, glutamate 2×10^{-2} M, cytochrome 2×10^{-5} M, cozymase 1 mg./flask. Experiment duration, 1 hr.

The system is saturated by a cytochrome concentration of about $1\cdot5\times10^{-5}$M. The addition of nicotinamide to the system rarely had any effect, suggesting that cozymase degrading enzymes are absent or of very low activity. The marked stimulation of the oxidation of malate by glutamate implies the presence of an active glutamic oxalacetic

aminopherase. The relatively small effect of cozymase suggests that the enzyme is normally fairly saturated with the coenzyme and is in accord with the postulated absence of cozymase destroying enzymes.

(d) Miscellaneous Hydrolytic Enzymes.

The presence in egg homogenates of a number of hydrolytic enzymes is indicated in Table 16. It will be seen that the activity of acid and alkaline phosphatase, proteinase and lipase is fairly low. The activity of apyrase is rather high for invertebrate tissue, being of the same order as that found in the adductor muscle of the present species (Humphrey, 1949) and higher than that found in Arbacia eggs (Connors and Scheer, 1947).

The amylase activity is unexpectedly high and is not accompanied by any phosphate uptake. It is very improbable that the activity is due to contamination of the egg suspensions with small digestive diverticula fragments, since the latter have never

TABLE 16.
Hydrolytic Enzymes in Egg Homogenates.

Enzyme.	Substrate.	Concentration.	Buffer.	pH.	Activator.	Method.	Typical Activity (per 0·1 ml. Eggs/½ hr.).
Alk. phosphatase.	Glycerophosphate.	$\frac{M}{20}$	Veronal.	9·0	Mg $(\frac{M}{100})$	△Pinorg.	25 μg.
Acid phosphatase.	,,	,,	Acetate.	4·8	—	,,	,,
Apyrase ..	ATP.	$\frac{M}{100}$	Veronal.	7·5	—	,,	140 μg.
Lipase ..	Tween 80.	5%	Bicarb.-CO_2.	,,	—	CO_2 displaced.	1·8 μM.
Proteinase	Haemoglobin.	2%	Acetate.	3·5	—	Anson (1938).	35 μg.
Amylase	Glycogen.	0·4%	Phosphate.	7·5	NaCl (1%).	△Glycogen.	4·5 mg.

Correction for changes in the blank (without substrate) was applied in all cases.

been recognized in suspensions, and the activity is unchanged on further washing of the eggs; the enzyme is soluble and not bound to cell granules (Cleland, 1950*b*). Furthermore, although the alkaline phosphatase activity of the digestive diverticula is high (Cleland, 1947) that of egg homogenates is low.

The question of the histochemical detection of the alkaline phosphatase in the egg has been considered elsewhere (Cleland, 1950*c*).

Complex Enzyme Systems in Homogenates.

(a) Glycolysis.

The experiments on whole eggs indicated that lactic and pyruvic acids were produced under aerobic and anaerobic conditions. In order to study the system further, reconstruction experiments were carried out with homogenates. As in the case of whole eggs, non-metabolic acid production vitiated manometric estimates of glycolysis. The three typical experiments incorporated in Table 17 indicate the following: (*a*) that in both whole homogenate and in homogenate supernatant, pyruvic acid is the main acid formed; (*b*) that both adenylic acid (or ATP) and cozymase are coenzymes for the glycolysis; (*c*) that when the granular components of the homogenate are eliminated by high-speed centrifugation a considerable apparent stimulation of glycolysis occurs in the reconstructed system, a fact suggesting that some mechanism of anaerobic pyruvate removal is present. Little pyruvate accumulates in the complete system under aerobic conditions in homogenates, although, owing to removal of the aerobic pyruvate utilizing system, considerable amounts may accumulate in extracts incubated aerobically.

The effect of the classical glycolytic inhibitors (cf. Stotz, 1945) on the reconstructed system is shown in Table 18. It will be seen that the oyster egg system behaves similarly to the mammalian system.

TABLE 17.
Anaerobic Glycolysis in Egg Homogenates and Extracts.

	System.	Whole Homogenate.		Supernatant.*	
		μg. Pyruvic.	μg. Lactic.	μg. Pyruvic.	μg. Lactic.
1	Enzyme preparation	8	4·1	0	0
2	(1)+glycogen	13	4·3	11	1·6
3	(2)+cozymase	18	6·0	13·5	1·2
4	(2)+ATP	32	6·3	26	2·3
5	(2)+adenylic acid	27	5·8	22	2·3
6	(4)+cozymase	52	9·2	36·5	3·2
(6)	Homologous enzyme	26·5	6·1	83	3·4

* After centrifugation for 5 mins. at 10,000 xg.

Three experiments are delimited by the thick lines. The final volume was 1 ml. in each experiment which contained phosphate buffer (pH 7·5) of final concentration $\frac{M.}{25}$ The additions were as follows: glycogen 6 mg., cozymase, ATP, and adenylic acid 1 mg. The enzyme preparation in the whole homogenate experiment was 0·5 ml. of 40% homogenate, in the supernatant experiment 0·5 ml. of 20% homogenate supernatant and in the homologous enzyme experiment 0·5 ml. of 30% homogenate or the supernatant therefrom. The duration of all experiments was 2 hours and the flasks were gassed with nitrogen.

In order to analyse the anaerobic pyruvate degrading mechanism further, the removal of added pyruvate by homogenates and extracts was studied. The experiment detailed in Table 19 indicates the following: (*a*) that anaerobic pyruvate removal is quite high, being only slightly less than that found under aerobic conditions; (*b*) that the removal is stimulated by the presence of carbon dioxide and/or bicarbonate; and

TABLE 18.
Effect of Inhibitors on Glycolysis in Extracts.

Inhibitor.	Concentration. (M.)	μg. Acid Formed.	
		Pyruvic.	Lactic.
——	——	65	3·2
Iodoacetate	10^{-2}	8·5	1·3
Phenyl mercuric nitrate ..	10^{-3}	0	0
Fluoride	10^{-2}	0	0·8
Phloridzin	10^{-2}	5	0

Each flask contained 0·5 ml. of 40% homogenate supernatant and 0·2 ml. $\frac{M}{5}$ phosphate buffer (pH 7·5) in a final volume of 1 ml. The additions were identical to those in system (6) of Table 17. The experiment duration was 2 hours, the flasks being gassed with nitrogen.

(*c*) that the removal does not involve a mere conversion to lactate; (*d*) that the system is present on the granular component of the homogenate.

The system, or part of it, is evidently labile, since the CO_2 and bicarbonate stimulations were usually less than shown in Table 19, as also were the rates of removal of

pyruvate. The experiments, however, suggest that CO_2 fixation plays at least some role in pyruvate removal. Experiments were therefore conducted in which changes in the bicarbonate and CO_2 content were followed. The results of the two extreme experiments are shown in Table 20. Owing presumably to the lability of the CO_2 fixing mechanism, the results, while not really satisfactory, do indicate that in some preparations (Expt. 1) some CO_2 fixation occurs, while in others the removal is due entirely to decarboxylation (Expt. 2).

TABLE 19.
Removal of Added Pyruvate by Homogenates.

Enzyme.	Gas Phase.	Bicarbonate (Final Concentration).	Pyruvate Removed. (μg.)	Lactate Formed. (μg.)
Homogenate	Air.	—	107	0
,, ...	N_2	—	67	3·1
,, ...	,,	0·005 M	85	1·4
,, ...	5% CO_2 in N_2	0·025 M	91·5	2·1
Supernatant* ..	N_2	—	110	2·8

* Centrifuged for 5 minutes at 10,000 × g.

Each flask contained 0·5 ml. of 30% phosphate saline homogenate or the supernatant therefrom and 0·2 ml. of $\frac{M}{5}$ phosphate buffer (pH 7·5) in a final volume of 1 ml. At the beginning of the experimental period, which was 60 mins., 110 μg. of pyruvate was added from the siderarm.

The experiments detailed in Table 5 on the acid production of whole eggs are not sufficiently precise to decide whether CO_2 fixation or decarboxylation is responsible for the lack of accumulation of pyruvate in whole eggs. Unfortunately, owing to failure of the supply of eggs, it has not yet been possible to perform precise experiments to decide this issue. The lability of the homogenate system, however, suggests that CO_2 fixation may play a considerable role in whole eggs.

TABLE 20.
The Mechanism of the Anaerobic Pyruvated Utilization.

	Experiment I.		Experiment II.	
	Control.	Experimental.	Control.	Experimental.
Pyruvate added (μg.) ..	—	223	—	226
,, recovered (μg.) ..	—	185	—	163
,, used (μg.)	—	38	—	63
CO_2 evolved (μl.)	8·9	7·25	24	31·5
Bicarbonate recovered (μl.) ..	56	53·5	42·5	47
Total (μl.)	64·9	60·75	66·5	78·5
Difference (μl.)	−4·15		+12	
Pyruvate used/CO_2 produced	−9·1		+5·25	

Each flask, of 5 ml. capacity, contained 0·5 ml. of phosphate saline homogenate (20% in Experiment I and 32% in Experiment II) and 0·1 ml. $\frac{M}{40}$ bicarbonate in a final volume of 0·7 ml. The side arm contained 0·1 ml. of 100% trichloracetic acid which was tipped at the end of the experimental period (90 minutes in Experiment I and 120 minutes in Experiment II) in order to recover the bicarbonate. The flasks were gassed with a mixture of 1% CO_2 in N_2. The pyruvate $\left(0{\cdot}1 \text{ ml. of } \frac{M}{50} \text{ into the main chamber}\right)$ and bicarbonate were added from opsonic pipettes.

(*b*) *Tricarboxylic Acid Cycle.*

The tricarboxylic acid cycle was formulated on the following three basic observations (Krebs, 1943): (1) ability of the posulated intermediates to cause a marked stimulation of respiration; (2) a synthesis of citrate from added oxalacetate; (3) oxidative formation of succinate from oxalacetate or fumarate in malonate poisoned systems. The last of these is regarded by Krebs as the most important.

The first of these conditions appears to be fulfilled in the present material (Table 11) in those intermediates which were tested. Glutamate and, to a much less extent, aspartate also cause increases in the oxygen consumption of homogenates.

In view of the considerably lower respiratory rate and the low. efficiency of malonate as an inhibitor of succinoxidase in the present material as compared with pigeon breast muscle, it seemed unlikely that the second and third conditions could be fulfilled.

The tricarboxylic acid cycle appears to be the only mechanism known in animal tissues whereby pyruvate is completely oxidized. If it could be shown that pyruvate oxidation approached completion in the present material the evidence in favour of the operation of the cycle would therefore be strengthened.

When pyruvate was added to phosphate saline homogenates (4 experiments) the extra oxygen consumed varied from 28% to 70% of the theoretical requirement for complete combustion.

It has been shown that the enzymes of the cycle are present on the large granule fraction of mammalian liver and kidney (Kennedy and Lehninger, 1949, Green *et al.*, 1948) and in this material the oxidation of pyruvate is complete. In the oyster egg the tricarboxylic acid cycle enzymes have been shown to be present on the egg granules (Cleland, 1950*b*). Because of the low activity of the respiration in eggs it seemed possible that the low efficiency of oxidation of pyruvate in egg homogenates might be due to loss of cycle intermediates from the granules to the medium during the oxidation of the pyruvate.

If this were so, some improvement in the efficiency of utilization of pyruvate might be expected if pyruvate and some other cycle intermediate were oxidized together. This was found to be the case in three experiments, one of which is shown in Table 12.

TABLE 21.
Oxidation of Pyruvate under Different Conditions.

	System.	μl. O_2.	Excess O_2.	Pyruvate Used. (μg.)	Completeness of Oxidation.
1	Homogenate	132	—	—	—
2	(1)+pyruvate	151	19	110	38%
3	(1)+succinate	272	—	—	—
4	(3)+pyruvate	305	33	74	90%
5	(1)+citrate	193	—	—	—
6	(5)+pyruvate	217	21	48	98%
7	(1)+malate	224	—	—	—
8	(7)+pyruvate	247	23	70	80%

Each flask contained 1 ml. of 35% phosphate saline homogenate, 0·4 ml. $\frac{M}{5}$ phosphate buffer (pH 7·5) and 0·2 ml. 2×10^{-4} M cytochrome C in a final volume of 2 ml. Succinate, citrate and malate concentrations were $\frac{M.}{100}$ Pyruvate (110 μg./flask) was added from the side bulb at the beginning of the experimental period which was 82 minutes.

When either citrate, succinate or malate is present during the oxidation of the pyruvate the rate of pyruvate utilization is reduced but the efficiency of oxidation approaches 100%. Citrate causes the most marked depression of pyruvate utilization and gives the most complete combustion, as would be expected on the above hypothesis. Kennedy and

Lehninger (1949) have described an inhibition of pyruvate utilization by malate in the rat liver granule system.

(c) *High Energy Phosphate Formation.*

That some high energy phosphate formation occurs during the respiration of homogenates is indicated in the experiment shown in Table 22. Under anaerobic conditions or in dinitrophenol treated homogenates a considerable decrease in the seven-minute hydrolysable P occurs. The endogenous respiration of homogenates is almost capable of maintaining the P_7 and when tricarboxylic acid cycle intermediates are being oxidized, the P_7 content shows a slight increase.

TABLE 22.

High Energy Phosphate Changes in Homogenates under Different Conditions.

	System.	Gas Phase.	Oxygen Used. (µl.)	P_0. (µg.)	P_7. (µg.)	P_{0+7}. (µg.)
1	Homogenate (at beginning) ..	—	—	87	22·5	109·5
2	(1) at end	Air	18·5	88	22	110
3	(2)+10^{-5} M dinitrophenol ..	Air	22	99	15	114
4	(2)+succinate and pyruvate ..	Air	42	82	26	108
5	(2)	N_2	0·5	95	9	104

Each flask, of 5 ml. volume, contained 0·5 ml. of 50% phosphate saline homogenate and 0·1 ml. of 2×10^{-4} M cytochrome in a final volume of 0·8 ml. Succinate and pyruvate were added in a final concentration of $\frac{M}{100}$. The experiment duration was 30 minutes. P_0 refers to inorganic P, P_7 to 7 minute hydrolysable P.

Keltch *et al.* (1949) have shown that a particulate enzyme system from Arbacia eggs is capable of oxidative phosphorylation when using a number of tricarboxylic acid cycle intermediates.

DISCUSSION.

The experiments on the penetration of substrates (Table 4) indicated that invertebrate cells do not necessarily have the same permeability properties as mammalian cells. The impermeability may be confined to free living marine invertebrate cells, since various preparations from earthworms appear to be freely permeable to a variety of substrates (O'Brien, personal communication).

The vertebrate cell or tissue is normally in a spatially limited, substrate containing environment and even when leaving the parent organism (e.g. sperm) the environment does not change greatly. In contrast, the spawned pelagic invertebrate egg is voided into an enormous substrate-free environment where intracellular substrate loss would be irrevocable.

The slight or absent permeability of the present material to natural substrates would, therefore, appear to have a definite survival value. Considerably larger molecules (respiratory and mitotic inhibitors) are able to exert their typical biological effect in the present material, which suggests that the impermeability is rather selective.

Impermeability to natural substrates has necessitated the use of acellular homogenates. This has considerably complicated the analysis of intermediary metabolism because of uncertainty about the relation of the homogenate respiratory mechanisms to those of the whole egg.

In Table 9 it was shown that glycogen caused a significant stimulation of homogenate respiration. Since glycolysis from glycogen occurs in homogenates (Table 17) and the end products of glycolysis are readily oxidized (Table 11) it may be concluded that the respiratory stimulation caused by glycogen proceeds over these pathways. Hexokinase is evidently present in the eggs, since added ATP enables a considerably increased respiration to be maintained by fructose and glucose (Table 9; Text-fig. 2).

It may therefore be concluded that whole eggs are capable of using in their oxidative metabolism both the polysaccharide and monosaccharide which they contain (Table 1).

The greater effect of fructose on uncomplemented homogenates may be due to different affinities of hexokinase for fructose and glucose (cf. Meyerhof, 1947).

In a previous paper (Cleland, 1950*a*) the respiratory quotient of whole eggs was shown to be approximately 0·80, a value which appeared to result from oxidation of carbohydrate and lipide. As already indicated, the maximal glycolytic rates found in extracts would yield sufficient pyruvate to maintain this quotient; the glycogen and monosaccharide content found is sufficient to allow the quotient to be maintained for about eight hours.

Some of the lower fatty acids are able to stimulate the oxygen consumption of homogenates (Table 10). The mechanism was not defined, but the variability of the results suggests that the reactions involved may be complex. As in the uncomplemented mammalian preparations, higher fatty acids at higher concentrations caused inhibitions of homogenate respiration, and even with added ATP and a low fatty acid concentration, under which conditions mammalian liver preparations actively oxidize the fatty acid (Lehninger, 1945, 1946), no increase in respiration could be demonstrated. In view of the presence of large amounts of fat in the present material (Cleland, 1947) and of a respiratory quotient suggestive of considerable lipide oxidation (cf. Table 23) the failure of homogenates to oxidize higher fatty acids may probably be ascribed to lability of the system (cf. Lehninger, 1945, 1946) or to a saturating concentration of fatty acid being already present in the uncomplemented homogenate. Acetoacetic acid is a product of fatty acid oxidation in the mammalian liver system, and either it or some precursor or product is believed to condense with oxalacetate and thus to enter the tricarboxylic acid cycle (cf. Breusch, 1948, Grafflin and Green, 1948). In contrast to pyruvate, acetoacetate is without effect on egg homogenate respiration. It cannot be decided whether this is due to a real qualitative difference between the oyster and mammalian material or whether it is due to lability of the condensation mechanism.

The experiments with amino acid additions indicate that no d-amino acid oxidase is present in the eggs. Apart from an active glutamic enzyme and an aspartic metabolizing system of low activity, amino acids do not appear to be normal respiratory substrates for homogenates. Whole eggs, however, show a slightly increased respiratory rate in the presence of glycine (Cleland, 1950*a*), which suggests that some of the homogenate inactivity may be due to the lability of the enzymes concerned. In view of the low ammonia production of the whole eggs it is not improbable that the rather large amounts of free amino acids present (Table 1) are used in synthetic reactions (e.g. chromosome synthesis) rather than serving as energy sources.

A superficially typical cytochrome oxidase of relatively high activity has been found in the present material (Table 12) and cyanide causes inhibition of the respiration of whole eggs comparable in magnitude with that found in mammalian material. It is fairly certain, therefore, that the link with oxygen in whole eggs is through the cytochrome system. There do not appear to be many reports of cytochrome oxidase in invertebrates. In cyanide inhibition studies, Robbie (1949) has indicated that the respiration of a wide range of invertebrate tissues is inhibited by cyanide in greater or lesser degree. Vanadium-containing invertebrates are evidently an exception. The presence of cytochrome oxidase in sea urchin eggs is well established not only by substrate and inhibitor studies on whole eggs but also by isolation of an enzyme system (Krahl *et al.*, 1941).

Succinoxidase (Table 13) has been described in few invertebrates. The literature has been considered by Humphrey (1917). It is also present in several tissues of the earthworm (O'Brien, personal communication) and is probably present also in the sea urchin egg (Crane and Keltch, 1949; Keltch *et al.*, 1949). As in the case of the adductor muscle of the present material and in the earthworm preparations, malonate causes relatively small inhibitions of the enzyme (Table 14). This fact indicates that considerable caution must be exercised in the interpretation of inhibitor experiments on unknown material.

Malic oxidase (Table 15) does not appear to have been described previously in invertebrate material, although malate has been shown to stimulate the respiration of an enzyme preparation from sea urchin eggs (Keltch *et al.*, 1949). The inferred presence of a glutamic-oxalacetic transanimase is interesting inasmuch as this system is very widespread and active in mammalian material (Herbst, 1944).

The activity of the hydrolytic enzymes, proteinase, amylase and lipase (Table 16) is high enough to yield considerably more breakdown products than could be removed by the oxidative metabolism of the intact egg. Increases in the activity of these enzymes, which is evidently controlled in the intact egg, would have to be considered among possible factors responsible for the increases in respiratory rate of eggs during development (Cleland, 1950*a*). Increases in their intracellular activity may also contribute to the increased respiration found in injured eggs.

It is not difficult to envisage a physiological role for the proteinase and lipase in the eggs, but the significance of the high amylase activity is difficult to assess. One possibility would be an insoluble and inaccessible glycogen-amylase complex capable of yielding substrate to the glycolytic system, but no evidence of this possibility has been found in studies of the intracytoplasmic distribution of amylase or glycogen (Cleland, 1950*b*).

Owing to false leads arising from unexpected features of the system, analysis of the glycolytic mechanism proved difficult. The facts emerging from the analysis are as follows: (*a*) Whole eggs and homogenates produce considerable amounts of acid, of which only a very small part can be ascribed to the lactic and pyruvic acids accumulating (Table 5). (*b*) Experiments on cytolytic acid production, some of which are summarized in the present paper, suggested beyond reasonable doubt that most of the acid production was non-metabolic. A considerable fraction of uncytolysed whole-egg acid production and the acid production of homogenates was viewed in the same light. (*c*) The effect of glycolytic inhibitors on whole-egg acid production (Table 6) suggested that the true glycolytic acid production is still greater than can be accounted for by the lactic-pyruvic acid analyses. (*d*) In the homogenate studies (Table 19) it was shown that pyruvate, the main glycolytic acid produced, disappears in sufficiently large amounts to account for the discrepancy noted in (*c*).

Unfertilized and recently fertilized sea urchin eggs produce considerable amounts of acid under anaerobic conditions, and, at least on fertilization, under aerobic conditions (e.g. Laser and Rothschild, 1939). Large amounts of acid are produced on injury or actual cytolysis of the egg (e.g. Runnstrom, 1936; Rothschild, 1939). Apart from the absence of acid production on fertilization in the oyster, there are points of similarity to the situation in the sea urchin egg. Small amounts of lactic acid also occur in the sea urchin egg (Perlzweig and Barron, 1926; Hutchens *et al.*, 1942).

Carbohydrate metabolism in the sea urchin egg is still unsatisfactorily known. Lindberg (1943, 1946) has presented evidence that carbohydrate is broken down, not through the usual glycolytic cycle but through phosphogluconic acid and pentoses. The more recent investigations of Lindberg and Ernster (1948) are not inconsistent with this view.

Some of the conclusions about sea urchin carbohydrate metabolism have been based on the lack of effect of iodoacetate. In view of the work of Barron and Tahmisian (1948), Harting (1947) and Humphrey (1949) this lack of effect must be interpreted with caution.

The glycolytic system in the present material is similar to mammalian material in its coenzyme needs and reaction to inhibitors. It differs quite markedly, however, in the lactate pyruvate equilibrium: pyruvic acid far exceeds the lactic acid accumulation. Accumulation of both acids is probably not uncommon in invertebrates (cf. Humphrey, 1949). In earthworm muscle both acids accumulate (O'Brien, personal communication). The oyster egg appears to be the most extreme case of pyruvate accumulation yet encountered.

The possibility that the pyruvate accumulation is an artefact must be considered. The phenomenon would be possible if some active system capable of oxidizing reduced cozymase were present. The latter possibility is considered unlikely for the following reasons: (*a*) the pyruvate accumulation is unaffected by rigorous exclusion of the final traces of oxygen from the nitrogen (passage over copper at 360°C.); (*b*) the cozymase oxidizing systems are evidently present on the granular fractions, since malate and glutamate oxidation is found to be associated with the granules (Cleland, 1950*b*) while the glycolytic system is found to be most active in homogenate supernatants where it is qualitatively similar to the system in whole homogenates (Table 17); (*c*) competition for reduced coenzyme by malic dehydrogenase in a CO_2 fixing mechanism would seem to be ruled out by the fact that qualitatively similar results are obtained in whole homogenates and in supernatants therefrom, while the mechanism is known to be present on the granules (Table 19). Since lactic dehydrogenase appears to be present (Table 11), it seems reasonable to infer that the pyruvate accumulation is due to the equilibrium properties of this enzyme.

Meyerhof and Geliazkowa (1947) have shown that the differences found in the glycolytic mechanism of mammalian homogenates and homologous extracts is due to the activity of the apyrase which is present mainly on the cell granules. This factor is unlikely to play any part in the difference between whole homogenate and the supernatant therefrom in the present material for the following reasons: (*a*) Glycogen is used as substrate rather than glucose. (*b*) Adenylic acid is almost as active as ATP (Table 17). (*c*) The apyrase activity is relatively low and is evenly distributed between granules and ground cytoplasm (Cleland, 1950*b*). (*d*) The alternative explanation proposed is quite adequate to account for the facts.

Evidence suggestive of CO_2 fixation has been presented (Tables 19 and 20). The fact that the malic, fumaric and succinic utilizing systems, like the system using pyruvate anaerobically, are on the granules (Cleland, 1950*b*) is consistent with this view, as also is the fact that these substances would all be capable of rapid oxidation when aerobic conditions returned.

If the CO_2 fixing mechanism operates in whole eggs, as seems likely on general grounds, it would provide a mechanism of oxidizing the reduced cozymase produced during glycolysis and would also yield considerably more energy than mere breakdown to pyruvate (Ochoa, 1947).

The data shown in Table 21 have indicated that homogenates are capable of oxidizing pyruvate completely under certain conditions, and the conclusion was drawn that this oxidation took place via the tricarboxylic acid. Owing to the relative insensitivity of the methods available and the low metabolic rate of the present material the experiments considered critical by Krebs (1943) did not seem feasible. However, the evidence adduced for the cycle (utilization of cycle intermediates, complete oxidation of pyruvate), together with the fact that the enzymes concerned are bound to cell granules and behave as part of a complex in the centrifugal field (Cleland, 1950*b*), as does the cyclophorase complex in mammalian material (Green *et al.*, 1948), seems adequate.

The respiratory metabolism of whole eggs and homogenate is compared in Table 23.

It will be seen that the respiratory rate of homogenates may exceed that of homologous whole eggs. The impression has been formed that this is found in eggs with low initial respiratory rate (i.e. eggs with unbroken germinal vesicles; cf. Cleland, 1950*a*) and is, further, more direct evidence that the respiratory rate of whole eggs is far from maximal.*

The respiratory quotient has been found to be slightly lower in homogenates than in whole eggs. This fact and the insignificance of the difference between the cyanide inhibitions indicates that no new oxygen transport mechanisms or oxygen-consuming systems (e.g. lipide auto-oxidation) arise on cytolysis.

* A phenomenon first observed by Warburg (*Arch. ges. Physiol.*, 1914, 158: 189).

The difference in methylene blue stimulation is probably due to the fact that in whole eggs there is usually insufficient penetration to cause visible staining of the egg interior (Cleland, 1947) while in homogenates strong staining of the granules occurs, a situation not unlikely to cause some disruption of the enzyme complex contained thereon. The fact that any stimulation occurs with methylene blue may be interpreted in either of two ways: (*a*) that the cytochrome-cytochrome oxidase system is limiting; the high activity of the cytochrome oxidase (Table 12) suggests that it is the cytochrome which is deficient; or (*b*) that some of the dehydrogenases are present in soluble form in the ground cytoplasm while all of the normal oxygen transport mechanisms are present on granules; methylene blue enables the soluble dehydrogenases to function maximally and eliminates the need for electron transport from enzyme to granule.

The strong succinoxidase and glycolytic inhibitor phenylmercuric nitrate (Tables 14 and 18) causes a marked inhibition of whole-egg respiration. That the main inhibition in whole eggs is due to inhibition of succinoxidase acting in the tricarboxylic cycle is suggested by: (*a*) the magnitude of the inhibition (pyruvate catabolism accounts

TABLE 23.
Comparison of the Respiration of Whole Eggs and Homologous Homogenate.

Characteristic.	Whole Eggs.	Homogenate.	Remarks.
Respiratory rate	—	Increase or decrease.	—
„ quotient	0·80	0·76	
Oxidative phosphorylation	Marked.	Slight.	Tables 7 and 22.
Respiration in the presence of (as percentage control):			
HCN (10^{-3} M)	5·4	8·5	No cytochrome added.
Methylene blue (3×10^{-3} M) ..	256	165	„ „
Dinitrophenol (10^{-5} M)	180	118	No P added.
Phenyl mercuric nitrate (10^{-3} M) ..	18	47	Data from single egg batch.
Iodoacetate (10^{-2} M)	35	61	„ „ „
Malonate (10^{-1} M)..	No penetration	65	„ „ „
Arsenite (10^{-2} M)	67	76	„ „ „

for only a portion of the total respiration); (*b*) the smaller inhibition in homogenates does not indicate that lipide oxidation is much affected by this inhibitor; (*c*) iodoacetate and arsenite which are less effective inhibitors of succinoxidase than phenylmercuric nitrate give less inhibition of egg respiration; (*d*) fluoride, a strong inhibitor of glycolysis at M/100 (Table 18) causes much less inhibition of respiration than the above two compounds. The phenylmercuric nitrate residual respiration would thus appear to give a crude estimate of the respiration not mediated by the tricarboxylic acid cycle. If this were so, other succinoxidase inhibitors would be expected to give inhibition of the phenylmercuric nitrate sensitive respiration of whole eggs approximately proportional to their effect on succinoxidase (Table 14). This appears to be so for arsenite but not for iodoacetate.

In homogenates the phenylmercuric nitrate inhibition is considerably less than in whole eggs, suggesting that only about 50% of the total homogenate respiration proceeds through the tricarboxylic acid cycle. The inhibitions caused by arsenite and malonate in whole homogenates are consistent with this hypothesis, but the inhibition by iodoacetate, as in the case of whole eggs, is greater than would be expected.

That the phenylmercuric nitrate residual respiration is due to oxidation of lipides to fragments small enough to enter the tricarboxylic acid cycle seems worth considering. The greater residual respiration together with the lower respiratory quotient in homogenates as compared with whole eggs is consistent with this.

Dinitrophenol causes a much smaller stimulation of homogenate than of whole-egg respiration. This may be due either to injury to the phosphorylating mechanisms in the

former, making these less obligatory in the oxidation, or to differences in the availability of inorganic phosphate at the enzyme sites in homogenates and whole eggs. The differences in high energy phosphate formation in homogenates and whole eggs (Tables 7 and 22) suggest that the former factor may be more important; however, until more is known about the intracellular distribution of inorganic phosphate, and its rate of diffusion in cytoplasm, the latter factor cannot be excluded.

The magnitude of the respiratory stimulation caused by dinitrophenol (Tables 3 and 23) strongly suggests that the availability of inorganic phosphate normally plays a role in limiting the respiratory rate of whole eggs. During the development of the egg the rising respiratory rate may thus be due to a greater rate of hydrolysis of the ATP arising during respiration. This might be caused by more rapid utilization in the endergonic cellular syntheses involved (e.g. chromosome, cell and nuclear membrane syntheses) and/or increases in apyrase activity. The effects of colchicine on the respiration of developing eggs (Cleland, 1950*a*) could also be interpreted on the former hypothesis. Owing to the arrest of the cellular syntheses by colchicine, phosphate turnover is diminished and the respiratory rate is depressed to a lower constant level.

Summary.

In order to provide a background for analysis of the metabolic changes during development and for the use of inhibitors in studies of the mitotic process, attempts were made to outline the intermediary metabolism of oyster eggs. The following points were elicited.

Whole Eggs:

(1) Glycogen, monosaccharide, phospholipide and neutral fat are present; free amino acids are present in considerable amount but not do appear to be metabolically active.

(2) Fructose phosphates, phosphoglyceric acid, cozymase, adenylic acid and ATP are present. Small quantities of lactic and pyruvic acid occur.

(3) Some 90–95% of the respiration is mediated by a cytochrome-cytochrome oxidase system. Phenylmercuric nitrate causes an 80% inhibition of respiration, a fact interpreted in terms of its inhibition of the tricarboxylic acid cycle.

(4) Eggs are impermeable to natural substrates, a fact making the use of homogenates obligatory for study of intermediary reactions.

(5) Anaerobic acid production far exceeded that expected on the grounds of lactic and pyruvic acid analyses. Experiments on eggs during cytolysis strongly suggested that much of this acid production was non-metabolic. A rough estimate of true glycolytic rate was made by the use of glycolytic inhibitors.

(6) The high energy phosphate content of eggs is depleted by cyanide inhibition, anaerobic conditions and dinitrophenol, indicating that phosphate bond energy is generated during respiration.

Homogenates.

(7) Glycogen and fructose stimulate the respiration; with ATP added, glucose does also.

(8) Some of the lower fatty acids and phospholipide stimulate the respiration; none was found with higher fatty acids.

(9) Of six amino acids tested only aspartate and glutamate caused respiratory stimulation.

(10) Citrate, α-ketoglutarate, succinate, fumarate, malate, pyruvate and lactate cause marked respiratory stimulation.

(11) Typical cytochrome oxidase, malic oxidase and succinoxidase systems have been demonstrated. The latter shows a relatively small malonate inhibition.

(12) Lipase, proteinase, amylase, acid and alkaline phosphatase, and apyrase have been demonstrated. The amylase is very active.

(13) The glycolytic system requires addition of cozymase and adenylic acid or ATP for maximal activity. Pyruvic acid is the main end product and greater accumulation is found when the granules are centrifuged from the homogenate.

(14) The latter fact is due to an anaerobic pyruvate removing mechanism on the granules. At least part of the removal seems due to a CO_2 fixing mechanism.

(15) Evidence for the operation of a tricarboxylic acid cycle is presented. The most important evidence is the complete oxidation of pyruvate.

(16) Some phosphate bond energy is formed during the respiration of homogenates.

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A FURTHER CONTRIBUTION ON THE LIFE HISTORY OF PHEROSPHAERA.

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(*Communicated by Dr. Patrick Brough.*)

(Plates ix–xii and Twenty-two Text-figures.)

[Read 29th November, 1950.]

This paper describes the development of the gametes and the proembryo of the Tasmanian conifer *Pherosphaera hookeriana* Archer, which features were not adequately dealt with by Lawson (1923*b*). The development of the embryo from the stage at which the prosuspensor begins to elongate has been the subject of a previous communication (Elliott, 1948).

Fertility and Pollination.

The material of *Pherosphaera hookeriana* used in the present investigation was collected in the Mt. Field National Park, Tasmania, in February, 1948. In my previous paper figures were given for the fertility of the material containing the youngest embryos collected on 22nd March, 1947. Similar figures have been worked out for some of the material collected from the same plants on 13th February, 1948. The site of these two collections is near tree line at 4,000 feet on Mt. Mawson, on a shelf just below the top of the range, which affords a slight but not very great amount of protection from the prevailing weather. Another collection was made on 15th February at Eagle Tarn, a small lake nestling at the foot of Mt. Mawson in a sheltered position (altitude 3,390 feet). The figures for all three lots of material are presented in Table 1.

The winter of 1946 was unusually severe, and as Table 1 shows, the fertility of the Mt. Mawson material in the following summer, 1947, was less than in 1948; the winter of 1947 was not severe.

Table 1.

Numbers of Ovules, their Fertility, and Stage of Abortion of Unpollinated Ovules in Pherosphaera hookeriana.

Material.	Number of Cones Examined.	Total Number of Ovules.	Percentage Fertile.	Percentage Unpollinated Ovules Aborting Early.
Mt. Mawson, 22 Mar., 1947	62	213	25·0	86·25
Mt. Mawson, 13 Feb., 1948	40	180	36·7	79·8
Eagle Tarn, 15 Feb., 1948	70	315	33·3	12·9

For the purpose of these tables, a fertile ovule is simply one that contains a well developed prothallus. As a rule unpollinated ovules abort, and do not develop large prothalli. Only very occasionally is a well developed prothallus found when there is no evidence of any pollen tube in the nucellus. The nucellus and embryo sac, and more particularly the integument, in unpollinated ovules may, however, continue to enlarge for a time before ceasing to grow. Some abortive ovules were very small. Others were nearly full sized, but both contained a mere shell of a nucellus or a very degenerate prothallus. A distinction could be drawn at about half the normal size of the fertile ovules. The stage at which the abortion took place is also indicated in Table 1. It will be seen that in the exposed situation a much higher proportion of ovules aborts at an early stage than at the more sheltered Eagle Tarn.

No attempt has been made to determine the fertility of ovules at different stages of development. It is to be expected that even in the presence of pollen tubes fertilization

might not take place, and hence the difference in "fertility" recorded between the two lots of Mt. Mawson material and between the two 1948 collections may be too great as the Mt. Mawson 1948 material was mostly in pre-fertilization stages.

In Table 2 the distribution of fertile ovules among cones is shown. In both lots of material from the exposed situation the actual distribution does not differ significantly from a Poisson series, that is to say, ovules are pollinated at random. However, this is not true of the sheltered situation, where significant excesses above expected numbers are found in the class 0 fertile ovules per cone, and also in the classes 3, 4 and 5 fertile ovules per cone. The average total number of ovules per cone is 4·5. Apparently pollen blows in a cloud in the sheltered situation, and some cones are in a more favourable position than others in respect to the passing pollen grains.

Technique.

Material was fixed in the field in formalin-acetic-alcohol. For examination, the ovules were removed from the cones and their integuments dissected off. The nucellus and the prothallus within it were then embedded using butyl alcohol as dehydrating

Table 2.

Distribution of Fertile Ovules among Cones on Pherosphaera hookeriana, *Compared with that Expected on Random Distribution.*

Material.		Number of Cones with n Fertile Ovules.							P
		$n=0$	1	2	3	4	5	6	
Mt. Mawson, 22 Mar., 1947	Found	22	28	10	2	0	0		0·16
	Expected	26·0	22·6	9·8	2·9	0·6	0·1		
Mt. Mawson, 13 Feb., 1948	Found	5	12	16	6	1	0	0	0·12
	Expected	7·7	12·7	10·4	5·8	2·4	0·8	0·2	
Eagle Tarn, 15 Feb., 1948	Found	21	19	12	12	4	2	0	0·04
	Expected	15·6	23·4	17·6	8·8	3·3	0·8	0·2	

P = probability that the numbers found constitute a Poisson series.

agent and solvent for paraffin. Microtome sections were stained with iron haematoxylin and counterstained with Bismarck brown. Squash preparations were also made, these being stained with carmine or by the Feulgen technique.

Stages of Development in the Material.

All the collecting in 1948 was done within a few days, but in different situations different stages were found, and each collection contained a wide range of stages. In these natural populations pollination apparently continues for a long time, and hence a tremendous number of ovules would have to be examined to study any one stage very intensively. In the present investigation some 200 fertile ovules have been examined. Most abundant in the material from Mt. Mawson (13th February) were archegonia prior to the ventral canal division and pollen tubes with body cells. The first division of the zygote illustrated in Pl. xi, fig. 22, came from this material, but such an advanced stage was exceptional. The cones collected at Eagle Tarn (15th February) yielded ovules in which developing and mature gametes, fertilization, and all proembryo stages were observed, together with embryo systems in which the prosuspensors were elongating and embryos beginning to grow. Similar and later stages were found in cones collected near Boronia Moor on 13th February (not referred to in the section on pollination and fertility).

Development of the Male Gametes.

According to Lawson (1923*b*) the pollen grains of *Pherosphaera* at the time of shedding contain two nuclei—the tube nucleus and the generative nucleus. The pollen tubes growing through the nucellus contain three nuclei—the tube nucleus, and the products of the division of the generative nucleus: the body cell and the stalk or sterile nucleus. The body cell has a densely staining sheath and contains a large nucleus, generally somewhat excentrically placed, with a conspicuous nucleolus, the centre of which does not stain so heavily (Text-fig. 1). The other two nuclei are generally found close to the body cell. In Pl. ix, fig. 4, the tube nucleus is unusually far from the body cell. The stalk nucleus may be found either ahead of, or behind, or sometimes to the side of, the body cell. Sterling (1948) reports that in *Taxus* the stalk nucleus moves round the side of the body cell, so the same is probably the case in *Pherosphaera*. It is generally considerably flattened against the body cell (Pl. ix, fig. 2). In this respect it resembles that of *Saxegothea* (Looby and Doyle, 1939, p. 111 and Pl. 3, fig. 22). It also has the appearance of the nuclei figured by Sinnott (1913) in *Podocarpus dacrydioides* (his Pl. 9, fig. 50) and *P. ferrugineus* (Pl. 5, fig. 2), although these were considered to be non-functional male gametes. However, the gametes in *Saxegothea* (Looby and Doyle) and *Pherosphaera* (as will be shown below) are very different from the stalk nucleus, and Sinnott's interpretation is in error. In fact the adherence of the stalk nucleus to the body cell in this fashion seems to be a feature of most podocarps.

The division of the body cell nucleus is shown in Pl. ix, fig. 3. The division results in two nuclei of equal size and each with a large nucleolus (Text-fig. 2). They resemble the body cell nucleus except in size. One of them is generally much closer to the edge of the body cell than the other. The more centrally placed nucleus enlarges considerably, while the other nucleus does not enlarge so much. At maturity the male cells are generally unequal, but the degree of inequality is variable. Text-figure 3 shows a case of extreme inequality in the two nuclei, although this is a young stage. Text-figures 5 and 7 and Pl. ix, fig. 7, may be regarded as typical, but the two nuclei may be more equal in size. The larger nucleus is surrounded by a densely staining sheath which becomes less homogeneous as development proceeds (Pl. ix, figs. 7, 8; Text-figs. 5, 6). Text-figure 9 shows part of the sheath of the male complex illustrated in Pl. ix, fig. 7. There are dark staining vesicular portions in protoplasm which does not stain so heavily. The smaller male nucleus is pushed well to the side of the protoplast, so that its outer side is quite naked, being covered by no cytoplasm at all as far as can be seen. In Pl. ix, fig. 6, part of the sheath has come off, but this exceptional male complex is interesting, as it shows the rest of the cytoplasm clearly, and also demonstrates that in *Pherosphaera* two male *cells* are not formed—there is no membrane of any sort dividing the protoplast, such as occurs in *Saxegothea* (Looby and Doyle, 1939) and *Phyllocladus* (Young, 1910).

As development proceeds the male nuclei stain more and more intensely. In the very young male nuclei small chromomeres only are visible. At a later stage, represented in Text-figure 6, heavily staining chromosomes are seen, and they are thrown into coils of large and varying diameter (Pl. ix, fig. 5 and Text-fig. 8). The full length of the chromosome is not so intensely stained at this stage, but the less heavily stained portions are also coiled (Text-fig. 8).

Up to this time the nucleolus still stains intensely, but as the chromosomes become intensely staining throughout their length the nucleolus loses its black colour with hæmatoxylin and appears reddish. Its fragmentation is not the reason for the dark staining of the male nuclei. The nucleolus in Text-figure 5 is red and the chromosomes are more closely coiled, apparently staining heavily throughout their length.

In the mature gametes (Pl. ix, fig. 8) the chromosomes are more regularly coiled, and the diameters of the gyres considerably less than in the earlier stage described. The appearance of the mature gamete, which has been described by Haupt (1941) and

Sterling (1948) as coarsely granular, is due to the coiled chromatids lying close together.

The chromosomes of the extruded nucleus are always less heavily stained than those of the nucleus near the centre. The difference in appearance between the two male nuclei is shown in Text-figures 5, 6 and 7 and Pl. ix, figs. 6 and 7.

Although they stain very intensely with haematoxylin, the gametes contain little or no desoxyribosenucleic acid. Even in material kept in alcohol for two years, metaphase and anaphase chromosomes in the prothallial tissue and proembryo are well stained in the Feulgen technique following 10 minutes' hydrolysis at 60°.

The coiling seen in the young gametes presumably represents a relic coiling, but the mature gametes seem to show a narrower, and therefore newly developing, spiral. The cytology of the male gametes of conifers deserves further study.

The male gametes of *Pherosphaera* are thus rather different from those of most podocarps hitherto described. The two male nuclei are unequal, both in size and the degree to which their chromosomes are coiled (or uncoiled ?) and capable of staining with haematoxylin. The male nuclei are equal in size in *Saxegothea* (Looby and Doyle, 1939) and in *Microcachrys* (Lawson, 1923*a*); and where they are unequal, as in *Podocarpus andinus* (Looby and Doyle, 1944*a*) and *Phyllocladus* (Young, 1910), the smaller nucleus is not extruded from the body cell protoplast. In each of these cases also the male nuclei are separated by a membrane into two *cells*. Stiles (1911) claimed the gametes of *Dacrydium* were unequal, but one cannot say whether his drawing represents a gamete or a stalk nucleus. There is, however, considerable resemblance between the gametes of *Pherosphaera* and the *Eupodocarpus* species described by Coker (1902).

The Ovule and Development of the Archegonia.

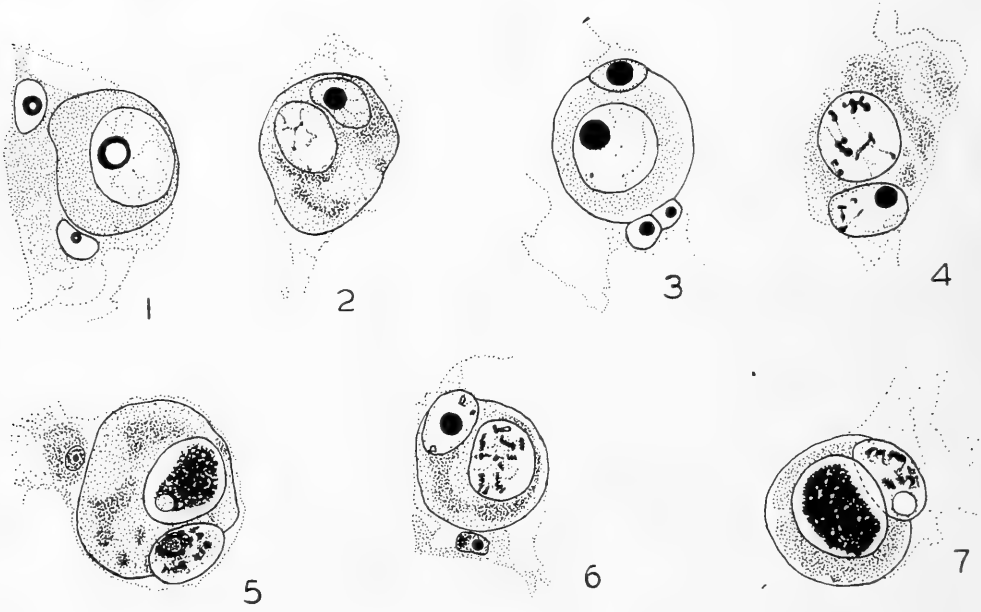
The structure of the ovule may be seen from Pl ix, fig. 1. The peculiar lips at the micropylar end are noteworthy. These appear to be present from early stages (Lawson, 1923*b*, Text-figs. 7–9). Lawson did not suggest they had any function in pollination. The nucellus is free from the integument right to the base. Occasionally the nucellus is found to contain two prothalli placed side by side, as in Text-figure 14, but this phenomenon is very rare—frequency less than 1%. The female prothallus has no "tent-pole".

One of the most significant features of the female gametophyte of *Pherosphaera hookeriana* is that the mature archegonia are deeply buried by the growth of the prothallus. This was overlooked by Lawson, although his Text-figures 26 and 27 show buried necks. Lawson said that "each archegonium has its own small and shallow depression over the neck" (pp. 511–2). This burial of archegonia is an important resemblance between *Pherosphaera* and other podocarps, in all of which this occurs.

Lawson records "three or occasionally four" as the most frequent number of archegonia. I have found a wider range. In 100 prothalli the number of archegonia ranged from 2 to 6; the mean was $3{\cdot}56 \pm 0{\cdot}09$.

Text-figure 10 shows the neck of an archegonium still in a superficial position, but the cells of the prothallus are just beginning to encroach over it. This stage resembles that in *Saxegothea* in Looby and Doyle's (1939) Pl. 2, fig. 10. The nucleus in the young archegonium has a characteristic structure, which is shown in Text-figure 10 and Pl. ix, fig. 9. It has a comparatively large and conspicuous nucleolus, and chromosomes can barely be seen in it. The nucleus is situated near the neck (Text-fig. 10 and Pl. ix, fig. 9), a position which it retains until the ventral canal division, which takes place close to the neck. The very young archegonium has a large central vacuole and very little cytoplasm, but the amount of cytoplasm rapidly increases, and in Pl. ix, fig. 9, there are numerous small vacuoles as well as what remains of the large central one. At this stage the neck is already deeply buried. The buried neck is also well shown in Pl. ix, fig. 10. The neck generally consists of four cells, but eight are sometimes found. In one prothallus a six-celled neck was observed.

The ventral canal nucleus is seen in Pl. ix, fig. 10. It is found near the neck, while the egg nucleus takes up a position in the centre of the archegonium. As the ventral canal nucleus was not often seen it is concluded that it degenerates quickly, although in several archegonia which had not been entered by a pollen tube, three or even four nuclei could be seen. The amount of cytoplasm continues to increase and appears to radiate in strands from the egg nucleus, but apart from this asteroids are not evident. Finally, the archegonium is nearly full of cytoplasm, and the appearance of the stained egg nucleus is somewhat similar (Pl. ix, fig. 11; Pl. x, fig. 12). In favourable cases chromosomes can be seen in the egg nucleus showing relic (?) coiling, as illustrated in Looby and Doyle's (1944*a*) photographs of *Podocarpus andinus* (their Pl. 11, figs. 4, 5 and 8). A nucleolus surrounded by chromocentres is generally seen in the egg nucleus (Pl. ix, fig. 11); the structure is especially well shown in carmine stained preparations.



Text-figures 1-7. *Pherosphaera hookeriana.*

1. Body cell with stalk and tube nuclei. 2. Young male gametes shortly after division of body cell nucleus. 3. Male gametes in early stage of development but showing marked inequality of size. Stalk and tube nuclei together in front of male complex. 4. Early stage of development of intense staining of chromosomes of gametes. 5. Well-advanced stage in development of gametes. 6. Gametes in mid-stage of development, similar to those shown in Text-fig. 8 and Pl. ix, fig. 5. 7. Typical male gametes. Magnifications, all figures, × 500.

The egg nucleus is not surrounded by a broad sheath of differentiated cytoplasm. In this respect *Pherosphaera* resembles *Saxegothea* (Looby and Doyle, 1939) and presumably also *Dacrydium* (Sinnott, 1913, Pl. 5, fig. 3), and differs from *Podocarpus andinus* (Looby and Doyle, 1944*a*) and *P. spicatus* and *P. dacrydioides* (Sinnott).

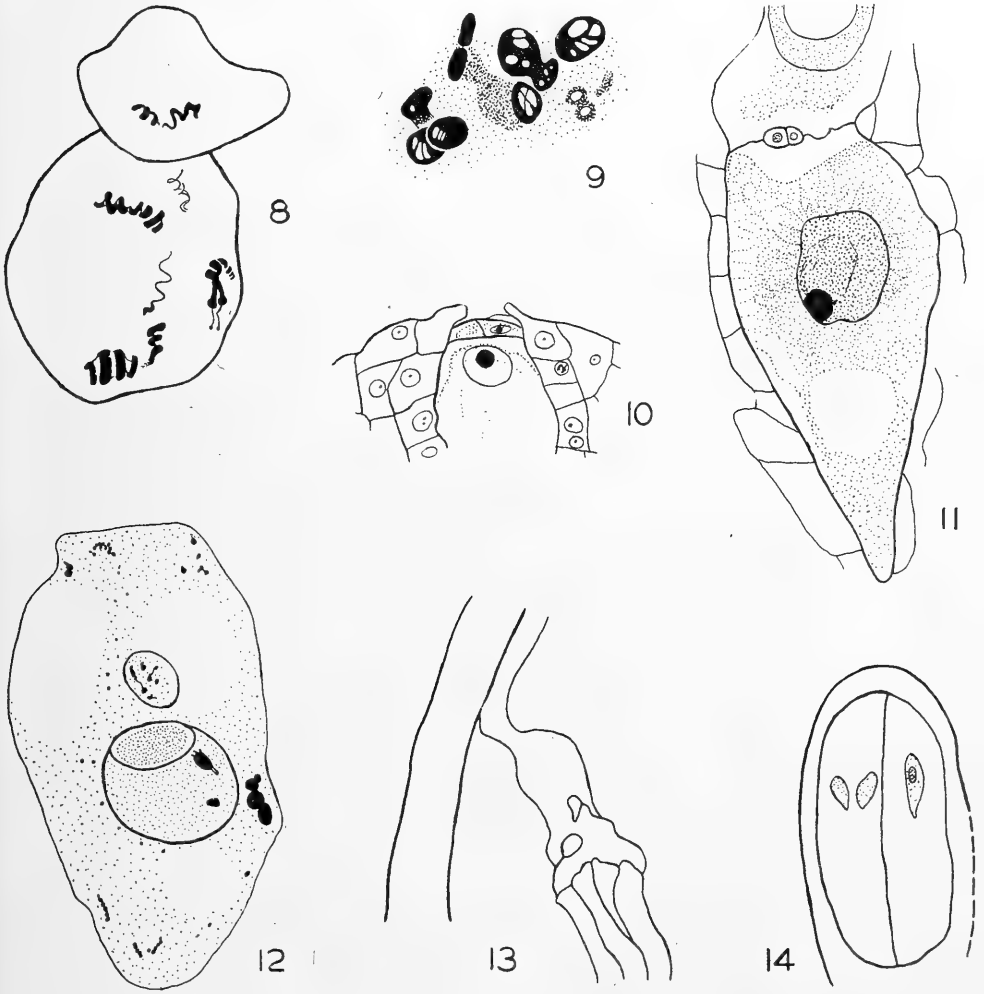
The cells of the prothallus surrounding the archegonium are usually differentiated as a jacket layer (Pl. ix, fig. 10; Pl. x, figs. 13, 16, 17). Archegonia are usually separated from one another by at least one layer of cells, but occasionally two are in direct contact (Pl. ix, fig. 11). In older archegonia, especially unfertilized ones, the outline of the egg nucleus becomes highly irregular.

Relation of Pollen Tube to Archegonium, and Fertilization.

The pollen tubes grow through the nucellus towards the apex of the prothallus (Pl. ix, fig. 4) and spread out over it, passing to the sides. They then grow between

the nucellus and prothallus to approximately the level of the archegonia. Lawson (1923*b*) mentions their being outside the megaspore membrane, but since this is very thin, hardly any thicker than many cell walls in the surrounding tissue, and is often much buckled, it is very difficult to be certain which side of the membrane the pollen tube is on. However, the archegonia are buried at an early stage, and the pollen tube must therefore penetrate some prothallial tissue to reach them. A pollen tube growing in prothallial tissue is shown in Pl. ix, fig. 1. In Text-fig. 13 the pollen tube has grown between the nucellus and prothallus and then inwards to reach the archegonia.

Lawson considered that the pollen tubes "have their full growth before the archegonia are organized" and that their course is "well established before the archegonia



Text-figures 8-14. *Pherosphaera hookeriana.*

8. Some of the chromosomes in male gametes showing coiling and intense staining (× 1,200). 9. Detail of part of sheath of male gamete shown in Pl. ix, fig. 7 (× 1,200). 10. Archegonium prior to ventral canal division, with prothallial tissue just beginning to grow over the neck (× 350). 11. Archegonium with pollen tube above resulting in "suspension" of neck cells. Details from several sections included (× 350). 12. Section of archegonium showing male gamete fusing with egg nucleus, and the second male nucleus just above it (× 350). 13. Course of pollen tube between nucellus and prothallus, thence inwards to neck (ruptured) of archegonium. Top of prosuspensor shown (× 200). 14. Two prothalli side by side in same ovule (× 50).

appear". But there is no evidence of any correlation between pollen tube growth and archegonium initiation, since the differences between the mean number of pollen tubes per ovule for different numbers of archegonia (Table 3) are not significant. When the number of pollen tubes is in excess of the archegonia, all body cells may form gametes, and in one instance as many as three male complexes were found outside the same archegonium; or some pollen tubes may grow very little and remain some distance from any archegonia, and the body cell nucleus does not then divide. But in several cases, where the number of pollen tubes was equal to or less than the number of archegonia, a small body cell was still found high up in the nucellus.

The pollen tubes generally reach the archegonia about the time of the ventral canal division. The pollen tube presses down on the neck, resulting in the "suspension" of the neck cells (Text-fig. 11), but owing to the small size of the archegonia, about two-fifths the diameter of those of *Podocarpus andinus*, this phenomenon is not so striking as in other podocarps. The occurrence of the ventral canal division is not related to the presence of pollen tubes, however, as the structure of the nucleus of old unfertilized archegonia is that of an egg nucleus. The body cell nucleus may divide before or after the ventral canal division, since in one case in which the body cell was dividing, the nuclei of the nearby archegonia had "egg nucleus" structure, while in two others the archegonium nucleus had a large nucleolus and little else visible in it.

TABLE 3.

Numbers of Pollen Tubes in Relation to Number of Archegonia in Sixty-five Ovules of Pherosphaera hookeriana.

Pollen Tubes.	0	1	2	3	4	5	6	7	8	9	10	11	Mean.
Archegonia													
2		1		2									
3	1	5		5	3	1	1					1	3·176
4	1	2	7	10	6	3	1	1					3·161
5		2	6	2	1	1		1	1				3·143

The entry of the male nuclei into the archegonium is always through the neck. The rupture of the neck probably takes place from within. Pl. x, fig. 12, shows cytoplasm protruding from an archegonium with male gametes outside. It does not protrude as far as in *Podocarpus andinus* (Looby and Doyle, 1944*b*). In Pl. x, fig. 13, there is no sign of a pollen tube outside, but the neck is ruptured all the same. In Pl. x, fig. 15, the male nuclei are about to enter the archegonium, and necks through which male cells have entered may be seen in Text-figs. 15 and Pl. x, figs. 14, 17 and 18.

When the functional male nucleus comes in contact with the egg nucleus its chromosomes still stain intensely (Pl. x, fig. 16). Before the membrane separating the two fusing nuclei disappears, however, the male nucleus assumes the appearance of the female nucleus (Pl. x, fig. 17). (One of the male nuclei in Pl. x, fig. 14, is seen to be in contact with the egg nucleus in another section.) Similar phenomena have been described by Haupt (1941) and Allen (1946).

The number of nuclei of the pollen tube which enter the archegonium seems to be variable in such cases where it was possible to identify them with certainty. The second male nucleus generally enters (Text-fig. 12 and Pl. x, fig. 14). It appears to have remained outside in Text-fig. 15. Possibly one of the stalk and tube nuclei has entered the archegonium in Pl. x, fig. 17, but neither has in Pl. x, fig. 14. The latter would appear to be the general rule. The sheath of the male gametes can generally be distinguished at this stage.

DEVELOPMENT OF THE PROEMBRYO.

In its general features the development of the proembryo of *Pherosphaera* resembles that of other podocarps. The first division of the zygote takes place in the centre of

the archegonium, but later free nuclear divisions take place in the lower part, while the cytoplasm in the upper part of the archegonium becomes rather diffuse (Pl. xi, figs. 23–7). Four divisions normally take place, giving sixteen free nuclei. The cytoplasm cleaves into uninucleate protoplasts beginning from below, and this distinguishes what I shall call the *primary embryo units* from a tier above (Text-fig. 18), which divides (Text-figs. 20, 19) to give the prosuspensors and the open cell tier (Text-figs. 21, 22). The nuclei of the primary embryo units divide to give binucleate embryo units (Text-fig. 21). This feature is characteristic of podocarps, but in *Pherosphaera* the binucleate cells, instead of remaining binucleate, immediately proceed to form two-celled units. The binucleate cell phase is of very short duration, and if binucleate embryo units persist, as reported previously (Elliott, 1948), it can only be regarded as highly exceptional.

In the third and fourth nuclear divisions, at metaphase the spindle is surrounded by a clear area (Pl. xi, figs. 25, 27), which is still evident at anaphase on occasions (Pl. xi, fig. 26). By late anaphase (Pl. xii, fig. 28) all appearance of an intranuclear nature has been lost. However, in the first two divisions an intranuclear nature may

TABLE 4.

Numbers of Nuclei (Resting, and up to Prometaphase) in Proembryos of Pherosphaera hookeriana *up to 8-nucleate Stage.*

Large Nuclei in Proembryonic Cytoplasmic Mass.	Extra Small Nuclei within Proembryonic Cytoplasmic Mass.	Extra Small Nuclei Extruded from Proembryo.	Number of Observations.
2	1	—	2
4	—	—	4
4	1	—	1
4	2	—	1
4	3	—	1
4	—	1	1
4	—	2	8
8	—	—	1
9*	—	—	1
8	—	1	2
8	—	2	2
7†	—	2	1
8	—	4	1

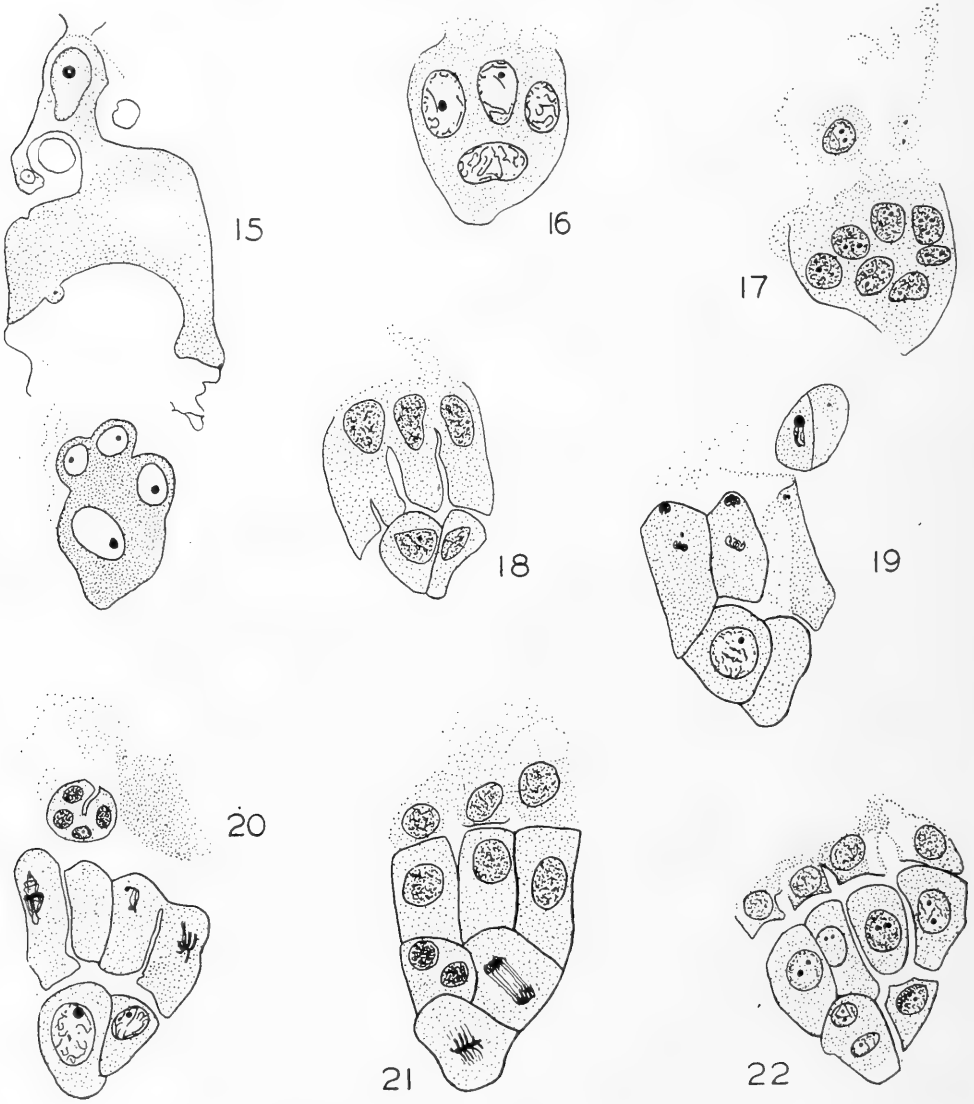
* Nuclei even sized, but not very large.
† Uncertain.

not be apparent even at metaphase (Pl. xi, fig. 22). There is no question here of the great disparity between size of spindle and fusion nucleus visible at the first division in *Podocarpus andinus*. The situation is similar to that in *Sciadopitys* (Tahara, 1937), where the first division is not intranuclear, while in later divisions (his Fig. 9) the chromosomes seem to be surrounded by a clear zone.

The base of the archegonium is not very long and pointed, hence tiering of nuclei is not marked. At the 16-free nuclear stage there are two layers of nuclei well defined, but the lower group may itself be two-tiered. Text-figure 17 is typical. As a result the number of prosuspensors and primary embryo units, and arrangement of the latter, is variable. Sometimes only two primary embryo units are formed; the numbers three and four are most frequent, but only where there are four or more embryo units are they in two tiers. There is no evidence of a "phragmoplast" during wall formation; cell formation is entirely by cleavage of the protoplast, beginning from below.

The division to form the prosuspensor tier takes place before that in the primary embryo units. In Text-figure 20 the upper tier is near metaphase while the nuclei in the embryo units are in prophase. The division in the primary embryo units is less

advanced in Text-figure 19, where the upper tier division is in telophase, and in Pl. xii, fig. 29, the open cell tier has been formed and the nucleus in the embryo units is in prophase. Where there is more than one tier of primary embryo units the division of nuclei in the cells nearer the prosuspensors takes place ahead of that below. In Text-



Text-figures 15-22. *Pherosphaera hookeriana.*

15. Archegonium with pierced neck and proembryo. The second male nucleus is outside the neck. Proembryo contains four large nuclei (two in this section) and three small (two visible) being constricted off. 16. Four of eight free nuclei in prophase of fourth free nuclear division. 17. Sixteen-free nuclear stage, showing a "rosette" above. 18. Proembryo in which primary embryo units are delimited, but division to give prosuspensor and open cell tiers has not yet taken place. 19. Primary embryo unit nucleus in early prophase and upper tier division in telophase. Two-celled "rosette" present. 20. Mid-prophase of division of primary embryo units, and prometaphase of upper tier division. Four-nucleate rosette above. Detail from two sections. 21. Prosuspensor and open cell tiers formed, with stages in formation of binucleate units from primary embryo units. Drawing made up from several adjacent sections. 22. Binucleate embryo unit, prosuspensor and open cell tier. Magnifications: Text-fig. 15, × 350; all others, × 500.

figure 21 the upper cells show a binucleate cell and late anaphase of division, while the terminal cell shows metaphase.

Unfortunately in no case were the two nuclei of a binucleate cell sufficiently in the same plane of focus to make a good photograph possible. Pl. xii, fig. 30, is the best example, though this is a stage in formation of a two-celled unit, as a cleavage plane is being formed across the centre of the cell. In Pl. xii, fig. 32, two two-celled units may be seen.

Although Tahara's (1937) account of the proembryo of *Sciadopitys* is not as clear as could be desired, there are some features which seem to be similar to those in *Pherosphaera*. After the fifth division, wall formation is confined to the lower parts (cf. *Pherosphaera*, Text-fig. 18) with relict nuclei above; and the sixth division consists of simultaneous formation of prosuspensor and open cell tier above and two-celled units below. In *Pherosphaera* these two processes are not so closely synchronized. Tahara's Figure 11 shows prophase in several primary embryo units, in the tier which gives rise to prosuspensor and open cell tier, and in several relict nuclei above.

The Origin of "Rosette" Embryos.

"Rosette" embryos are of general occurrence in *Pherosphaera* (Elliott, 1948). It is unfortunate that this term has to be used, for the embryos here referred to are in no way comparable with those of certain Pinaceae. However, a better name has yet to be thought of.

In the free nuclear stages it would be expected that the numbers of nuclei found would be 2, 4, 8 and 16. In *Pherosphaera* other numbers, such as 3, 6, 7 and 9, also occur.

In Text-figure 15 seven nuclei, of which four are seen in the section drawn, are found in the denser protoplasm at the base of the archegonium—four of them large, and three much smaller. The smaller ones appear in the process of being constricted off. Plate xi, figs. 23 and 24, are two sections through the same proembryo in which there are four large nuclei (three visible in fig. 23 and the fourth in fig. 24) and a small one (fig. 24) already cut off from the main embryonic mass of cytoplasm. The difference in size is significant. The observations of proembryo nuclei up to the eight-nucleate stage are set out in Table 4.

Supernumerary spindles have been found in several archegonia (Table 5). A series of three spindles is shown in Pl. x, figs. 19, 20, 21. In all such cases two have been

Table 5.

Spindles (Metaphase and Anaphase) Seen in Proembryonic Cytoplasmic Mass in Pherosphaera hookeriana.

Division Number, and Number of Spindles Expected.	Number of Observations.		
	Expected Number of Spindles Only.	One Extra Spindle.	Two Extra Spindles.
1 (1)	1		
2 (2)	1	3	
3 (4)	1	1	1
4 (8)	4	—	—

found to be much larger than the other. It has not been possible to count exactly the number of chromosomes on each, but the evidence is very suggestive that two spindles are diploid (Pl. x, figs. 19, 20) and one haploid (fig. 21). On this presumption, the small nuclei in Text-figure 15 and Pl. xi, fig. 24 may be considered haploid. Their origin is apparently from the non-functional male gamete, or one of the other male nuclei, or possibly from one of the cells of the female gametophyte which happen to

enter the archegonium: the cells of the jacket layer about the time of the first division of the zygote appear indistinct from the archegonium, and their nuclei divide synchronously with the first two free nuclear divisions. However, in no case did the supernumerary male nuclei appear to be about to divide. Few studies have been made of the fate of the supernumerary male nuclei, but Allen (1946) found in *Pseudotsuga* that, though they sometimes may divide, they remain in the upper part of the archegonium. Their identification is always difficult, since at this time numerous bodies of nucleus-like appearance (as stained with haematoxylin) are found in the archegonium (cf. Looby and Doyle, 1939, p. 112).

Thus in the proembryo of *Pherosphaera* the nuclei derived from the zygote are frequently accompanied by smaller nuclei, which are probably haploid and therefore of gametophytic origin, although this has not been definitely ascertained. These small nuclei divide synchronously with those of the embryo while they are in a common cytoplasmic mass, but later they are separated off from the proembryo and lead an independent existence in the upper part of the archegonium, and their divisions generally cease to be synchronous.

Relict nuclei (diploid nuclei from the zygote which do not contribute to the cellular proembryo) occur very rarely, if at all, in *Pherosphaera*. Where it is possible to count the number of nuclei with certainty, as it is up to the eight-nucleate stage, it appears that no nucleus from the zygote (so determined by its large size, especially towards prophase of the next division) is constricted off from the cytoplasm containing the proembryo. Eight spindles only occur in the dense cytoplasm in every case where this fourth division was observed, and though in the 16-nucleate stage it is generally too difficult to count the nuclei to be quite sure about the presence of relicts, counts, when certain, of the numbers of prosuspensors and primary embryo units indicate that all 16 nuclei had taken part in the formation of these structures. In two cases, however, only seven prosuspensors and one embryo unit could be found, and since rosettes were absent in these proembryos, only three instead of four free nuclear divisions could have taken place. In Text-figure 17 the nucleus by itself looks very like those in the protoplast below it, and it may be a relict.

It is unlikely that any rosette embryos come from the open cell tier, as these nuclei appear to degenerate very quickly. On the other hand, nuclei resembling the male nuclei may be found in the archegonium at a late stage (e.g., Pl. xii, fig. 31, cf. Pl. x, fig. 15).

Some Features of the "Early" Embryo.

The main features of the development of the embryo have already been described (Elliott, 1948). The prosuspensor elongates and pushes the two-celled units some distance into the prothallial tissue before any further development takes place. Food reserves are built up in the prothallial cells as soon as the prosuspensor begins to elongate. Starch, however, is confined to the central zone of tissue, as in *Saxegothea* (Doyle and Looby, 1939, p. 132). Each two-celled unit develops as an independent embryo which produces embryonal tubes. The development is a good example of determinate cleavage polyembryony.

It was recorded that developing embryos regularly contain binucleate cells, quite apart from the binucleate embryo unit phase. In Pl. xii, fig. 35, three embryos are seen. The nuclear division in the cell marked with an arrow is at telophase, but there is no trace of a wall being formed across the equator of the spindle, which is itself scarcely evident. Pl. xii, figs. 33 and 34 (two photographs of the same section in different planes of focus) show two embryos. Each of the two cells in the right-hand embryo is binucleate. In the right-hand cell of this embryo, however, a furrow is being formed on the outside of the protoplast, but has not yet reached the centre. In fig. 33 the furrow appears as a definite cleavage plane right across the centre of the cell. In fig. 34, in the plane of focus of one of the nuclei, the furrow is not nearly so evident, and does not extend between the nuclei. The fixative used would tend to enlarge, but hardly to create, such furrows, and I regard them as significant and

tangible evidence of cleavage plane formation. Moreover, the evidence goes to show that similar cleavage planes develop in the binucleate cells leading to two-celled units, just as in *Podocarpus andinus* (Looby and Doyle, 1944*b*) leading to four-celled units from a quadrinucleate protoplast.

It is of interest to record that during the elongation of the prosuspensor the two-celled units pass through a stage during which they are difficult to fix and stain well. They emerge from this condition before further development takes place in the embryo units, the lower embryo units emerging before those nearer the prosuspensor. A similar phenomenon has been described for the binucleate embryo units in *Podocarpus andinus* (Looby and Doyle, 1944*b*, p. 261).

Discussion.

In some features of its life history described in this paper *Pherosphaera hookeriana* shows close similarities to other well-known podocarps, as well as considerable differences. The structure of the body cell, the burial of the neck of the archegonium at an early stage, and the general features of embryogeny up to the binuclate stage are features shared with other podocarps. On the other hand, the form of the male gametes and the presence of two-celled embryo units, as well as the absence of male prothallial cells (Lawson, 1923*b*) are important differences. *Pherosphaera* also has unique epidermal structures (Florin, 1931).

I previously suggested that in respect of cone morphology *Pherosphaera* has close affinities with *Dacrydium*; and I would now stress what I observed before, that we can hardly estimate the significance of what we now know about *Pherosphaera* until more is known about the life history of *Dacrydium*. Little enough is known of *D. cupressinum*, and in leaf form it differs widely from the group including *D. bidwillii* and *D. franklinii*, which in external appearance at least bear a closer resemblance to *Pherosphaera hookeriana*. The present paper, though by no means an exhaustive account, can provide some more substantial basis for comparison when the life history of *Dacrydium* is better understood.

Summary.

Comparisons of the numbers of fertile ovules, stage of abortion of unpollinated ovules, and randomness of distribution of fertile ovules in three collections of *Pherosphaera hokeriana* suggest that severity of season affects fertility, while in sheltered situations, as contrasted with exposed, unpollinated ovules grow large and ovules are pollinated at random.

The male nuclei are usually unequal in size and the smaller one is extruded from the protoplast that contained them, but is not delimited therefrom by a membrane. During development of the male gamete relic coiling of the chromosomes is apparent. The chromosomes increase greatly in their intensity of staining with haematoxylin, the length of chromosome so staining developing progressively.

The archegonia are buried at an early stage before pollen tubes reach them. In such features as the number of archegonia, and the nuclear divisions in them, the female gametophyte is independent of the stage of development of the male gametophyte.

The development of the proembryo up to the binucleate stage resembles that in other podocarps, but the binucleate cells immediately form two-celled units.

Rosette embryos probably arise from haploid nuclei which occur in the protoplast with diploid nuclei, but from which they are constricted off. Diploid relict nuclei have not been observed.

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EXPLANATION OF PLATES.

PLATE IX.

1. Section of ovule showing integuments, micropyle, nucellus, and prothallus with archegonium and pollen tube. × 50.

2. Body cell with stalk nucleus. × 400.

3. Division of body cell nucleus. × 400.

4. Pollen tube growing through nucellus, showing body cell and tube nucleus. × 300.

5. Coiled and heavily staining chromosomes in young male gamete. Section adjacent to that shown in Text-figure 8. × 1,000.

6. Male complex with peculiar sheath. × 400.

7. Typical male gametes in mid-stage of development. × 400.

8. Mature male gamete. × 400.

9. Young archegonium with buried neck. Small arrows (top and bottom left) indicate boundary between prothallus and nucellus. × 300.

10. Archegonium with ventral canal nucleus, showing buried neck cells. × 300.

11. Two archegonia in direct contact, showing characteristic appearance of mature egg nucleus. × 300.

PLATE X.

12. Archegonium with cytoplasm protruding through neck. Male gametes above, the smaller nucleus in focus. × 300.

13. Archegonium showing egg nucleus and jacket cells, with cytoplasm protruding from neck, but no male complex outside. × 300.

14. Two male nuclei inside archegonium (one of them in contact with egg nucleus in another section), ruptured neck through which they entered, and stalk and tube nuclei outside. × 300.

15. Male gametes about to enter archegonium through neck; functional gamete out of focus, nucleolus of second male nucleus visible. × 300.

16. Male nucleus just in contact with egg nucleus. × 300.

17. Later stage of fusion of nuclei. Two other male nuclei visible in archegonium above. × 300.

18. Archegonium in which second proembryo division is taking place. Shows neck through which male gametes entered. × 300.

19, 20, 21. Three spindles visible in single proembryo. Those in 19 and 20 presumed diploid, the smaller in 21, haploid. × 1,000.

PLATE XI.

22. First division of fusion nucleus. × 1,000.

23, 24. Four free nuclei; three of them in 23 and the fourth in 24, which also shows small nucleus forming rosette. × 400.

25. Metaphase of second free nuclear division. × 400.

26. Anaphase of third free nuclear division. × 400.

27. Late prophase of fourth free nuclear division, with a "rosette" in which two nuclei are dividing. × 400.

PLATE XII.

28. Late anaphase of fourth free nuclear division. × 400.

29. Primary embryo unit (nucleus in prophase), prosuspensors and open cell tier, with division of nucleus in a "rosette" above. × 400.

30. Proembryo showing binucleate unit in process of conversion into two-celled unit. × 500.

31. Supernumerary nuclei in archegonium with prosuspensors below. × 400.

32. Proembryo with two-celled units. × 400.

33, 34. Two views of embryo in different planes of focus, showing formation of cleavage plane in binucleate cell. × 850.

35. Embryos at tip of elongating prosuspensor. That marked by arrow shows absence of cell plate formation, leading to binucleate cell. × 485.

A NEW GENUS AND FOUR NEW SPECIES OF PHLOECHARINAE (COLEOPTERA, STAPHYLINIDAE) FROM THE AUSTRALIAN REGION.

By W. O. Steel.

(*Communicated by J. W. T. Armstrong.*)

(Twenty-nine Text-figures.)

[Read 25th October, 1950.]

Introduction.

Up to the present three species of Phloecharinae have been described from the Australian region, all of them being placed in the genus *Phloecharis*; these are *P. antipodum* Fauvel from Australia, *P. maori* Cameron from New Zealand, and *P. australis* Fauvel from New Caledonia. The first two of these, and presumably also the third (see below), differ, particularly in the form of the maxillary palpi, from true *Phloecharis* and cannot be retained therein. A new genus is necessary for these species and the description of this, together with those of four new species, is given below.

No specimens of *P. australis* have been available for study, but there seems to be no reason why this should not be congeneric with the Australian species. Bernhauer (Col. Cat. 129, 1933, 1024) records this species as occurring in Queensland, though no other reference to this has been found. There are, however, four specimens in the British Museum of Natural History from Queensland which bear Bernhauer's label "*Phloecharis australis* Fauv.", and these would appear to be the insects on which the Australian record is based. These specimens are referable to *P. maori* Cam., with which, from Fauvel's description (Rev. d'Ent., 21, 1902, 257), *australis* could well be conspecific. Against this contention, however, there is the fact that Fauvel knew the New Zealand species, to which he gave the manuscript name *maori*, besides implying (l.c.p. 257) that it was different from *autstralis*. It appears advisable, therefore, not to synonymize *australis* and *maori* until such time as authentic *australis* can be examined and also, until then, to delete this species from the Australian list.

P. maori, though known to Fauvel from New Zealand and described by Cameron from that country, appears to be very rare there. It is shown here, however, to be widely distributed and apparently common in Australia, and it would appear, therefore, that the species is an Australian one which has, by some means or other, spread to New Zealand.

The figures illustrating this paper have all been drawn with the aid of a camera lucida. Those of the mouth-parts and aedeagi are from Euparal mounts and the rest from dried specimens. The aedeagi and mouth-parts are figured as transparencies, that is to say, internal chitinizations, etc., are shown, but in the case of the former, as the unextruded internal sac shows no features of taxonomic significance, this is omitted in all cases. The figures illustrating surface sculpture were made using a vertical illuminator.

I am greatly indebted to Mr. H. M. Hale and Mr. H. Womersley, of the South Australian Museum, for making available to me a large amount of material, together with Mr. A. M. Lea's notes on the specimens. Thanks are also due to Mr. J. Balfour-Browne, of the British Museum of Natural History, for facilities for using the collections there, and to Messrs. J. W. T. Armstrong and K. M. Guichard.

Genus PSEUDOPHLOECHARIS, gen. nov.

In general facies, very similar to *Phloecharis* Mann.

Head narrower than the pronotum, about as long as broad (excluding eyes), the post-ocular portion (about posterior third) normally retracted into the thorax. The

eyes about one-third the total length of the head, rounded and rather prominent, so that the visible portion of the head appears more or less triangular (Text-figures 12–18). Posterior head suture* distinct on dorsal surface, almost absent on the ventral. Ventral head sutures well separated, converging from base to about the level of the middle of the eyes, then diverging towards front; distinct throughout their whole length.

Antennae with all the segments sparsely setose, the seventh to eleventh also with short pubescence.

Labrum (Text-figure 3) transverse, the front margin very lightly rounded, the anterior angles broadly rounded; the surface with a few punctures bearing long setae. Mandibles (Text-figures 2, 4) rather short and stout, pointed apically, the right with a small rounded tooth at about middle internally, the left internally with two very fine tubercles near apex and a third often absent, presumably abraded at about middle. Both just behind middle internally with a membranous area which bears a fringe of very fine, short setae; below this, at base, with a small file-like molar area. Inner lobe of maxillae (Text-figure 1) broader than the outer, at apex with numerous rather stout setae and, externally, a curved pointed spine. The outer with a brush of fine setae apically. Maxillary palpi with the first segment very small, a little longer than broad, the second much longer and broader than the first, widened apically, the third widened apically, shorter than the second, the fourth distinctly longer than and as broad as the third, elongate pyriform. All the segments with numerous setae. Mentum distinctly transverse, trapezoidal, narrowed in front. Glossae not distinguishable from the paraglossae, the ligula consisting of two lobes which are fused except in front and which bear one or two fine setae. Lobes of the hypopharynx well developed, densely setose internally. Labial palpi with the first segment distinctly longer than broad, the second much shorter and a little narrower, slightly transverse, the third narrower than and distinctly longer than the second.

Pronotum transverse, variable in shape. Prosternal process narrow and pointed, extending backwards between the anterior coxae for about one-third of their length. Mesosternal process narrow, rounded apically, extending backwards between the intermediate coxae for about three-quarters of their length and slightly overlapping the short, bluntly pointed metasternal process. Metasternum variable in length, without a process posteriorly. Elytra variable in length, the wings sometimes atrophied.

Third to sixth, first to third visible abdominal segments about equal in length, the seventh and eighth distinctly longer. Sternite of the third segment with a median longitudinal keel on basal half. Tergites of segments four and five on each side of middle, besides the normal puncturation and pubescence, with a diagonal row of fine, strongly curved setae. Posterior margin of the tergite of the seventh segment with a fine, whitish membranous fringe (even in the brachypterous species).

Posterior coxae transverse, strongly expanded laterally above dorsal to the femora, also with a small expansion below ventral to the trochanter. Tibiae finely and rather closely setose, with a few stouter setae on each side at apex. Tarsi about two-thirds as long as their respective tibiae, the second segment about twice as long as broad, inserted almost at the base of the first, which, from above, appears very short. It, however, extends below the second and is nearly as long as this (as in *Phloecharis*). The third segment distinctly shorter than the second, the fourth a little shorter than the third, the fifth much longer, about as long as the four preceding together.

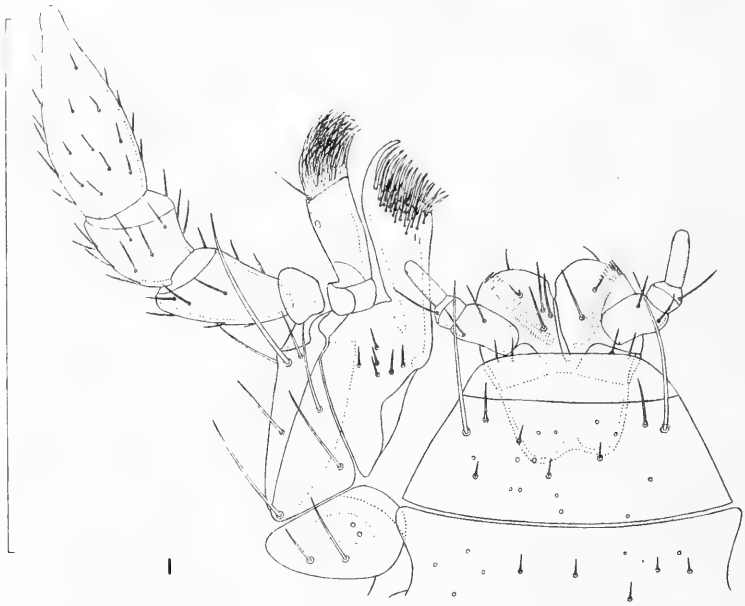
Genotype: *Phloecharis maori* Cameron.

Range: Australia, New Zealand and (presumably) New Caledonia.

Little is known as to the habits of the species of this genus, but they would appear to be similar to those of *Phloccharis* spp. The larvae are as yet unknown.

This genus differs from *Phloecharis* Mann. mainly in the structure of the maxillary palpi and ligula. In this latter genus the second segment of the maxillary palpi is longer and distinctly narrower, the third is a little longer than the second and much wider, while the fourth is much shorter than the third and less than half as wide,

* See Steel, *Ent. Mon. Mag.*, 85, 1949, 232.



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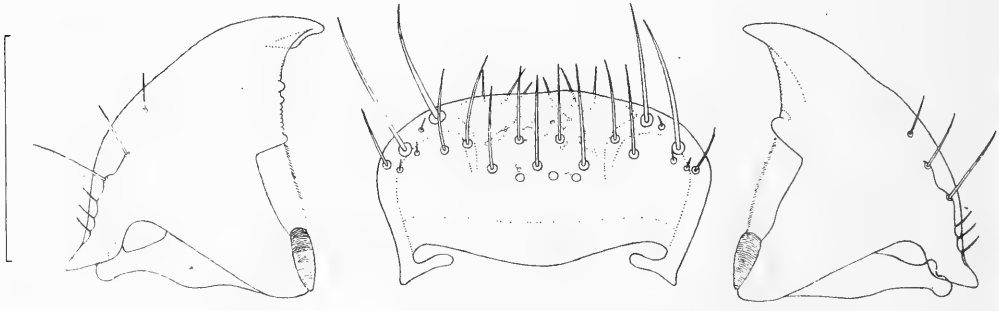

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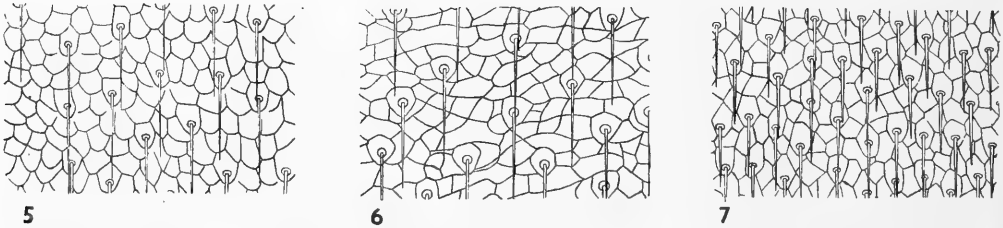

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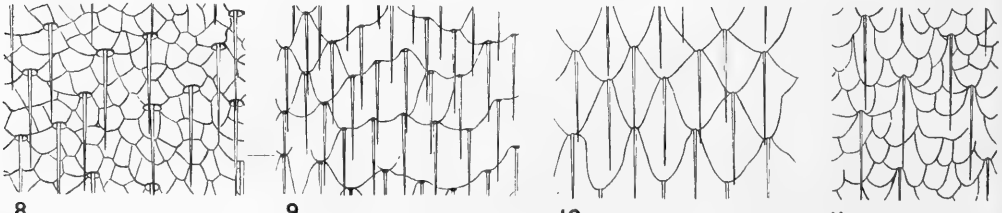

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Text-figures 1-11.

1. *Pseudophloecharis maori* (Cam.), maxillae and labium (scale = 0·2 mm.). 2. *Pseudophloecharis maori* (Cam.), left mandible (scale = 0·1 mm.). 3. *Pseudophloecharis maori* (Cam.), labrum (scale as 1). 4. *Pseudophloecharis maori* (Cam.), right mandible (scale as 2).

bluntly pointed apically, with the sides almost straight. The ligula has the glossae and paraglossae distinct, the former extending forwards some distance beyond the latter.

Besides the above, there are also some more obscure differences, at least from *Phloecharis subtilissima* Mann. (the only species of *Phloecharis* which has been available for dissection). In this species the scale-like setae on the ventral surface of the labrum are rather numerous and all small, whereas in *Pseudophloecharis* spp. these are distinctly fewer in number and the posterior two are about twice as large as the rest. These setae are shown by dotted lines in Text-figure 3. The left mandible of *P. subtilissima* has a small bifid tooth at about middle which is absent in *Pseudophloecharis*.

The ground sculpture of the head, pronotum and elytra varies somewhat in *Pseudophloecharis*, but that of the abdomen is more or less constant for all the species dealt with here. The tergites are finely and rather closely punctured and pubescent, the ground sculpture of those of the third to sixth (first to fourth visible) segments is more or less reticulate (Text-figure 10, though not always as regular as this), whereas on those of the seventh and eighth it is distinctly closer and alutaceous (Text-figure 11). The sternites are sculptured as the tergites.

KEY TO THE KNOWN SPECIES OF PSEUDOPHLOECHARIS.

1. Sutural length of elytra shorter than the pronotum ..... *brachyptera*, n. sp.
   Sutural length of elytra distinctly longer than the pronotum 2
2. Sides of the pronotum sinuate before the posterior angles 3
   Sides of the pronotum not sinuate before the posterior angles 4
3. Sinuation of the pronotal sides very marked (Text-fig. 13), elytra shorter in relation to the pronotum (pronotum : sutural length about 1 : 1·2) ..... 2. *sinuatum*, n. sp.
   Sinuation of the pronotal sides less marked (Text-fig. 14), elytra longer in relation to the pronotum (pronotum : sutural length about 1 : 1·4) ..... 3. *antipodum* (Fvl.)
4. Head, elytra and abdomen black or blackish, the pronotum lighter. Disc of pronotum rather flattened ..... 4. *fuscum*, n. sp.
   Colour yellowish-brown to brown, pronotum convex 5
5. Puncturation of pronotum very close, as fine as that of the abdominal tergites. Pronotum distinctly more narrowed in front than behind (Text-fig. 18). Aedeagus as Text-figures 27, 28 ..... 6. *maori* (Cam.)
   Puncturation of pronotum less close, coarser than that of the abdominal tergites. Pronotum (Text-figs. 16, 17) rather variable in shape, but less narrowed in front than in the preceding species. Aedeagus as Text-figures 25, 26 ..... 5. *occidentalis*, n. sp.

1. PSEUDOPHLOECHARIS BRACHYPTERA, n. sp. (Text-figures 5, 8, 12, 19–20).

Rather flattened. Dorsal surface of head yellowish red to pitchy red, pronotum and abdominal tergites yellowish red, elytra yellowish red with about the basal fourth and a narrow area along the suture black. Mandibles, palpi and legs yellowish red, antennae with the first three segments yellowish red, the rest pitchy. Ventral surface wholly yellowish red. Pubescence yellowish.

Length: 2·25–2·5 mm.

Tergites of abdomen moderately shining, the head, pronotum and elytra less so, greasy lustrous. Head distinctly, but not very closely punctured and pubescent, the punctures coarser than those on the abdomen; between the punctures with a well marked ground sculpture similar to that on the pronotum. Antennae with the third segment about as long as the second, the fourth and fifth subequal in length, longer than broad, the sixth to tenth about equal in length, but increasing gradually in breadth, the sixth a little transverse, the tenth rather strongly so, about twice as broad as long, the eleventh distinctly longer, as broad as and about twice as long as the tenth, bluntly pointed apically.

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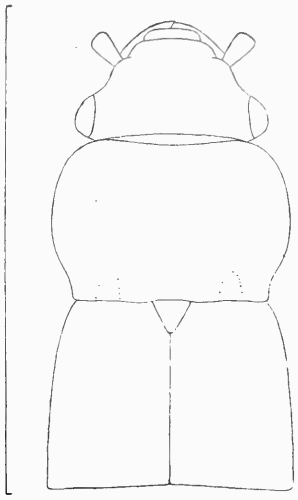
5. *P. brachyptera*, n. sp., ground sculpture of pronotum (× 225). 6. *P. sinuatum*, n. sp., ground sculpture of pronotum (× 225). 7. *P. maori* (Cam.), ground sculpture of pronotum (× 225). 8. *P. brachyptera*, n. sp., ground sculpture of elytra (× 225). 9. *P. maori* (Cam.), ground sculpture of elytra (× 225). 10. *P. antipodum* (Fvl.), ground sculpture of tergite of fourth abdominal segment (× 225). 11. *P. antipodum* (Fvl.), ground sculpture of tergite of seventh abdominal segment (× 225).

Pronotum about one and one-half times as broad as long, broadest at about middle, the sides rounded in front, distinctly sinuate behind, the anterior angles rounded, the posterior scarcely rounded, subrectangular, the anterior margin lightly emarginate, the posterior lightly sinuate. Puncturation and pubescence similar to that on head, but distinctly closer; between the punctures with a well-marked, more or less regular coriaceous ground sculpture (Text-fig. 5). On each side, at base, with a shallow impression.

Elytra very short, about one and one-quarter times as broad as long, the sutural length a little shorter than the pronotum. Puncturation and pubescence as close as that on the pronotum, but the punctures a little finer and somewhat raised. Ground sculpture well marked and more or less regular (Text-fig. 8).



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Text-figure 12. *P. brachyptera*, n. sp., head, thorax and elytra (scale = 1 mm.).

Tergites of abdomen with puncturation and pubescence a little closer than that of the pronotum, the punctures somewhat finer. Ground sculpture as noted above (see generic description).

Aedeagus as Text-figures 19–20.

Victoria: Dividing Range (ex coll. Blackburn).

Holotype (♂) and allotype in the South Australian Museum; no further specimens seen.

This species may be easily distinguished from all the others dealt with here by the short elytra. In these it is similar to *Phloecharis* spp. of the subgenus *Scotodytes* Saulcy, but, apart from the generic characters, differs in the normally developed eyes.

2. PSEUDOPHLOECHARIS SINUATUM, n. sp. (Text-figures 6, 13, 21–22).

Rather flattened. Dorsal surface of head blackish red to black, pronotum red, elytra red, with a black, triangular, basal area, tergites of abdomen blackish except apically, where they are reddish. Antennae, mandibles, palpi and legs yellowish red. Ventral surface of head and prosternum red, meso- and metasternum blackish, abdominal sternites reddish. Pubescence yellowish.

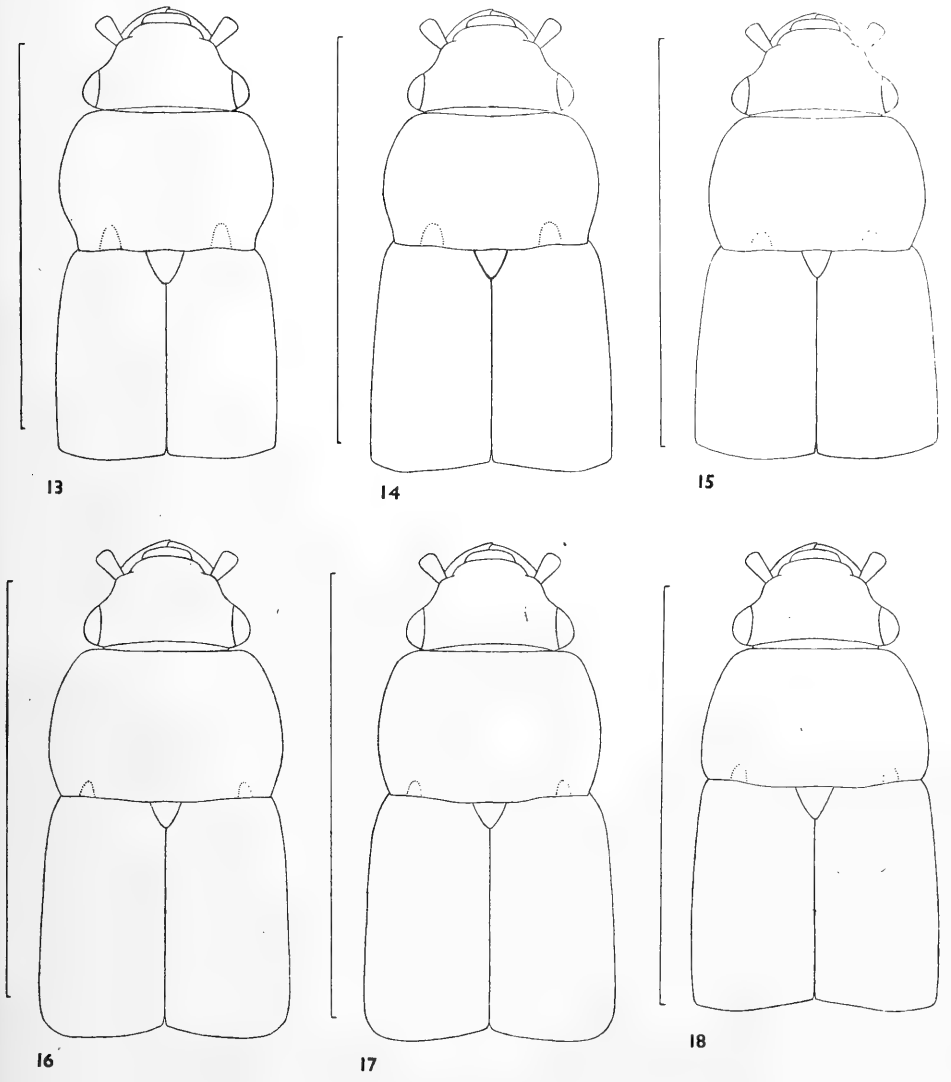
Length: 2·5–3 mm.

Greasy lustrous, the abdominal tergites a little more shining. Head distinctly, but not closely punctured and pubescent, the punctures coarser than those on the abdomen; between the punctures with a ground sculpture similar to that on the pronotum, but a little less marked. Antennae with the third segment about as long as the second, the fourth and fifth about equal in length, longer than broad, the sixth to tenth about equal in length, but gradually becoming broader, the sixth hardly transverse, the tenth trans-

verse, less than twice as broad as long, the eleventh distinctly longer, nearly twice as long as the tenth, bluntly pointed at apex.

Pronotum about one and one-half times as broad as long, broadest at about middle, the sides rounded in front, distinctly sinuate behind, the anterior angles rounded, the posterior subrectangular, hardly rounded, the anterior margin lightly emarginate, the posterior lightly sinuate. Puncturation and pubescence similar to that on head, but distinctly closer; between the punctures with a well-marked, rather irregular, coriaceous ground sculpture (Text-fig. 6). On each side, at base, with a shallow impression.

Elytra about as broad as long, the sutural length about one and one-quarter times as long as the pronotum. Puncturation and pubescence as close as that on the pronotum, but the punctures somewhat finer. Ground sculpture well marked and more or less regular (as *brachyptera*, Text-fig. 8).



Text-figures 13-18.

Head, pronotum and elytra of: 13. *P. sinuatum*, n. sp. 14. *P. antipodum* (Fvl.). 15. *P. fuscum*, n. sp. 16-17. *P. occidentalis*, n. sp. 18. *P. maori* (Cam.). The scale, in each case, = 1 mm.

Tergites of abdomen with puncturation and pubescence a little closer than that of the pronotum, the punctures finer. Ground sculpture as noted above (see generic description).

Aedeagus as Text-figures 21–22.

Victoria (Blackburn): Holotype.

Tasmania: Hobart, allotype; Mt. Wellington (Lea).

Holotype (♂) and one paratype (♂) in the South Australian Museum, allotype in the British Museum (Nat. Hist.).

This species is in some ways very close to *brachyptera*, the pronotum being practically identical in form and the puncturation very similar. It may, however, be easily distinguished from this by the longer elytra and the form of the aedeagus. From the other species of the genus it is easily separated by the form of the pronotum.

3. PSEUDOPHLOECHARIS ANTIPODUM (Fauvel) (Text-figures 10–11, 14, 23–24).

Phloecharis antipodum Fauvel, Ann. Mus. Civ. Gen., 13, 1878, 483. Blackburn, Trans. Roy. Soc. S. Aust., 1888, 191.

Rather flattened. Dorsal surface of head reddish to black, pronotum and abdomen red, the latter sometimes darker, elytra red, with a basal triangular mark blackish. Mandibles, antennae, palpi and legs yellowish red. Ventral surface red. Pubescence yellowish.

Length: 2–2·5 mm.

Greasy lustrous, the abdominal tergites slightly more shining. Head distinctly, but not very closely, punctured and pubescent, the punctures coarser than those on the abdomen; ground sculpture similar to that on the pronotum, but a little less marked. Antennae similar to those of *sinuatum*, q.v.

Pronotum a little more than one and one-half times as broad as long, broadest at about middle, the sides rounded, slightly sinuate before the posterior angles, which are subrectangular and not rounded, the anterior angles rounded, the anterior margin lightly emarginate, the posterior lightly sinuate. Puncturation and pubescence similar to that on the head, but distinctly closer; the ground sculpture well marked, very similar to that of *brachyptera* (Text-fig. 5). On each side, at base, with a shallow impression.

Elytra very slightly broader than long, the sutural length a little more than one and one-third times as long as the pronotum. Puncturation and pubescence as close as that on the pronotum, but the punctures somewhat finer. Ground sculpture distinct and more or less regular (as *brachyptera*, Text-fig. 8).

Tergites of abdomen with puncturation and pubescence a little closer than that on the pronotum, the punctures finer. Ground sculpture as noted above (see generic description).

Aedeagus as Text-figures 23–24.

Type Locality: Western Australia. Type in the British Museum (Nat. Hist.).

Specimens examined: 5, the type in the British Museum collection and four specimens from the South Australian Museum.

Records:

Western Australia.

South Australia: Lucindale with *Rhytidoponera forelli* Craw. (Lea); Adelaide (Blackburn).

New South Wales: Forest Reefs (Lea); Glen Innes (Lea).

In the shape of the pronotum this species is intermediate between *sinuatum* and *fuscum*. The aedeagus is, however, quite different from either of these, being longer in relation to its breadth than in any other known species of the genus.

4. PSEUDOPHLOECHARIS FUSCUM, n. sp. (Text-figures 15, 29).

Rather flattened. Dorsal surface of head black, pronotum blackish red to black, sometimes lighter (? immature), elytra black, sometimes reddish at apex, abdominal tergites black with the apices reddish. Mandibles brown, palpi and legs yellowish brown. Antennae brown with the basal segments yellowish brown. Ventral surface black with the apices of the abdominal sternites reddish. Pubescence yellowish.

Length: 2 mm.

Head and pronotum greasy lustrous, the elytra and abdomen a little more shining. Head distinctly, but not very closely, punctured and pubescent, the punctures coarser than those on the abdomen; ground sculpture similar to that on the pronotum. Antennae very similar to those of *sinuatum*, q.v.

Pronotum nearly one and two-thirds times as broad as long, broadest a little behind middle, not sinuate before the posterior angles, the sides rounded, the anterior angles rounded, the posterior angles scarcely rounded, obtuse, the anterior margin lightly emarginate, the posterior lightly sinuate. Puncturation and pubescence similar to that on the head, but closer; the ground sculpture well marked, more or less regular, similar to that of *brachyptera* (Text-figure 5). On each side, at base, with a shallow impression.

Elytra very slightly broader than long, the sutural length a little more than one and one-third times as long as the pronotum. Puncturation and pubescence as close as that on the pronotum, the punctures a little finer. Ground sculpture distinct, similar to that of *maori* (Text-fig. 9).

Tergites of abdomen with puncturation and pubescence a little closer than that on the pronotum, the punctures finer. Ground sculpture as noted above (see generic description).

Aedeagus as Text-figure 29.

South Australia: Port Lincoln (Blackburn), holotype; Myoponga (A. H. Elston).
Victoria (Blackburn): Allotype; Glenferrie (K. M. Guichard).
Holotype (♂), allotype and one paratype in the South Australian Museum, one paratype in the British Museum (Nat. Hist.) and one paratype in my collection.

In puncturation and general facies this species is very close to *antipodum* (Fvl.). It differs, however, in the colour, non-sinuate pronotum, the ground sculpture of the elytra and the aedeagus. The ground sculpture of the elytra of *fuscum* and *maori* is distinct from that of all the other species in that it is reticulate instead of alutaceous (cf. Text-figs. 8 and 9).

5. PSEUDOPHLOECHARIS OCCIDENTALIS, n. sp. (Text-figures 16–17, 25–26).

Rather convex, the disc of the pronotum scarcely depressed. Dorsal surface lighter or darker brown, the head and/or the elytra sometimes darker. Mandibles, palpi and legs yellowish-brown, antennae with the basal segments yellowish brown, the remainder darker. Ventral surface lighter or darker brown. Pubescence yellowish.

Length: 2–2·5 mm.

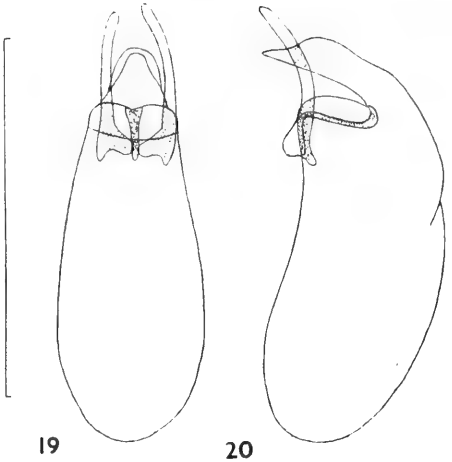
Tergites of abdomen moderately shining, the remainder less so, greasy lustrous. Head distinctly, though not very closely, punctured and pubescent, the punctures coarser than those on the abdomen; between the punctures with a ground sculpture similar to that on the pronotum. Antennae very similar to those of *sinuatum*, but with the penultimate segments a little less transverse.

Pronotum rather variable in shape, from one and one-half to one and five-eighths times as broad as long, broadest at or a little behind middle, the sides rounded, the anterior angles rounded, the posterior scarcely so, obtuse, the anterior margin lightly emarginate, the posterior lightly sinuate. Puncturation and pubescence similar to that on the head, but distinctly closer; ground sculpture more or less regular, similar to that of *brachyptera* (Text-fig. 5). On each side, at base, with a shallow impression, which is very indistinct and sometimes almost absent.

Elytra very slightly broader than long, the sutural length about one and one-third times as long as the pronotum. Puncturation and pubescence as close as that on the pronotum, the punctures a little finer. Ground sculpture more or less regular (as *brachyptera*, Text-fig. 8).

Tergites of abdomen with puncturation and pubescence slightly closer than that of the pronotum, the punctures a little finer. Ground sculpture as noted above (see generic description).

Aedeagus as Text-figures 25–26.



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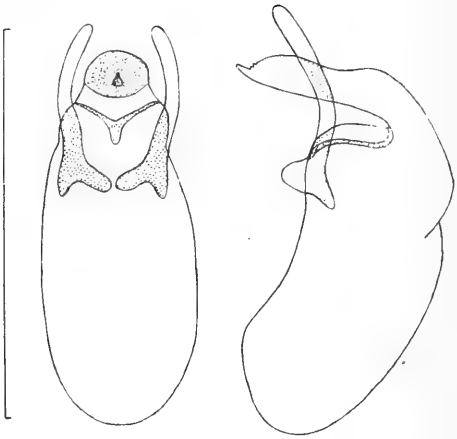

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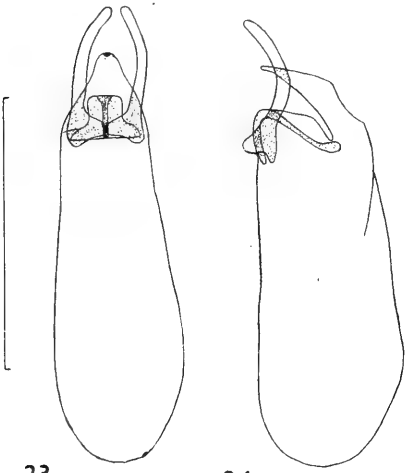

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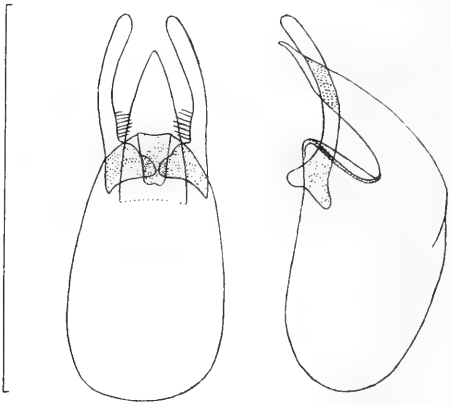

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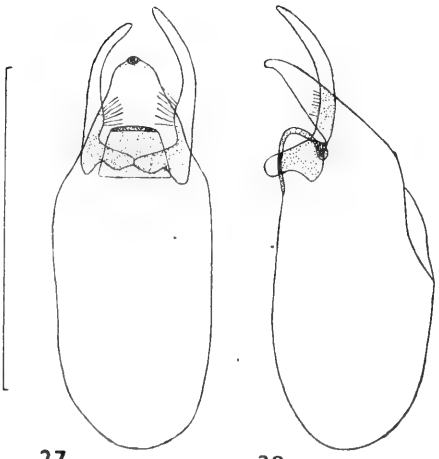

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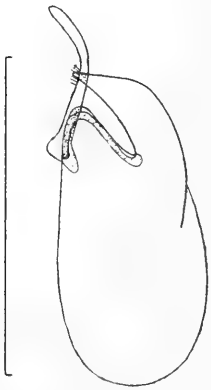

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Western Australia: Donnybrook (Lea) (holotype and allotype); Swan River (Lea); Newcastle (now known only as Toodyay) (Lea); Darling Ranges, on *Xanthorrhoea* (Lea); Yanchep, 13-23.xi.1935 (R. E. Turner).

Holotype (♂), allotype and four paratypes in the South Australian Museum, one paratype in the British Museum (Nat. Hist.), and one paratype in my collection. One further specimen in the South Australian Museum.

Occidentalis differs from the species previously described in the more convex form, particularly of the pronotum. The puncturation of the head and pronotum is also rather finer. In some ways (convex pronotum and colour) it is close to *maori*, but can be easily distinguished from this by the shape of and the coarser and less close puncturation of the pronotum, the ground sculpture of the elytra and the form of the aedeagus.

The pronotum (as noted above) varies somewhat in shape and it was at first thought that more than one species was represented in the material before me. Examination of the aedeagi, however, disproved this. In the material examined, the specimens with the less transverse pronotum have this broadest at about the middle of its length (Text-fig. 17), whereas those with this more transverse have it broadest behind the middle and a little more narrowed in front than behind (Text-fig. 16). Whether this is generally true for the species can only be shown by the examination of a much larger number of specimens.

The species appears to be more localized in its distribution than the others, being apparently restricted to Western Australia.

6. PSEUDOPHLOECHARIS MAORI (Cameron) (Text-figures 1–4, 7, 9, 18, 27–28).

Phloecharis maori Cameron, Ann. Mag. Nat. Hist., 11, 1944, 779.
? *Phloecharis australis* Fauvel, Rev. d'Ent., Caen, 22, 1903, 257.

Moderately convex, the disc of the pronotum not depressed. Dorsal surface lighter or darker brown, the head often a little darker. Mandibles, palpi and legs yellowish brown, antennae brown, slightly lighter basally. Ventral surface lighter or darker brown. Pubescence yellowish.

Length: 1·75–2·25 mm.

Head, pronotum and elytra rather dull, the abdomen somewhat more shining. Head moderately closely punctured and pubescent, the punctures as fine as those of the abdomen; ground sculpture similar to that of the pronotum. The eyes somewhat larger than in the other species. Antennae with the third segment a little shorter than the second, the fourth shorter than the third, slightly longer than broad, the fifth a little longer and wider than the fourth, longer than broad, the sixth to tenth about equal in length, but increasing gradually in breadth, the sixth very slightly transverse, the tenth about one and one-half times as broad as long, the eleventh nearly twice as long as the tenth, rounded apically.

Pronotum a little variable in shape, slightly more than one and one-half times as broad as long, broadest just in front of base, distinctly more narrowed in front than behind, the sides rounded; the anterior angles rounded, the posterior rather sharply rounded, obtuse, the anterior margin lightly emarginate, the posterior lightly sinuate. Puncturation and pubescence close, the punctures as fine and close as those on the abdomen; ground sculpture as Text-figure 7. On each side, at base, with an indistinct, shallow impression.

Text-figures 19-29.

19. *P. brachyptera*, n. sp., aedeagus, ventral view. 20. *P. brachyptera*, n. sp., aedeagus, lateral view. 21. *P. sinuatum*, n. sp., aedeagus, ventral view. 22. *P. sinuatum*, n. sp., aedeagus, lateral view. 23. *P. antipodum* (Fvl.), aedeagus, ventral view. 24. *P. antipodum* (Fvl.), aedeagus, lateral view. 25. *P. occidentalis*, n. sp., aedeagus, ventral view. 26. *P. occidentalis*, n. sp., aedeagus, lateral view. 27. *P. maori* (Cam.), aedeagus, ventral view. 28. *P. maori* (Cam.), aedeagus, lateral view. 29. *P. fuscum*, n. sp., aedeagus, lateral view. (This figure is more or less a reconstruction, as the only male specimen of this species available for study had the aedeagus partially extruded and somewhat damaged.) The scale, in each case, = 0·4 mm.

Elytra somewhat impressed on each side of the suture, a little broader than long, the sutural length about one and one-third times as long as the pronotum. Puncturation and pubescence similar to that on the pronotum, ground sculpture as Text-figure 9.

Tergites of abdomen closely and finely punctured and pubescent, ground sculpture as noted above (see generic description).

Aedeagus as Text-figures 27–28.

Type Locality: Glen Hope, New Zealand. Type in the Broun collection, British Museum (Nat. Hist.).

Specimens examined: 54, the type and six other specimens in the British Museum (Nat. Hist.), 46 specimens from the South Australian Museum, and one specimen from the collection of Mr. J. W. T. Armstrong.

Records:

Western Australia: Swan River, with *Iridomyrmex nitidus* Mayr. (J. S. Clark); Donnybrook on *Xanthorrhoea* (Lea); Darling Ranges (Lea).

South Australia: Lucindale (Feuerheerdt).

New South Wales: Galston (Lea); Muswellbrook (Lea); Sydney; Wollongong (Lea); Springwood (Mrs. L. Smith).

Queensland: Mt. Tambourine (Lea).

Tasmania: Hobart (Lea); Launceston (Lea); Mole Creek (Lea); Ulverstone (Lea); Waratah (Lea and Carter); Huon River (Lea); King Is. (Lea).

New Zealand: Glen Hope (Broun); Tairua (Broun); Ngaruaroahia (Broun).

?*New Caledonia*: Boulari (Savès).

This species is very easily recognized by the close and fine puncturation and pubescence of the head and pronotum, the punctures being as fine as those on the abdomen. The shape of the pronotum, though showing a slight variation, is also characteristic, this being always broadest a little in front of the base.

There is a greater variation in size than has been noted in any of the previous species, but this is no doubt partially due to the much larger number of specimens examined.

Maori appears to be the commonest and most widely distributed species of the genus. As previously noted, it agrees very well with the description of *Phloecharis australis* Fvl., and an examination of the New Caledonian species will probably show the two to be identical.

NOTES ON THE MORPHOLOGY AND BIOLOGY OF ANABARRHYNCHUS FASCIATUS MACQ. AND OTHER AUSTRALIAN THEREVIDAE (DIPTERA, THEREVIDAE).

By Kathleen M. I. English,

Department of Zoology, University of Sydney.

(Forty-seven Text-figures.)

[Read 29th November, 1950.]

Introduction.

The family Therevidae is of world-wide distribution and it is well represented in Australia. Tillyard (1926) gave the number of species as 56. Later, Mann (1928–1933) described new species and sank some names as synonyms, and he recognized 75 species in 14 genera; but he states: "It must be stressed that these are 'Revisional Notes' only, as insufficient material is available to allow a complete revision of the family."

No description has been found of the immature stages of any Australian species, and very little detailed information on the immature stages is available anywhere.

The earliest records I have seen of Therevidae larvae are by Bouché (1834) and Westwood (1840). The latter gives two other earlier references. Descriptions of larvae and pupae have been given by Brauer (1883), Williston (1896), Lundbeck (1908), Collinge (1909), Felt (1912), Malloch (1915), de Meijere (1916), Issac (1925), and Bhatia (1934).

In all these papers the descriptions of the larvae have family characteristics only, there are no details of the head or mouth-parts, and the text-figures by de Meijere, of mouth-parts, are the only ones which may have some generic characters.

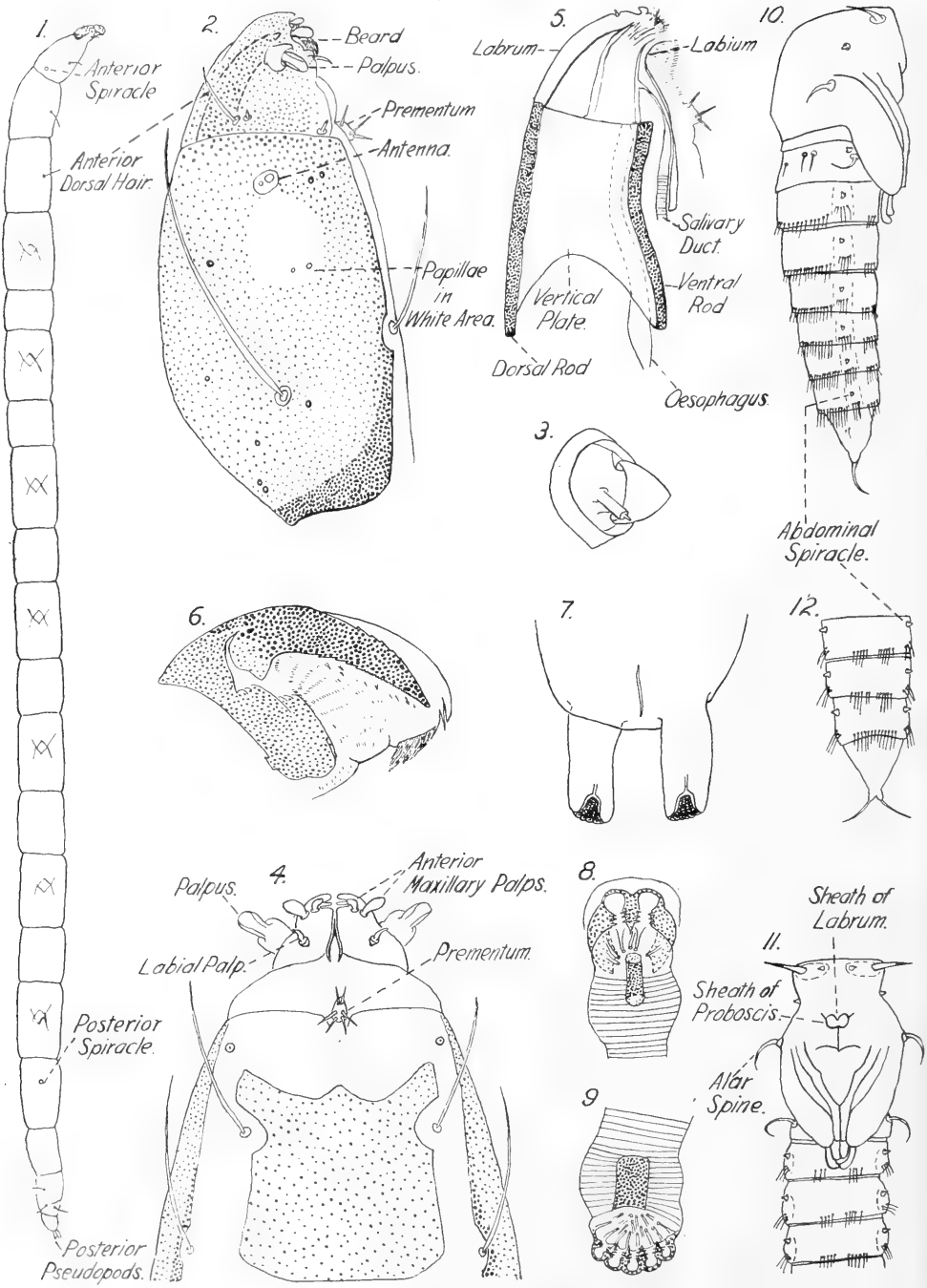
In this paper records are given of the occurrence of *A. fasciatus* Macq., and the larva and pupa are described in some detail. Records are given also of the occurrence of eight other species, and the larva and pupa of each is described briefly, it being considered necessary to describe only the features that distinguish the species.

Anabarrhynchus fasciatus Macquart. Text-figures 1–13.

A. fasciatus was described by Macquart (1848), but the decription is more accessible in Australia in Mann's Revisional Notes, pt. 1, p. 151 (1928). In 1947, when specimens were submitted to him for identification, Mr. Mann states: "It is a species that I know from Sydney only."

Occurrence.

Many larvae were found in the sandy soil of a small garden at Rose Bay, Sydney, N.S.W. They were particularly numerous in two small plots where fruit fell from the overhanging branches of a peach tree. The peaches were infected with fruit fly, and in January, 1947, the garden was left untended for some weeks, the fallen fruit accumulated and fruit fly and other larvae developed unhindered. In February, when this decaying fruit was being picked up, one large Therevid larva was seen on the soil under the remains of one peach; and in March, when the soil was being dug over where the fallen fruit had lain, nine Therevid larvae were turned up in a few minutes. In April, during a week-end of gardening, thirty larvae of various sizes were found, but only ten of the larger ones were kept. From March to October, 1947, 91 Therevid larvae were collected in various parts of the small garden and many more were seen but not taken. Many were found near the surface and were uncovered by just scratching the top soil; others were found deeper down when digging, but it was not possible to determine at what depth exactly they were in the soil. In colour most of them were white or cream, but some of those found in October had a distinct pink tinge;



Text-figures 1-12. *Anabarrhynchus fasciatus.*

1. Larva, lateral view, × 7½.
2. Head of larva, lateral view, × 120.
3. Antenna of larva, × 500.
4. Head of larva, ventral view, × 120.
5. Vertical section of larval head, showing labrum and labium, × 120.
6. Mandible and maxilla of larva, × 185.

others appeared to have alternating dark and light bands; this was evidently due to some dark-coloured foodstuff showing through the thin-walled part of the segments.

The larvae are carnivorous and it was necessary to keep each larva in a jar or tube by itself, for if several were left together overnight there would be only one surviving in the morning. No real attempt was made to supply the larvae with food. In the soil under the peach trees were many larvae and pupae of fruit fly and another Acalyptrate fly. Some of these were given to a few of the Therevid larvae and were apparently acceptable, as the larvae were consumed and some of the pupae were pierced and the contents extracted.

When found many of the larvae had small mites attached to them; and some had brown spots on the skin, evidence of some diseased condition, for these did not survive long; others, after having been kept in captivity for a time, developed this condition and later died.

Of the larvae collected at Rose Bay in 1946 and 1947, 48 pupated, and from these there emerged 31 of *A. fasciatus* and four of other species. Seven pupae were parasitized by a Bombyliid, of which four adults emerged and three were dissected out in the pupal stage. One adult of *A. fasciatus* was obtained from a larva collected in 1946 in the Hornsby valley, and seven adults were obtained from larvae collected at Woolwich in 1949.

The pupal period varied from 26–28 days in early September to 14–15 days in the latter part of October, and 10 days for one specimen in February.

Some adults were collected in the garden at Rose Bay, but not as many were seen as might have been expected from the number of larvae in the soil. No eggs were found nor was egg laying observed.

Larva. Text-figures 1–9 and 13.

The larva (Text-fig. 1) is about 25 mm. long and about 1 mm. in width. It has a dark brown chitinized non-retractile head and a long slender body consisting of three thoracic and ten abdominal segments. The thoracic segments each bear a pair of latero-ventral hairs, the first segment tapers anteriorly to the small head, and near its posterior border is situated the anterior spiracle. The first six abdominal segments are each divided by a constriction into two parts, and the first seven segments have, laterally, a pattern of depressed lines. The eighth segment bears the posterior spiracles. The tenth segment is divided by a constriction into two parts: the proximal part bears three pairs of hairs and the distal part bears the anus on the ventral surface and two pseudopods at the apex.

The Head.—The head (Text-fig. 2) is about $\frac{1}{2}$ mm. long, it is broad posteriorly and tapers to a point anteriorly, it is rounded on the dorsal surface and is almost flat on the ventral surface. The epicranium is a shining brown, the posterior edge is much darker, and laterally there is a white or colourless area of an irregular shape. On each side of the epicranium are ten or more papillae, two of which occur, one above the other, in the posterior end of the white area. At the anterior edge of this area is the antenna (Text-fig. 3), a curious structure of two very dissimilar parts, the larger part shaped like a pointed dome on a short thick stalk, the smaller part shaped like a candle, with a pointed palp at the top, and the whole encircled by a ridge. On the posterior portion of the epicranium is a large forwardly directed bristle or hair.

On the ventral surface of the epicranium (Text-fig. 4) there is a large chitinized ventral plate, and on each side of it are two long bristles and one papilla.

Internally the box-like "pharynx support" through which runs the œsophagus (Text-fig. 5) is very similar in arrangement to that described for *Apiocera maritima* Hardy (These PROCEEDINGS, lxxi, p. 298, 1947).

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7. Posterior end of larval exuvia with pseudopods, × 120.
8. Anterior spiracle of larva, surface view, × 185.
9. Posterior spiracle of larva, surface view, × 185.
10. Pupa, lateral view, × 7½.
11. Pupa, anterior end, ventral view, × 7½.
12. Pupa, posterior end, ventral view, × 7½.

The anterior portion of the head contains the mouth-parts and bears bristles and palps externally. On each side near the lateral posterior edge are three bristles (Text-fig. 2). The one nearest the median line I have called the anterior dorsal hair; it is much longer than the one immediately below; near the ventral edge is another short bristle. In front of these is a large two-jointed palpus. On the ventral surface (Text-fig. 4) is a centrally situated fleshy lobe armed with six spines, called by de Meijere (1916) the prementum. Further forward are a pair of slender labial palps, and near the anterior edge are two pairs of lobes or palps, which I have called the anterior maxillary palps.

Mouth-parts. The labrum (Text-fig. 5) curves downwards and the anterior part is hidden by the mandibles when the head is viewed from the side. In the anterior part of the labrum there is a pair of small spines set in a depression; nearer the apex the edge forms an upturned peak, and below this the chitin is armed with spines and hairs. Below the labrum is the hypopharynx, articulated posteriorly to the ventral rods, and from it the salivary duct runs back below the ventral rods. Below the hypopharynx is the labium, a laterally compressed structure composed of a chitinous rod above with fleshy lobe below covered with fine hairs or spines. The arrangement and shape of the hypopharynx and labium is very similar to that described for *Apiocera maritima* Hardy. On each side of the labrum are the strongly chitinized pointed mandibles (Text-fig. 6) with a fine saw-tooth lower edge and some small barbs on the upper edge. The maxillae are set outside the mandibles, they are not heavily chitinized, and, as well as the small anterior palps, they are provided with a beard, or thick tuft, of long hairs.

Behind the head is the capsule rod (Text-fig. 13). It articulates with the posterior dorsal peak of the epicranium and extends through the prothorax into the anterior part of the mesothorax and can be seen through the skin; in the live larvae it can be seen moving actively with the movements of the head. The rod is about 1 mm. in length, it is strongly chitinized and further strengthened by very heavy chitin in the shaft and in the distal portion of the wider part.

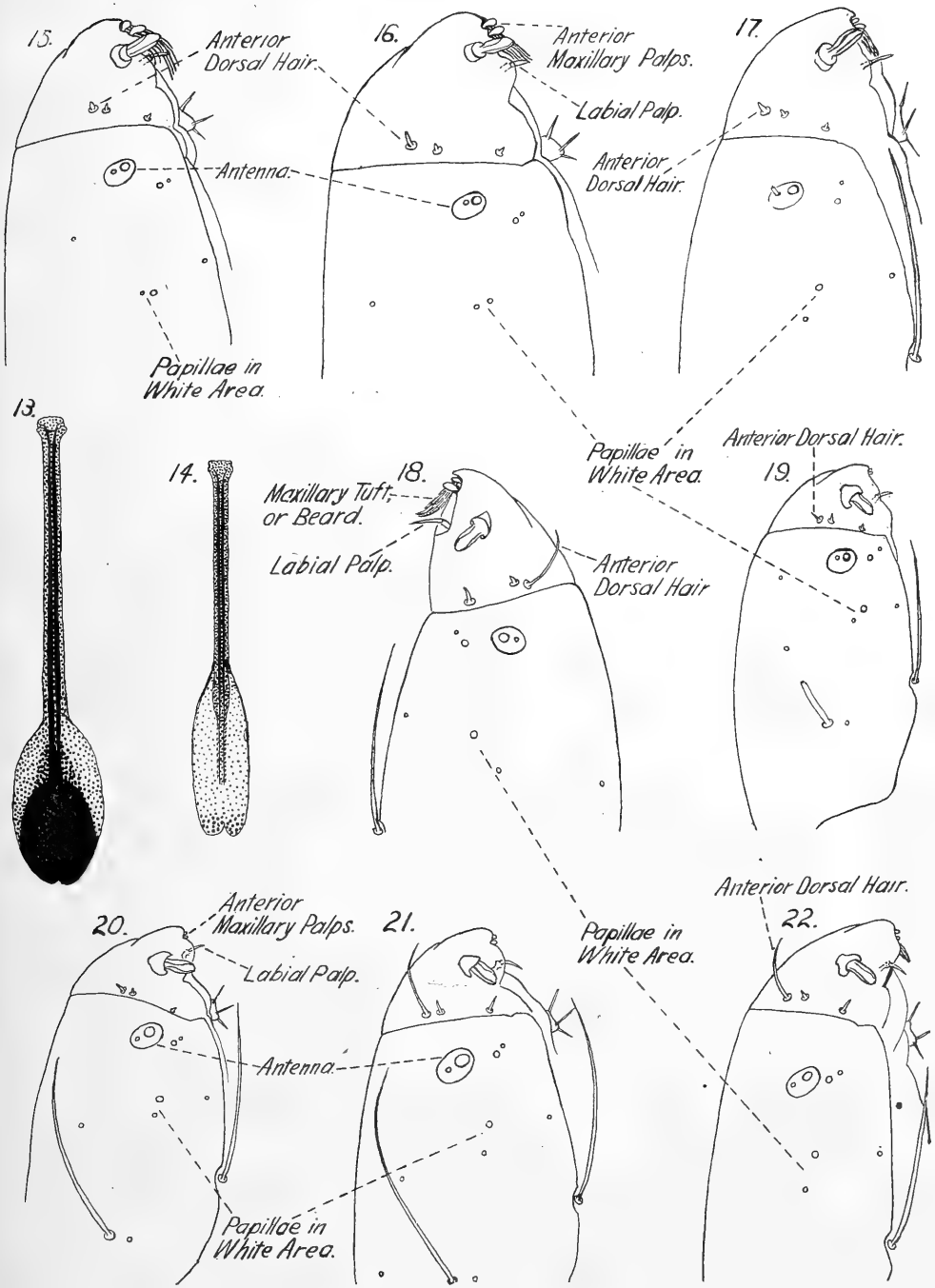
The posterior pseudopods are used continuously for locomotion when they are turned downwards at right angles to the body; they are about half the length of the distal part of the last segment. In the larva itself no structure is visible in the pseudopods, but in the larval exuvia a very definite chitinized structure is visible (Text-fig. 7); this probably enables the larva to grip surfaces by suction.

The spiracles (Text-figs. 8 and 9). The anterior pair are situated on the posterior part of the prothorax, and the posterior pair are on the eighth abdominal segment. Both pairs are strongly chitinized and show up on the live larva as almost black spots.

Pupa. Text-figures 10, 11 and 12.

Pupal exuviae vary in length from 8–9 mm. The head bears a pair of slender antennal processes, each ending in a slender spine. The sheath of the labrum is very short and does not extend to the edge of the sheath of the proboscis. On the thorax there is a strong alar spine on each side at the base of the wing sheath; and each spiracle is placed on a small high tubercle. The first abdominal segment bears, on each side, one long spine laterally near the spiracle, and three small dorso-lateral spines. The second and succeeding abdominal segments, except the last, each have a single incomplete row of fine bristles near the posterior edge; the bristles are mostly short on the second segment and they increase in length on each succeeding segment. On the dorsal surface the bristles are numerous; on the ventral surface there are six or seven on the second segment and they increase on each succeeding segment to twelve or thirteen in the last row. Laterally there are four or five bristles below the spiracle on each lateral prominence. The last segment bears on each side two very small bristles, and it ends in two tubercles, each bearing a long spine. The abdominal spiracles are on small high tubercles.

No difference could be found between the male and female pupae of this species, though there are very slight differences noted in the other species described in this paper.



Text-figures 13-22.

13. *Anabarrhynchus fasciatus*, capsule rod of larva, × 60.
14. *Acupalpa semiflava*, capsule rod of larva, × 60.
15. *Platycarenum quinquevittata*, head of larva, lateral view, × 120.
16. *Anabarrhynchus maritimus*, head of larva, lateral view, × 120.
17. *Ectinorrhynchus variabilis*, head of larva, lateral view, × 120.
18. *Taenogera superbus*, head of larva, lateral view, × 120.
19. *Acraspisa trifasciata*, head of larva, lateral view, × 150.
20. *Acuplpa semiflava*, head of larva, lateral view, × 120.
21. *Agapophytus albobasalis*, head of larva, lateral view, × 120.
22. *Agapophytus aterrimus*, head of larva, lateral view, × 120.

PLATYCARENUM QUINQUEVITTATA Macquart. Text-figures 15, 23–26.

Recorded by Mann from N.S.W. and Tasmania.

Occurrence.

Adults were collected at Narooma in January, 1937 and 1938, on beach sand; and at Cronulla, on 5th October, 1947, among grasses on the sand-dunes behind the beach, where they were very numerous, and there were many mating couples; three pairs were caught.

Larvae were collected at Narooma, Cronulla and Yarra Bay (Botany Bay). The larvae are found in sand, either in the beach above high-water mark or in the dunes and sand-hills behind the beach. They travel at times just below the surface of the sand and leave behind a fine line, or track, of raised sand in a very minute "rick-rack" pattern. These fine tracks, about one or two mm. in width, sometimes extend for long distances and are often very numerous. There was no opportunity to observe whether these tracks occur at any time of the year or only at certain seasons; nor was it possible to determine for how long they would remain on the sand if not levelled by rain or blown away by wind.

Narooma: In November, 1938, two large larvae were found in surface sand on the beach.

Cronulla: In May, 1947, six larvae were collected in the sand-hills behind the beach; they were found by scraping away the top sand at the ends of the "rick-rack" tracks.

Yarra Bay: In July, 1948, one larva was found among the roots of the spinfex growing in the sand at the back of the beach. It had its mouth-parts sunk into a small beetle pupa, and it continued to feed even after being uncovered. In October, 1948, five larvae were found among spinifex roots in the sand. Many Lepidoptera and some Coleoptera larvae were present also in the sand among the grass roots. Four adults were obtained from these larvae—two males and two females—and the pupal period was obtained for two. One emerged in early December (pupal period 18 days); one emerged in early February (pupal period 16 days).

Larva. Text-figure 15.

The larvae are large, up to 30 mm. in length and 1·5 mm. in width. On the epicranium the two papillae in the white area are placed close together and one above the other. The anterior dorsal hair is short, the anterior maxillary palps large, and maxillary tuft long and strong. The labrum has a small indentation, but it lacks the distinct upturned peak of *A. fasciatus*.

The capsule rod is heavily chitinized and shaped as in *A. fasciatus* (Text-fig. 13). The posterior pseudopods are minute, less than one-fifth of the length of the distal part of the last segment. The spiracles are not very heavily chitinized.

The dark capsule rod shows very conspicuously through the skin of the thorax; this and the minute posterior pseudopods make it possible to determine the species of the live larva if the habitat is known.

Pupa. Text-figures 23–26.

Pupal exuviae vary in length from 8 to 9 mm. The head bears slender antennal processes, each terminating in a long spine. The sheath of the labrum is short. The thorax bears a long alar spine on each side; the spiracles are each on a small high tubercle.

The first abdominal segment bears on each side one long spine laterally, near the spiracle, and three long dorso-lateral spines. The second and succeeding abdominal segments, except the last, bear fine bristles near the posterior edge. On the dorsal surface the bristles are in two rows: an anterior row of shorter bristles which become shorter on each succeeding segment, and a posterior row of longer bristles which become longer on each succeeding segment. On the ventral surface the bristles are long and short, irregularly placed; they become longer on each succeeding segment. On the lateral prominences there are short and long bristles; the number and arrange-

ment is irregular. The last segment is divided by a constriction into two parts. The proximal part bears laterally ten or twelve bristles, short and long; the distal part bears at the apex two small thin bristles. The abdominal spiracles are borne on very small tubercles.

In the male pupa (Text-fig. 25), on the last segment, in addition to the lateral bristles, there are nine or ten short bristles on the ventral surface.

ANABARRHYNCHUS MARITIMUS Hardy. Text-figures 16 and 27–29.

Recorded by Mann from Tasmania, N.S.W. and Queensland.

Occurrence.

Adults were collected at Narooma in January, 1937, 1938, 1939, and in November, 1938. More were collected at Cronulla on 5th October, 1947, where they were numerous on the tall grasses on the sand-dunes behind the beach. Some mating couples were seen and one pair was caught.

One larva was found at Yarra Bay, 10th July, 1948, among the roots of spinfex growing in sand at the back of the beach. On 13th September it had pupated and on 9th October a male emerged before 8 a.m. This was the only specimen obtained from a larva.

Larva. Text-figure 16.

No measurement was made of the larva before it pupated. On the epicranium the two papillae in the white area are placed close together, one above and a little behind the other. The anterior dorsal hair is short, anterior maxillary palps large, and maxillary tuft long and strong. The labrum has a very small prominence near the apex, but it has not the distinct peak of *A. fasciatus*. The capsule rod is heavily chitinized and shaped as in *A. fasciatus* (Text-fig. 13). In the larval exuvia the posterior pseudopods are about the same length as in *A. fasciatus* and they have the same structure at the extremity (Text-fig. 7), though not quite so heavily chitinized. The spiracles are fairly heavily chitinized.

Pupa. Text-figures 27, 28 and 29.

The pupal exuvia is 9 mm. long. The head bears slender antennal processes, each ending in a slender spine. The sheath of the labrum is short. The thorax bears a long alar spine on each side; the spiracles are each on a small tubercle.

The first abdominal segment bears, on each side, a long bifid spine near the spiracle, and two long dorso-lateral spines. The second and succeeding abdominal segments, except the last, bear a single incomplete row of fine bristles near the posterior edge, and most of these become longer on each succeeding segment. On the dorsal surface the bristles are numerous on the second segment, but the number is reduced a little on succeeding segments. On the ventral surface there are seven to nine bristles on each side of a central space. On the lateral prominences there are seven to nine bristles on segments two to six, and five on the seventh. The last segment is divided by a constriction, the proximal part bears laterally two short bristles, and on the ventral surface four short bristles. The distal part bears, at the apex, two very small spines. The abdominal spiracles are borne on very small tubercles.

No female pupa was obtained for comparison.

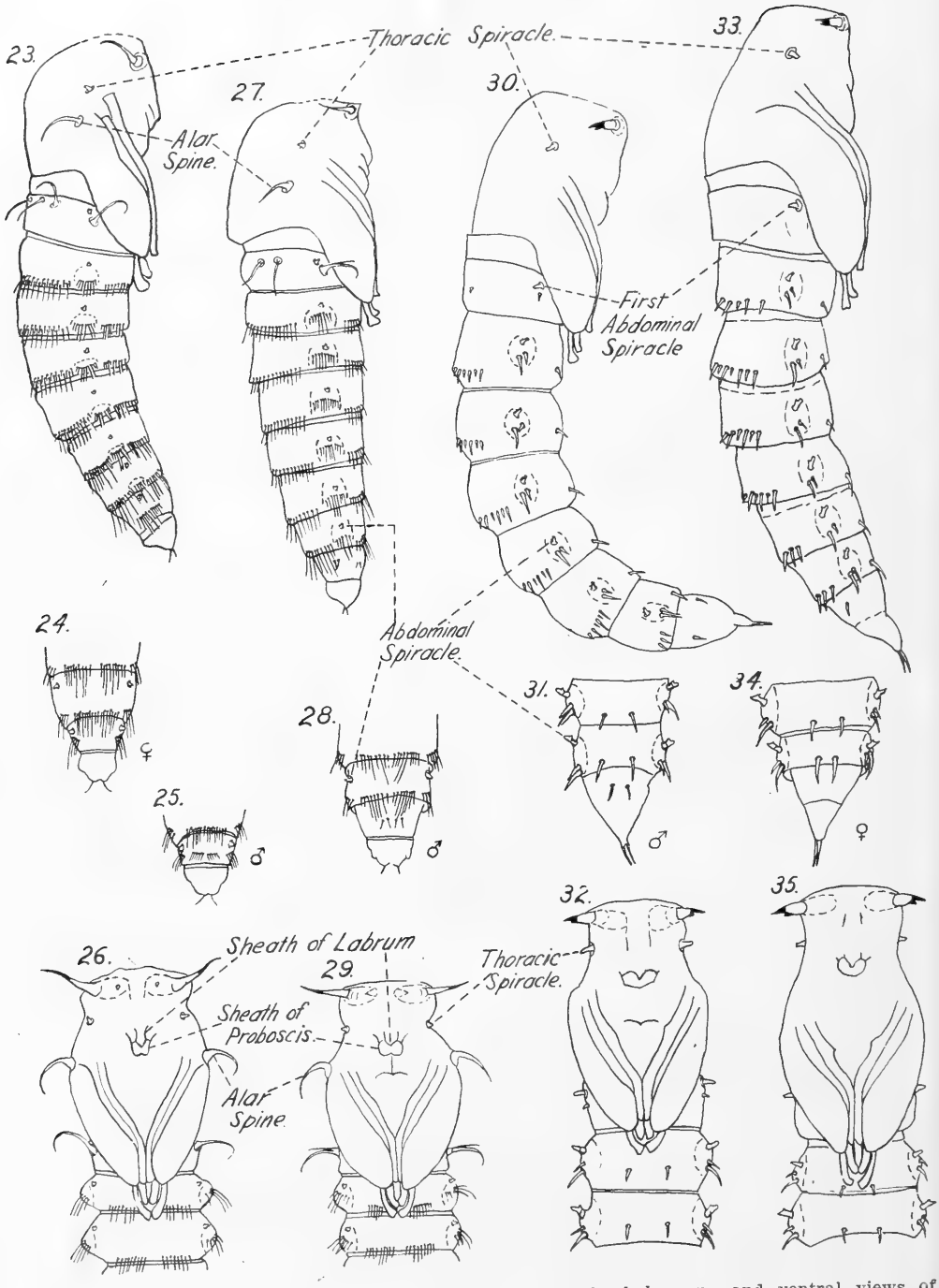
ECTINORRHYNCHUS VARIABILIS Macquart. Text-figures 17 and 30–32.

Recorded by Mann from Tasmania, N.S.W., Queensland and Western Australia.

Occurrence.

No adults were collected.

Among the larvae of *A. fasciatus* collected in garden soil at Rose Bay in April, 1947, was one from which a male of this species emerged in September; and two males emerged from larvae collected in garden soil at Woolwich, one in September, 1949 (pupal period 33 days), and one in August, 1950. These were the only specimens obtained.



Text-figures 23-35. Pupae, showing lateral view of whole pupa and ventral views of anterior and posterior ends of pupae.

23, 24, 25, 26. *Platycarenum quinquevittatd*, × 7½.

27, 28, 29. *Anabarrhynchus maritimus*, × 7½.

30, 31, 32. *Ectinorrhynchus variabilis*, × 7½.

33, 34, 35. *Taenogera superbus*, × 7½.

Larva. Text-figure 17.

No measurements were made of these larvae before they pupated. On the epicranium the two papillae in the white area are placed well apart, one behind and slightly above the other. The anterior dorsal hair is short. The anterior maxillary palps are smaller than in the previous species described, but they are as long as the basal segment of the labial palp. In the maxillary tuft the hairs are sparse. The labrum has no peak near the apex.

The capsule rod is shaped as in *A. fasciatus* (Text-fig. 13), but not quite so heavily chitinized. In the larval exuvia the posterior pseudopods appear to be nearly as long as in *A. fasciatus*, and they have the same heavily chitinized structure (Text-fig. 7). The spiracles are fairly heavily chitinized.

Pupa. Text-figures 30, 31 and 32.

The pupal exuviae are 10–11 mm. in length. The head bears short thick antennal processes, each bearing a short strong spine with a very short spine at its base.

The sheath of the labrum is short. There are no alar spines on the thorax; the spiracles are each on a long slender tubercle.

The first abdominal segment bears on each side one very small thorn-like spine laterally near the spiracle, and one very small dorso-lateral spine, but in one specimen there were no spines on this segment. The second and succeeding segments, except the last, are armed with spines near the posterior edge. On the dorsal surface, on segments two to five, there are about twelve spines, long and short; segment six may be like segment five or more like segment seven; on segment seven there are six long spines. On the ventral surface on segments two to seven are two spines placed well apart; they increase in length on each succeeding segment. On the lateral prominences, on segments two to six, are two spines, one long and one short; on segment seven they are both long. The last segment bears, on the anterior portion, one short spine laterally and two short spines ventrally; the distal portion bears at the apex two long spines. The abdominal spiracles are borne on long slender tubercles, and on segments three to seven these bear a small peak on the anterior edge.

No female pupae were obtained for comparison.

TAENOGERA SUPERBUS Schiner. Text-figures 18 and 33–35.

Recorded by Mann from Queensland and N.S.W.

Occurrence.

Two adults, a mating couple, were collected at Yass, N.S.W., in December, 1928.

Three larvae were found in soil at Yass in August, 1930, and from these emerged two females and one male in November. The pupal period was not obtained.

Larva. Text-figure 18.

The largest larva was 24 mm. in length. On the epicranium the two papillae in the white area are placed well apart, one behind and slightly above the other. The anterior dorsal hair is long. The largest of the anterior maxillary palps are as long as the basal segment of the labial palp. This species appears to have an extra pair of maxillary palps covered with hairs instead of the maxillary tuft of the other species in this paper. The labrum has a very small peak near the extremity. The capsule rod is shaped as in *Acupalpa semiflava* (Text-fig. 14), but it is not so heavily chitinized. In the larval exuvia there is no chitinized structure at the extremity of the posterior pseudopods. The spiracles are not heavily chitinized.

Pupa. Text-figures 33, 34 and 35.

The longest pupal exuvia was 12 mm. in length. The head bears short thick antennal processes, each terminating in a strong spine with a very small spine at its base.

The sheath of the labrum is short. On the thorax there are no alar spines; the spiracles are each on a long slender tubercle.

There are no spines on the first abdominal segment. The second and succeeding abdominal segments, except the last, are armed with strong spines near the posterior edge. On the dorsal surface, on segments two to five, there are ten to fourteen spines, long and short; on segments six and seven there are six long spines. On the ventral surface, on segments two to seven, there are two spines placed apart; on the seventh segment the spines are longer and placed closer. On the lateral prominences, on segments two to seven, there are two spines, one long and one short. The last segment is divided by a constriction into two parts; on the proximal part, on each side, is one small dorso-lateral spine. The distal part bears at the apex two long spines. The abdominal spiracles are borne on long slender tubercles, and on segments three to seven these bear a small peak on the anterior edge.

The male pupa bears, in addition, on the last segment two small spines on the ventral surface.

ACRASPISA TRIFASCIATA Krober. Text-figures 19 and 36–38.

Recorded by Mann from N.S.W. and Northern Territory of Australia.

Occurrence.

Adults were collected at Yass, N.S.W., where they were numerous in October, 1932.

Two larvae were found in soil at Yass in August and October, 1930; from these emerged a male and a female in November. The pupal period was not obtained.

Larva. Text-figure 19.

The larvae were about 14 mm. in length. On the epicranium the two papillae in the white area are placed close together, one behind and a little above the other. The anterior dorsal hair is short. The anterior maxillary palps minute, maxillary tuft scant or absent. The shape of the labrum could not be determined. The capsule rod is shaped as in *Acupalpa semiflava* (Text-fig. 14), but is not so heavily chitinized. In the larval exuvia the posterior pseudopods have some structure at the extremity but it is not chitinized and its formation could not be determined. The spiracles are not heavily chitinized.

Pupa. Text-figures 36, 37, 38.

The pupal exuviae are 6 mm. in length. The head bears short thick antennal processes, each terminating in a slender spine which may have a minute spine at its base.

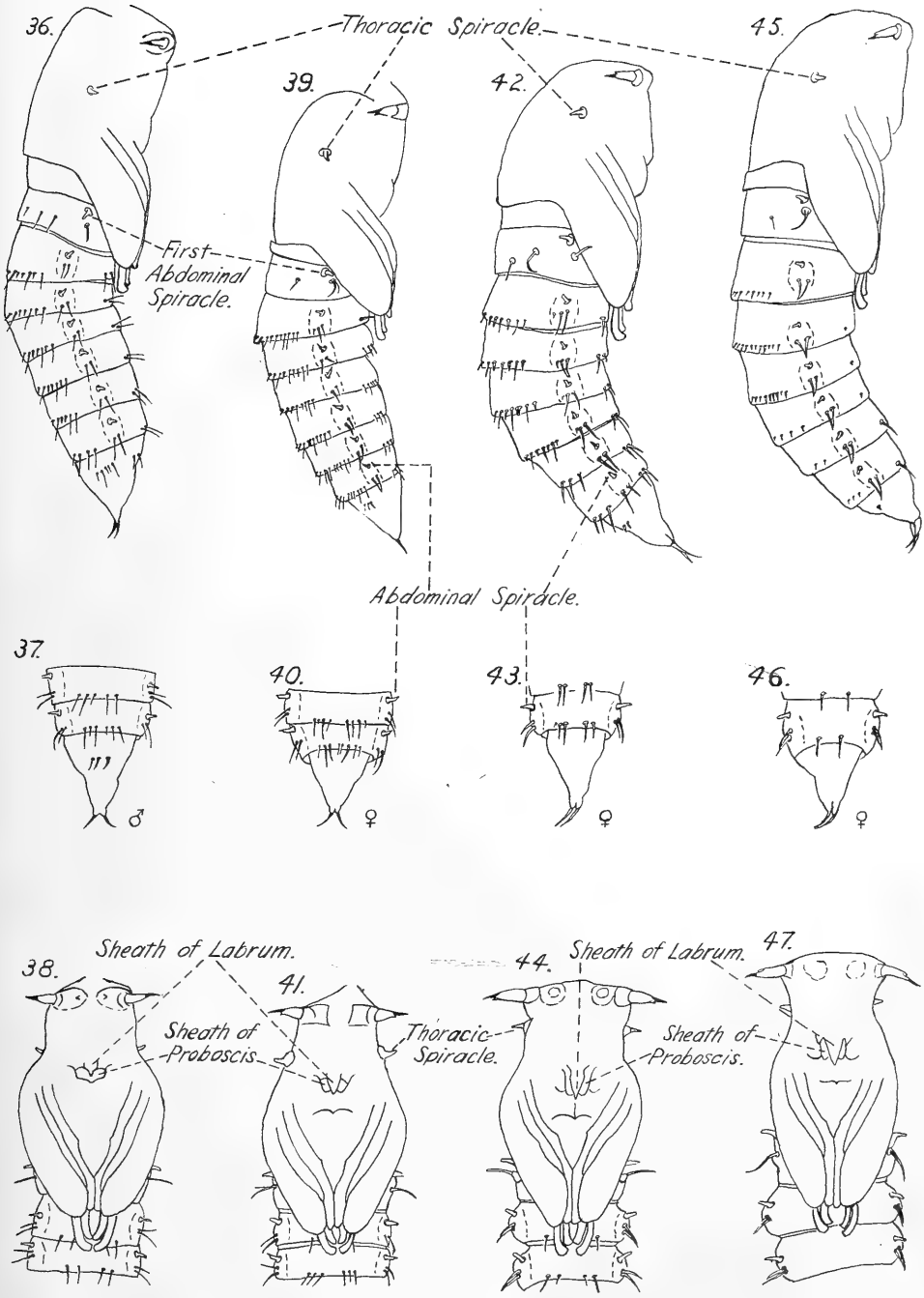
The sheath of the labrum is short. On the thorax there are no alar spines; the spiracles are each on a long slender tubercle.

The first abdominal segment bears, on each side, one strong bristle laterally near the spiracle, and three very slender dorso-lateral bristles. The second and succeeding segments, except the last, are armed with long bristles and short spines near the posterior edge. On the dorsal surface, on segments two to six there are six slender bristles and eight short spines; on the seventh segment are six bristles but only two or three very small spines. On the ventral surface, on segments two to seven there are four long bristles and one or two short bristles also on segments six and seven. On the lateral prominences, on segments two to seven are two long bristles. The last segment bears, on each side, four or five long and short dorso-lateral bristles, and on the ventral surface in the male pupa are three strong bristles. Apically the last segment ends in two tubercles, each ending in a spine. Abdominal spiracles are borne on tall slender tubercles.

In the female pupa the short spines are more numerous on the dorsal surface of all abdominal segments. The last segment bears two or three long dorso-lateral bristles on each side, and there are no bristles on the ventral surface.

ACUPALPA SEMIFLAVA Mann. Text-figures 14, 20, 39–41.

Recorded by Mann from Queensland.



Text-figures 36-47. Pupae, showing lateral view of whole pupa and ventral views of anterior and posterior ends of pupae.

36, 37, 38. *Acraspisa trifasciata*, ×12.

39, 40, 41. *Acupalpa semiflava*, ×7½.

42, 43, 44. *Agapophytus albobasalis*, ×7½.

45, 46, 47. *Agapophytus aterrimus*, ×7½.

Occurrence.

One adult was collected at Yass, N.S.W., in October, 1932. Two larvae were found in soil at Yass in September, 1932, and from these two females emerged in November and December. The pupal period was not obtained.

Larva. Text-figures 14, 20.

No measurements were made of the larvae before they pupated. On the epicranium the two papillae in the white area are placed close together, one behind the other. The anterior dorsal hair is short, the anterior maxillary palps minute, maxillary tuft scant or absent. The curved labrum has no peak or depression. The capsule rod (Text-fig. 14) is shorter than in *A. fasciatus*, the shaft is not so heavily chitinized, the wide part is longer and narrower and has no heavily chitinized portion at the posterior end. In the larval exuvia the posterior pseudopods are very short and the extremity is not chitinized. The spiracles are not heavily chitinized.

Pupa. Text-figures 39, 40, 41.

Length of pupal exuviae 8 mm. The head bears short thick antennal processes, each bearing a slender spine, with a small basal spine. Sheath of the labrum is long; it reaches beyond the sheath of the proboscis. On the thorax there are no alar spines; the spiracles are each on a high slender tubercle on a rounded base.

The first abdominal segment bears, on each side, one slender bristle (sometimes bifid) near the spiracle, and one dorso-lateral bristle. The second and succeeding segments, except the last, bear bristles and spines near the posterior edge. On the dorsal surface, on segments two to seven, are long bristles and short spines, irregularly placed. On the ventral surface, on segments two to seven, are long and short bristles, irregularly placed. On the lateral prominences, on segments two to seven, are two long bristles, and in some cases some short bristles also. The last segment bears, on the proximal part, three small dorso-lateral bristles on each side; the distal part divides at the apex into two small tubercles, each ending in a spine. The abdominal spiracles are borne on long slender tubercles and, on segments two to seven, these terminate in a small spine. There is no male pupa for comparison.

AGAPOPHYTUS ALBOBASALIS Mann. Text-figures 21, 42–44.

Recorded by Mann from Queensland, N.S.W. and South Australia.

Occurrence.

Two adults, a mating pair, were collected at Yass, N.S.W., in December, 1929.

Larvae were found in 1930 at the same locality, and from these seven adults emerged, four females and three males. Pupal periods obtained were 22–23 days for two females which emerged on December 6, and 17 days for a male which emerged on December 10.

Larva. Text-figure 21.

Larvae varied in length from 20 to 23 mm. On the epicranium the two papillae in the white area are placed well apart, one behind the other. Anterior dorsal hair is long, anterior maxillary palps minute. The maxillary tuft scant or absent. The labrum has no peak or depression. The capsule rod is shaped as in *Acupalpa semiflava* (Text-fig. 14). In the larval exuvia the posterior pseudopods are very short and they have no chitinized structure at the extremity. The spiracles are not heavily chitinized.

Pupa. Text-figures 42, 43, 44.

Length of pupal exuviae 9 mm. The head bears thick antennal processes, each terminating in a strong spine which may have a minute spine at its base. The sheath of the labrum is long. On the thorax there are no alar spines; the spiracles are each on a long slender tubercle on a round base.

The first abdominal segment bears, on each side, a long strong bristle near the spiracle and two strong dorso-lateral bristles. The second and succeeding segments,

except the last, bear short spines and long bristles near the posterior edge. On the dorsal surface, on segments two to five, are short spines and long bristles, irregularly placed; on segments six and seven are six long bristles. On the ventral surface, on segment two are two long bristles; on segment three are two long and two short; and on segments four to seven are four long bristles. On the lateral prominences, on segments two to seven are two long bristles and in some cases a short one also. The last segment bears, in the female pupa, two small dorso-lateral bristles on each side; the distal part divides into two tubercles, each bearing a long spine. The spiracles are borne on long slender tubercles and, on segments two to seven, these each end in a very small spine. The male pupa bears on the proximal part of the last segment two very small spines on the ventral surface.

AGAPOPHYTUS ATERRIMUS Mann. Text-figures 22, 45–47.

Recorded by Mann from Queensland, N.S.W. and Victoria.

Occurrence.

No adults were collected.

Larvae were found at Yass, N.S.W., in 1930, mostly in the soil at the base of hollow rotting tree stumps, and from these three females and one male emerged in December. Pupal periods obtained were: 25 days for one male, which emerged on 5th December, and 18 days for one female, which emerged on 13th December.

Larva. Text-figure 22.

The largest larvae were 27 mm. in length. On the epicranium the two papillae in the white area are placed well apart, one behind the other. The anterior dorsal hair is long, anterior maxillary palps minute, maxillary tuft short and thick. The labrum has no peak or depression. The capsule rod is shaped much as in *Acupalpa semiflava* (Text-fig. 14), but in some specimens it is more heavily chitinized at the posterior end. In the larval exuvia the posterior pseudopods are short and there is no chitinized structure at the extremity. The spiracles are fairly heavily chitinized.

Pupa. Text-figures 45, 46, 47.

Length of pupal exuviae 9–11 mm. The head bears thick antennal processes, each ending in a thin spine with a minute spine beside it. The sheath of the labrum is long. On the thorax there are no alar spines; the spiracles are each borne on a long slender tubercle.

The first abdominal segment bears, on each side, one long strong bristle near the spiracle and one short slender dorso-lateral bristle. On the dorsal surface, on segments two to four, near the posterior edge, are numerous short strong spines; on segments five to seven the spines are short, slender and few in number. On the ventral surface segments two to five may bear two minute spines or none; segments six and seven bear two long strong spines. The lateral prominences each bear two long strong spines. The last segment bears, on each side, one very small dorso-lateral spine in the female pupa; the distal part ends in two tubercles, each bearing a spine. The spiracles are borne on long slender tubercles.

The male pupa bears on the proximal part of the last segment two small strong spines on the ventral surface, and there may be an additional very small latero-dorsal spine on each side.

NOTES ON HABITS.

In the prepupal stage the larvae of the Therevidae assume a very characteristic position. The larva lies in the soil in a curved position somewhat like the letter U, or almost in a circle; if it is uncovered the larva straightens itself and slowly makes its way below the surface of the soil, then re-assumes its curved position. The hard skin of the larva does not allow it to contract very much, the thoracic segments are slightly shortened and become bead-like in appearance, and the abdominal segments contract a little.

Adult Therevidae are fairly numerous, but not as many are to be seen as might be expected from the prevalence of the larvae; they fly lazily, and some species fly with the legs hanging downwards, in the manner of certain wasps.

Tentative Keys for the Genera Described in this Paper.

Larvae.

1. Anterior maxillary palps as long as, or longer than, the basal segment of the labial palp, as in Text-figure 16 2
   Anterior maxillary palps minute, as in Text-figure 20 5
2. Papillae in lateral white area of head placed one above the other, or nearly so, as in Text-figures 15 and 16 3
   Papillae in lateral white area of head placed one well behind the other, as in Text-figure 17 4
3. Posterior pseudopods minute, less than one-fifth of the length of the distal part of the last segment ..... *Platycarenum*
   Posterior pseudopods long, about half the length of the distal part of the last segment (Text-fig. 1) ..... *Anabarrhynchus*
4. Anterior dorsal hair short (Text-fig. 17) ..... *Ectinorrhynchus*
   Anterior dorsal hair long (Text-fig. 18) ..... *Taenogera*
5. Anterior dorsal hair short, as in Text-figure 19 6
   Anterior dorsal hair long (Text-figs. 21 and 22) ..... *Agapophytus*
6. Papillae in lateral white area of head about as far apart as papillae below antenna (Text-fig. 19) ..... *Acraspisa*
   Papillae in lateral white area of head about twice as far apart as papillae below antenna (Text-fig. 20) ..... *Acupalpa*

Pupae.

1. Thorax with alar spine, as in Text-figure 23 2
   Thorax without alar spine 3
2. Dorsal abdominal bristles in two rows on segments two to seven (Text-fig. 23) ..... *Platycarenum*
   Dorsal abdominal bristles in one row on segments two to seven (Text-figs. 10 and 27) ..... *Anabarrhynchus*
3. Sheath of labrum short, not reaching to edge of sheath of proboscis, as in Text-figures 29 and 38 4
   Sheath of labrum long, reaching beyond edge of sheath of proboscis, as in Text-figure 41 5
4. First abdominal segment with very small spines or without spines; on segments 2-7 abdominal spines strong (Text-figs. 30 and 33) ..... *Ectinorrhynchus* / *Taenogera*
   First abdominal segment with long spines; on segments 2-7 abdominal spines slender (Text-fig. 36) ..... *Acraspisa*
5. Abdominal spines strong (Text-figs. 42 and 45) ..... *Agapophytus*
   Abdominal spines slender (Text-fig. 39) ..... *Acupalpa*

Conclusions.

Therevid larvae are found frequently in gardens and bushland soil, but they are apparently of no economic importance, and the adult flies are inoffensive; these facts probably explain why there is so little published information on their life history.

The keys given for the larvae and pupae described in this paper are tentative only, for one cannot say whether the characters selected will be of use for other genera. For the larvae it was necessary to find very small differences to distinguish them, and it would be difficult, if not impossible, to identify the living larvae. The pupae, on the whole, are more readily distinguished, but it was impossible to separate satisfactorily the genus *Ectinorrhynchus* from the genus *Taenogera*. I hesitated to use the small differences in the abdominal spines because these vary considerably in different individuals of the same species and they even vary on the two sides of the same individual.

The specimens used in the preparation of this paper, e.g. the adult flies with pupal exuviae and slide mounts of the larval exuviae, have been deposited in the Macleay Museum at the University of Sydney.

Acknowledgements.

The writer is indebted to Professor P. W. F. Murray and Mr. A. R. Woodhill, Department of Zoology, University of Sydney, who made available laboratory accommodation at the Department; and to Mr. J. S. Mann, of the Biological Section, Department of Public Lands, Queensland, who identified the adult flies.

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ABSTRACT OF PROCEEDINGS.

ORDINARY MONTHLY MEETING.

31st May, 1950.

Dr. Lilian Fraser, Vice-President, in the Chair.

Dr. Fraser announced that the Council had elected Professor P. D. F. Murray to fill a vacancy on the Council.

Dr. Fraser congratulated Miss Muriel Morris, Linnean Macleay Fellow in Zoology on the award of an International Federation of University Women Fellowship for study overseas. Miss Morris had arranged to continue her studies on plankton at Oxford University.

Mr. F. K. Rickwood, B.Sc., Sydney University, was elected an Ordinary Member of the Society.

Library accessions amounting to 39 volumes, 182 parts or numbers, 22 bulletins, 3 reports and 6 pamphlets, total 252, had been received since the last meeting.

PAPERS READ.

1. Cytological Studies in the Myrtaceae. III. Cytology and Phylogeny in the Chamaelaucoideae. By S. Smith-White.

2. A Review of and Contribution to Knowledge of *Phylloglossum Drummondii*. By Frances M. V. Hackney.

3. A Revision of the Australian Species of the Genus *Lagenophora* Cass. By Gwenda L. Davis.

ORDINARY MONTHLY MEETING.

28th June, 1950.

Mr. D. J. Lee, President, in the Chair.

Mr. A. A. Day, Bexley, was elected an Ordinary Member of the Society.

Library accessions amounting to 9 volumes, 119 parts or numbers, 3 bulletins, 7 reports and 3 pamphlets, total 141, had been received since the last meeting.

PAPERS READ.

1. Notes on the Aphodiinae of Australia (Coleoptera, Scarabaeidae, Aphodiinae). By B. B. Given. (*Communicated by P. B. Carne.*)

2. The Morphology of the Immature Stages of *Aphodius howitti* Hope (Coleoptera, Scarabaeidae, Aphodiinae). By P. B. Carne.

3. Notes on Australasian Simuliidae (Diptera). II. By M. J. Mackerras and I. M. Mackerras.

NOTES AND EXHIBITS.

Coral Genera of Heron Island (Barrier Reef). By K. E. W. Salter and M. A. Besley.

Situated practically on the Tropic of Capricorn (23° 27′ S.), Heron Island is close to the southern limit of the Great Barrier Reef. A careful study of the coral fauna of the reef immediately north of this island shows that there are at least fifty-one species of Madreporaria. Preparatory to completion of all identifications, the following twenty-five genera are placed on record as occurring in this region:

PERFORATA.
Acropora (seven species)
Turbinaria (one species)
Astraeopora (one species)
Montipora (three species)
Porites (two species)*
Goniopora (one species)
Dendrophyllia (one species)

FUNGIDA.
Fungia (two species)†
Psammocora (two species)
Coscinaria (one species)

IMPERFORATA.
Pocillopora (one species)*
Stylophora (one species)
Seriatopora (one species)
Acrhelia (one species)
Galaxea (one species)
Cyphastrea (one species)
Echinopora (one species)
Favia (seven species)
Favites (four species)
Coeloria (four species)
Platygyra (one species)
Merulina (one species)
Hydnophora (three species)
Lobophyllia (two species)
Symphyllia (one species)

Coral growth is inhibited by certain factors such as the covering of their polyps by sand and by the active movements of the sea. Once identification is established, statistical data can be compiled and comparative counts made to show the relative abundance of particular species in relation to the above factors in the environment. The fauna and its growth as it occurs under such inhibitors contrasts with its distribution in deep pools where both sanding and wave action are reduced to a minimum.

ORDINARY MONTHLY MEETING.

26th JULY, 1950.

Mr. D. J. Lee, President, in the chair.

Miss Elise E. Sellgren, B.Sc., Coogee, was elected an Ordinary Member of the Society.

The President announced that following a request from the Information Service, C.S.I.R.O., which had resulted from one of the recommendations of the Royal Society Scientific Information Conference (July, 1948), the Council had decided that a synopsis of each paper published in the PROCEEDINGS would appear below the title of the paper. Such a synopsis will not interfere in any way with the arrangement of the paper but will facilitate the reading of the articles in the PROCEEDINGS. The synopses will be used as abstracts by Australian and overseas abstractors.

The President drew members' attention to the fact that Dr. Yao-tseng Tchan, Macleay Bacteriologist to the Society, would be arriving in Sydney shortly.

The President wished Miss Morris *bon voyage* on the eve of her departure to England.

Library accessions amounting to 6 volumes, 131 parts or numbers, 35 bulletins, 4 reports, total 176, had been received since the last meeting.

PAPERS READ.

1. A Classification of the Bacteria, with Special Reference to Non-pathogenic Eubacteria. By E. J. Ferguson Wood.

2. Australian Polyporaceae in Herbaria of Royal Botanic Gardens, Kew, and British Museum of Natural History. By G. H. Cunningham. (*Communicated by N. A. Burges.*)

3. A Revision of the Genus *Solenogyne* Cass. By Gwenda L. Davis.

NOTES AND EXHIBITS.

Mitotic Poisons. By Miss M. Hindmarsh.

A large number of very different chemical substances are known to inhibit mitosis in plant cells, but the inhibition is not always the same. Many act like colchicine and prevent spindle formation. However, they are usually not as efficient as colchicine in producing polyploids, since they also inhibit the entry into prophase and so gradually

* Additional species are thought to occur.

† Only two species were found at Heron Island; but a third was recorded from the Capricorns at One Tree Island.

suppress mitosis. Other substances produce effects similar to X-ray induced abnormalities which result in a permanent alteration to the chromosomes of the cell.

Mr. A. Musgrave exhibited specimens of *Stephanitis queenslandensis* Hacker, 1927, a member of the family Tingidae (Lace Bugs). The insect was first described by Hacker in the *Mem. Q'land Mus.*, ix, p. 28, from specimens from Tambourine Mountain and Brisbane, S.Q., on *Stephania hernandifolia* Walp., and in the following year he recorded it in the same publication from the Cairns district and Magnetic Island, N.Q. Specimens are in the Australian Museum collection from Lane Cove, Sydney, 28th April, 1946, collected by Mr. N. W. Rodd, on Azalea leaves, and also from Lindfield, 13th January, 1941, on Rhododendrons. Specimens have also been collected at Canberra, A.C.T., by Dr. A. J. Nicholson, February, 1944, and identified by Mr. Hacker as his species. This insect is well known to growers of Rhododendrons and Azaleas in the Sydney district, and references to the Lace Bug appear in salesmen's catalogues.

The genus *Stephanitis* is known from the Palaearctic and Oriental regions, and at least three species are known to attack Azaleas and Rhododendrons in Europe, United States of America, and South Africa, the best known being *Stephanitis* (*Leptobyrsa*) *rhododendri* Horvath, 1905, first recorded from Holland upon cultivated Rhododendrons. The Australian insect appears to be very closely related to this widespread European insect, which has been the subject of many papers and articles in scientific journals abroad. Nothing appears to have been written about the economic aspects of our Australian species, though it is understood that the N.S.W. Department of Agriculture has been carrying out experiments for its control.

ORDINARY MONTHLY MEETING.

30th August, 1950.

Mr. D. J. Lee, President, in the chair.

Miss Joy G. Garden, B.Sc.Agr., Sydney, and Mr. R. A. Oxenford, B.Sc., Lane Cove, were elected Ordinary Members of the Society.

The President congratulated Miss Adele Millerd, Linnean Macleay Fellow in Biochemistry, on the award of a research fellowship which would enable her to study at the California Institute of Technology.

Library accessions amounting to 14 volumes, 120 parts or numbers, 6 bulletins, 2 reports, 11 pamphlets, total 153, had been received since the last meeting.

The meeting then took the form of a number of short talks on various aspects of research in the New South Wales Department of Agriculture.

Dr. C. J. Magee introduced the work of the Department.

Mr. Grahame Edgar, Director of Veterinary Research, spoke on various aspects of the Division of Animal Industry. Investigations on problems of the animal industry of this State are centred in the Division of Animal Industry whose research centre is located at the Veterinary Research Station, Glenfield. The problems fall into three main categories, namely, animal health, nutrition and genetics. Whilst it is possible to make these broad differentiations, all three overlap and it is impossible to establish each as a separate entity. The importance of the work during the last ten years has been emphasized by the urgent necessity of increasing food production, especially of animal origin. Extraordinary inflated values for wool and leather have also stressed the significance of the animal industries in the national economy. The appraised price of wool during the war years was fixed at 1s. 3d. per lb., whereas the Australian Wool Clip during the 1949/50 selling season realized an overall price of nearly 5s. per lb. Animal health research will always remain paramount as maximum production from our flocks and herds can never be achieved unless a satisfactory standard of health is maintained. Disease in animals and birds is the greatest source of economic loss. Whilst Australia is happily situated in being free of many of the major livestock epizootics, the presence of Rinderpest, Foot and Mouth Disease, Rabies and many other animal plagues in the countries immediately north of Australia presents an ever present threat. The aims and objects of animal health research lie in the fields of preventive medicine.

The Veterinary Research Station, Glenfield, was established in 1923, and during the 27 years of its existence has developed and extended enormously in its field of activities. Since its inception over 400 scientific articles and papers have been published by the professional officers on the staff. The work of the Station falls into two categories, diagnosis and research. The diagnostic material received ranges from 20,000 to 30,000 specimens per annum, from animals and birds in various parts of New South Wales.

Research investigations on specific projects are located also at other centres in different parts of the State. For instance, at the Shannon Vale Nutrition Station, Glen Innes, a comprehensive series of experiments has been in progress for ten years whose aim is the investigation of the nutrition and breeding of Merino sheep on the granitic soils of New England. At Trangie Agricultural Experiment Station, blowfly investigations and progeny testing of Merinos are being intensively pursued and a new laboratory was opened early last year for studies and research on wool fibre. The Barooga Field Station located on the Murray River near Tocumwal is devoted solely to the investigation of the problem of Toxaemic Jaundice in sheep. This work is a co-operative effort with the Division of Animal Health and Production of C.S.I.R.O. Genetic and nutritional investigations of poultry are centred at the Seven Hills Poultry Experiment Farm.

Mr. Edgar then briefly mentioned investigations on tuberculosis and mastitis in cattle, contagious pustular dermatitis and blowfly strike in sheep, swine fever in pigs, fowl-tick fever in poultry. He also mentioned the new Nutrition Laboratory at Glenfield and experiments in the drought-feeding of merino sheep, the effects of diet on butterfat production, and under genetics, investigations on sheep and poultry.

Dr. S. L. Macindoe, Principal Research Agronomist, gave a brief account of the organization, progress and results of plant research conducted primarily on the ten Experiment Farms and two Agricultural Colleges in New South Wales. An active programme of plant introduction has been pursued as the basis of plant breeding investigations. The introduction of the rust resistant variety Walsh has enabled the linseed acreage in the State to increase from 500 acres in 1947-48 to an estimated 20,000 acres in 1950. Breeding for flag smut resistance in wheat has been more successful than breeding for resistance to stem rust. In 1942 and again in 1948 highly rust-resistant wheat varieties became susceptible to new races of stem rust. Varieties of better baking quality released by the University and the Department have resulted in one-third of the State's wheat acreage being sown to wheats of medium to strong baking quality. Three new potato varieties bred by the Department and two recently introduced are expected to be worth £150,000 annually in increased production. Eight new hybrid maize strains are expected to raise yields at least 20%. Considerable use has been made of genetic theory in the development of hybrid maize programmes. Experiments with crops and pastures are designed so as to determine the best varieties, fertilizers and methods of establishment in each region. Spectacular results have recently been obtained using molybdenum on cauliflowers, an expenditure of 3s. to 4s. per acre preventing losses of £100 to £300 per acre from Whiptail disease. Considerable success is being obtained in the use of selective weedicides on blackberries, and also on water hyacinth, Noogoora burr and Bathurst burr. In a New England rotation experiment the incorporation of red clover in an oats-maize cropping sequence has resulted in a greater total yield of oats and maize being obtained from four crops where Red Clover is included than from six where the red clover is omitted. Additional rotation experiments are being commenced to determine the best type of pasture, the length of pasture and the desirable cropping sequence in the arable phase. A rate of stocking experiment with deferred grazing is being conducted at Trangie in co-operation with the C.S.I.R.O.

Dr. F. T. Bowman, Special Fruit Research Officer, discussed research in Horticulture.

The research work of the Division of Horticulture deals with the problems of fruit production, handling and processing, and in organization it corresponds broadly with that of the Division of Plant Industry. The Division has a number of experiment orchards and other facilities for the carrying out of research, and often conducts its work on growers' properties, in packing houses, etc., where the problems occur.

The Division deals with perennial plants, and thus the problems of horticulture range from the propagation of plants, setting out the orchard, early care and training of the plants, to care of the mature and ageing plants. Other problems arise from the annual product, the fruit, from the stage of blossom bud initiation, through development and harvesting, to preparation for market or some form of storage or processing, as the case may be.

They were often concerned with cumulative effects, e.g., in irrigation practice, as well as with the effects of different seasonal influences, e.g., spray injuries. Hence, the results of research on fruit must usually be based on many years' work.

Some typical examples of research in the different sections of the subject are: Propagation research (introduction of rootstock for fruit trees), soil management of orchards (contour planting; suitable covercrops; and reduced tillage), irrigated soils, pruning, cropping (pollination; chemical thinning; stop drop sprays), fruit handling and processing.

Mr. S. L. Allman, Senior Entomologist, described the Entomological Branch which, as part of the Department of Agriculture, was formed in 1890, and for the past 60 years has been concerned with insect pest problems. Some of the earlier members specialized in systematic work, but in recent years the emphasis has been increasingly on the applied side. At the present time the staff consists of 14 entomologists, three of whom are permanently located in country districts. Most phases of pest control are undertaken, and this calls for co-operation with other Divisions of the Department of Agriculture, and also outside bodies, including the Department of Health.

Pests of fruit, vegetables and field crops, medical and veterinary pests, stored products and grain pests, industrial pests and quarantine safeguards against pest introductions have all received careful attention.

New insecticides and new equipment now tend to dominate the pest control field and present-day investigations are mainly concerned with reviewing pest control schedules from this viewpoint.

Dr. C. J. Magee, Chief Biologist, discussed research in the Biological Branch. The research work of the Biological Branch is directed mainly at preventing losses from plant diseases. In addition, some attention is given to dairy and food bacteriology and general biology.

The nature of the plant disease investigations varies but follows a conventional plan of determining the cause, exploring the conditioning factors and endeavouring to develop control measures. It usually happens that the control phase of the investigation is the most prolonged, consisting of a prescribed programme based on the researches and a series of modifications of the programme until a thoroughly satisfactory one that suits the disease, the grower and everybody else (i.e., the consumer and Health authorities) is evolved.

Examples of the nature of the work undertaken are indicated by the following investigations: Bunt, Black Spot of Apples, Whiptail of Cauliflower, Bean Scald, Exanthema (copper deficiency of Citrus), Phytophthora Rot of Citrus, Scaly Butt of Citrus, Stem-pitting of Grapefruit, Black Spot of Citrus, Bunchy Top of Bananas, and Foliar Nematode of Chrysanthemums.

ORDINARY MONTHLY MEETING.

27th September, 1950.

Mr. D. J. Lee, President, in the chair.

Professor H. N. Barber, M.A., Ph.D., Hobart, Tasmania; Mr. J. M. Hallinan, Bexley; Dr. Y. T. Tchan, Sydney, and Mr. J. L. Willis, B.Sc., Artarmon, were elected Ordinary Members of the Society.

The President referred to the death of Dr. G. A. Waterhouse, who had been a member of the Society since 1897 and was elected a Corresponding Member in 1943.

The President announced that the Council is prepared to receive applications for Linnean Macleay Fellowships tenable for one year from 1st January, 1951, from qualified candidates. Applications should be lodged with the Secretary not later than Wednesday, 1st November, 1950.

Library accessions amounting to 3 volumes, 33 parts or numbers, 3 bulletins, total 39, had been received since the last meeting.

PAPERS READ.

1. The Hair Tracts in Marsupials. V. A Contribution on Causation. By W. Boardman. (By title only.)

2. The Hair Tracts in Marsupials. VI. Evolution and Genetics of Tract Pattern. By W. Boardman. (By title only.)

3. The Hair Tracts in Marsupials. VII. A System of Nomenclature. By W. Boardman. (By title only.)

4. Further Notes on Experimental Crossing within the *Aëdes scutellaris* Group of Species (Diptera, Culicidae). By A. R. Woodhill.

5. A Note on Non-reciprocal Fertility in Matings between Sub-species of Mosquitoes. By S. Smith-White.

Professor N. A. Burges and Dr. N. C. W. Beadle gave talks on Recent Changes in the Vegetation of north-western New South Wales.

Dr. N. C. W. Beadle showed a number of kodaslides illustrating the striking contrast in the condition of the saltbush-bluebush country before and after the good rains of 1949 and 1950. The luxuriance of the regenerating communities, with their relatively high frequency of recently established perennials suggests that complete recovery of the communities will occur if stocking rates are judiciously controlled.

Professor Burges gave a brief account of the changes which have occurred in the Mulga country in the neighbourhood of Tibooburra since the abnormal rains of 1949. Kodachrome slides and a short length of coloured cinema film were used to illustrate the general appearance of the Mulga dune country in February and August, 1950.

ORDINARY MONTHLY MEETING.

25th October, 1950.

Mr. D. J. Lee, President, in the Chair.

Mr. K. R. Sharp, Cooma, N.S.W., was elected an Ordinary Member of the Society.

The President referred to the death of Dr. B. L. Middleton, of Murrurundi, who had been a member since 1937.

The President announced that the Council is prepared to receive applications for Linnean Macleay Fellowships tenable for one year from 1st January, 1951, from qualified candidates. Applications should be lodged with the Secretary not later than Wednesday, 1st November, 1950.

Library accessions amounting to 29 volumes, 60 parts or numbers, 47 bulletins, 1 report and 3 pamphlets, total 140, had been received since the last meeting.

PAPERS READ.

1. Respiration and Cell Division in Developing Oyster Eggs. By K. W. Cleland.

2. The Intermediary Metabolism of Unfertilized Oyster Eggs. By K. W. Cleland.

3. A New Genus and Four New Species of Phloecharinae (Coleoptera, Staphylinidae) from the Australian Region. By W. O. Steel. (*Communicated by J. W. T. Armstrong.*)

Professor P. D. F. Murray gave a short talk on The Fusion of Long Bones.

In the normal guinea-pig the tibia and fibula are not fused, but in guinea-pigs whose fibulae have been experimentally fractured one of the two halves comes into contact with the tibia and presumably rubs against it. In the region of contact the periostea fuse

and transform themselves into a pad of cartilage between the two large bones. Then blood vessels attack the cartilage from each of the two bones and resorb it, while bone is deposited in its place, as in ordinary "endochondral ossification".

Fusion between pairs of long bones occurs normally in many animals, and it seemed of interest to find out whether the fusion occurred by the same process where it was normal as in the guinea-pig, where it is abnormal. In the rat, the normal fusion of the tibia and fibula occurs just as in the guinea-pig, but in the metatarsals of the young chick, the tibia and fibula, and radius and ulna of the frog, the fusion occurs without the formation of cartilage.

Mr. B. R. A. O'Brien gave a short talk on Some Aspects of Regeneration in Earthworms.

ORDINARY MONTHLY MEETING.

29th November, 1950.

Mr. D. J. Lee, President, in the Chair.

The President announced that Miss Mary Hindmarsh had been reappointed to a Linnean Macleay Fellowship in Botany for 1951, and that Mr. N. C. Stevens and Mr. T. G. Vallance had been appointed Linnean Macleay Fellows in Geology for 1951.

Library accessions amounting to 7 volumes, 80 parts or numbers, 3 bulletins, 6 reports and 16 pamphlets, total 112, had been received since the last meeting.

PAPERS READ.

1. A Further Contribution on the Life History of *Pherosphaera*. By Charles G. Elliott. (*Communicated by P. Brough.*) (By title only.)

2. Notes on the Morphology and Biology of *Anabarrhynchus fasciatus* Macq., and other Australian Therevidae (Diptera, Therevidae). By Kathleen M. I. English.

Talks on northern Australia were given by Professor J. Macdonald Holmes, "The Geographical Background to the Problems of North Australia"; Dr. W. Kirkland, "Medical Problems of North Australia"; and Dr. N. W. G. Macintosh, "Comments on the Physical Types of the Aborigines of Arnhem Land". A talk prepared by Mr. W. Poggendorf on "Problems of Agriculture" was given by Mr. E. B. Furby.

SPECIAL GENERAL MEETINGS.

Two Special General Meetings were held on 8th and 22nd February, 1950, respectively, to alter Rule VI to read as follows:

Line 1: "The Annual Subscription shall be two guineas . . . ."

Lines 10–11: ". . . . , the sum of twenty guineas in lieu of further Annual Subscriptions."

Two Special General Meetings were held on 25th October and 29th November, 1950, respectively, to make an addition to Rule VI, as follows: "Any Ordinary Member who has paid the Annual Subscription for forty years shall be exempt from further payments."

LIST OF MEMBERS.

(15th December, 1950.)

ORDINARY MEMBERS.

1940 Abbie, Professor Andrew Arthur, M.D., B.S., B.Sc., Ph.D., c.o. University of Adelaide, Adelaide, South Australia.
1927 *Albert, Michel Francois, "Boomerang", 42 Billyard Avenue, Elizabeth Bay, Sydney.
1940 *Allman, Stuart Leo, B.Sc.Agr., M.Sc., Entomological Branch, Department of Agriculture, Farrer Place, Sydney.
1948 Anderson, Miss Beverley I., 19 Kareela Road, Chatswood, N.S.W.
1922 Anderson, Robert Henry, B.Sc.Agr., Botanic Gardens, Sydney.
1927 *Armstrong, Jack Walter Trench, "Callubri", Nyngan, N.S.W.
1938 Ashby, Professor Eric, D.Sc., D.I.C., F.L.S., Vice-Chancellor's Lodge, Lennoxvale, Belfast, N. Ireland.
1912 Aurousseau, Marcel, B.Sc., c.o. Mr. G. H. Aurousseau, 16 Woodland Street, Balgowlah, N.S.W.

1948 Baddams, Miss Greta, B.A., B.Sc., New England University College, Armidale, N.S.W.
1948 Balmain, Miss Judith Hope, M.Sc., Shinfield Dairy Research Institute, Reading, Eng.
1950 *Barber, Professor H. N., M.A., Ph.D., University of Tasmania, Hobart, Tasmania.
1935 *Beadle, Noel Charles William, D.Sc., Botany School, Sydney University.
1946 Bearup, Arthur Joseph, 66 Pacific Avenue, Penshurst, N.S.W.
1940 Beattie, Mrs. Joan Marion, M.Sc. (*née* Crockford), Bradley Street, Cobar, N.S.W.
1907 Benson, Professor William Noel, B.A., D.Sc., F.G.S., University of Otago, Dunedin, New Zealand.
1948 Besly, Miss Mary Ann Catherine, B.A., 7 Myra Street, Wahroonga, N.S.W.
1948 Birch, Louis Charles, B.Ag.Sc., M.Sc., Department of Zoology, Sydney University.
1941 Blake, Stanley Thatcher, M.Sc., Botanic Gardens, Brisbane, Queensland.
1929 Boardman, William, M.Sc., Department of Zoology, University of Melbourne.
1947 Bradhurst, Miss Peggy Joan, B.Sc., 25 Belgium Avenue, Roseville, N.S.W.
1946 Brett, Robert Gordon Lindsay, B.Sc., 7 Petty Street, West Hobart, Tasmania.
1923 Brough, Patrick, M.A., D.Sc., B.Sc.Agr., Botany School, Sydney University.
1950 Brown, Kenneth George, 6 Dolphin Street, Randwick, N.S.W.
1924 Browne, Mrs. Ida Alison, D.Sc., Department of Geology, Sydney University.
1949 Browne, Lindsay Blakeston Barton, 34 Kent Road, Rose Bay, N.S.W.
1911 Browne, William Rowan, D.Sc., Department of Geology, Sydney University.
1947 Browning, T. O., Waite Agricultural Research Institute, Adelaide, South Australia.
1943 Bryan, Clement, B.A., Intermediate High School, Corowa, N.S.W.
1949 Burden, John Henry, 1 Havilah Street, Chatswood, N.S.W.
1931 *Burges, Professor Norman Alan, M.Sc., Ph.D., Botany School, Sydney University.
1920 Burkitt, Professor Arthur Neville St. George Handcock, M.B., B.Sc., Medical School, Sydney University.

1949 Campbell, Mrs. Emily Mary, 22 Madeline Street, Hunter's Hill, N.S.W.
1927 Campbell, Thomas Graham, Council for Scientific and Industrial Research, Box 109, Canberra, A.C.T.
1934 *Carey, Professor Samuel Warren, D.Sc., Geology Department, University of Tasmania, Hobart, Tasmania.
1949 Carne, Phillip Broughton, B.Agr.Sc. (Melb.), Division of Entomology, C.S.I.R.O., Box 109, Canberra, A.C.T.
1905 Carne, Walter Mervyn, c.o. Department of Commerce and Agriculture, Reliance House, Flinders Lane, Melbourne, Victoria.
1947 Carroll, Miss Dorothy, B.A., B.Sc., Ph.D., D.I.C., Science House, 157 Gloucester Street, Sydney.
1936 *Chadwick, Clarence Earl, B.Sc., Entomological Branch, Department of Agriculture, Farrer Place, Sydney.
1899 Cheel, Edwin, 40 Queen Street, Ashfield, N.S.W.
1947 Christian, Stanley Hinton, Malaria Survey, Banz, Central Highlands, via Lae, New Guinea.
1932 *Churchward, John Gordon, B.Sc.Agr., Ph.D., 1 Hunter Street, Woolwich, N.S.W.
1946 Clark, Laurance Ross, M.Sc., C.S.I.R.O., Box 109, Canberra, A.C.T.
1901 Cleland, Professor John Burton, M.D., Ch.M., 1 Dashwood Road, Beaumont, Adelaide, South Australia.
1942 Cleland, Kenneth Wollaston, M.B., Department of Anatomy, Sydney University.
1931 Colefax, Allen Neville, B.Sc., Department of Zoology, Sydney University.

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* Life Member.

1946 Colless, Donald Henry, Borneo Malaria Research, Labuan, British North Borneo.
1942 Copland, Stephen John, B.Sc., Chilton Parade, Warrawee, N.S.W.
1947 Costin, Alec Baillie, 12 Barambah Road, Roseville, N.S.W.
1908 Cotton, Professor Leo Arthur, M.A., D.Sc., Department of Geology, Sydney University.
1928 Craft, Frank Alfred, B.Sc., 10 Bank Street, Wellington, N.S.W.
1950 Crawford, Lindsay Dinham, B.Sc., 4 Dalton Avenue, West Hobart, Tasmania.

1945 Davis, Mrs. Gwenda Louise, B.Sc., New England University College, Armidale, N.S.W.
1948 Davison, Miss Daphne Claire, M.Sc., "Carinya", Waratah Street, Palm Beach, N.S.W.
1950 Day, Alan Arthur, 13 Besborough Avenue, Bexley, N.S.W.
1936 Day, Maxwell Frank, Ph.D., B.Sc., C.S.I.R.O., Box 109, Canberra, A.C.T.
1934 Day, William Eric, 23 Gelling Avenue, Strathfield, N.S.W.
1925 de Beuzeville, Wilfred Alexander Watt, J.P., "Melamere", Welham Street, Beecroft, N.S.W.
1937 Deuquet, Camille, B.Com., 126 Hurstville Road, Oatley, N.S.W.
1927 *Dixson, Sir William, "Merridong", 586 Gordon Road, Killara, N.S.W.
1948 Drover, Donald P., Institute of Agriculture, University of Western Australia, Nedlands, W.A.
1937 Dulhunty, John Allan, D.Sc., Department of Geology, Sydney University.
1926 Dumigan, Edward Jarrett, 10 High Street, Toowoomba, Queensland.
1946 Durie, Peter Harold, B.Sc., C.S.I.R.O., Veterinary Parasitology Laboratory, Yeerongpilly, Brisbane, Queensland.

1948 Ealey, Eric H. M., 18 Ray Road, Epping, N.S.W.
1941 Edwards, Eric Thomas, Ph.D., M.Sc.Agr., National Press Pty. Ltd., 126-130 Phillip Street, Sydney.
1949 Elliott, John Henry, 8 Shellcove Road, Neutral Bay, N.S.W.
1947 Endean, Robert, M.Sc., 15 Milton Avenue, Eastwood, N.S.W.
1930 English, Miss Kathleen Mary Isabel, B.Sc., 1 Mt. Morris Street, Woolwich, N.S.W.

1947 Fenton, Miss Enid Grace, 45 Cecil Street, Gordon, N.S.W.
1948 Fraser, Ian McLennan, 131 Fox Valley Road, Wahroonga, N.S.W.
1948 Fraser, Miss Judith A., 14 Milray Avenue, Wollstonecraft, N.S.W.
1930 Fraser, Miss Lilian Ross, D.Sc., "Hopetoun", 25 Bellamy Street, Pennant Hills, N.S.W.
1939 Frohlich, Mrs. Frances Marie Veda, D.Sc., No. 5, 176 Ebley Street, Bondi Junction.

1950 Garden, Miss Joy Gardiner, B.Sc.Agr., Botanic Gardens, Sydney.
1935 *Garretty, Michael Duhan, M.Sc., "Surry Lodge", Mitcham Road, Mitcham, Vic.
1944 Greenwood, William Frederick Neville, c.o. Colonial Sugar Refining Co. Ltd., Lautoka, Fiji.
1946 Griffiths, Mrs. J. F. G., B.Sc. (*née* Crust), 81 Muston Street, Mosman, N.S.W.
1936 Griffiths, Mervyn Edward, M.Sc., Australian Institute of Anatomy, Canberra, A.C.T.
1939 *Gunther, Carl Ernest Mitchelmore, M.B., B.S., D.T.M., Bulolo, New Guinea.

1950 Hallinan, John Michael, "Quirindi", 65 Dunmore Street, Bexley, N.S.W.
1928 Hamilton, Edgar Alexander, 16 Hercules Street, Chatswood, N.S.W.
1917 Hardy, George Huddleston Hurlstone, "The Gardens", Letitia Street, Katoomba, N.S.W.
1947 Harker, Miss Janet Elspeth, B.Sc., Department of Zoology, University of Manchester, Manchester, Eng.
1932 Harris, Miss Thistle Yolette, B.Sc., 14 Pacific Street, Watson's Bay, N.S.W.
1947 Henderson, David Leonard Wylie, L.S.M.I.S., "Berida", R.M.B. 420, Bourke, N.S.W.
1930 Heydon, George Aloysius Makinson, M.B., Ch.M., Flat 5, 79 O'Sullivan Road, Rose Bay, N.S.W.
1938 Hill, Miss Dorothy, M.Sc., Ph.D., Department of Geology, University of Queensland, Brisbane, Queensland.
1943 Hindmarsh, Miss Mary Maclean, B.Sc., Botany School, University of Sydney, N.S.W.
1930 Holmes, Professor James Macdonald, Ph.D., B.Sc., F.R.G.S., F.R.S.G.S., Department of Geography, Sydney University.
1943 Horowitz, Benzoin, Engr.Agr.S., Dr.Agr.Sc. (Cracow, Poland), Waite Institute, Private Mail Bag, Adelaide, S.A.
1932 Hossfeld, Paul Samuel, M.Sc., 132 Fisher Street, Fullarton, South Australia.
1942 Humphrey, George Frederick, M.Sc., Department of Biochemistry, Sydney University.

1917 Jacobs, Ernest Godfried, "Cambria", 106 Bland Street, Ashfield, N.S.W.
1938 Jacobs, Maxwell Ralph, D.Ing., M.Sc., Dip.For., Commonwealth Forestry Bureau, Canberra, A.C.T.
1947 Johnson, Lawrence Alexander Sidney, 178 Beecroft Road, Cheltenham, N.S.W.
1945 Johnston, Arthur Nelson, B.Sc.Agr., Hawkesbury Agricultural College, Richmond, N.S.W.

* Life Member.

1907 Johnston, Professor Thomas Harvey, M.A., D.Sc., F.L.S., University of Adelaide, Adelaide, South Australia.
1948 Joklik, Wolfgang, M.Sc., 74 New South Head Road, Vaucluse, N.S.W.
1937 Jones, Mrs. Valerie Margaret Beresford, M.Sc. (*née* May), Mooloolabel Esplanade, Narrabeen, N.S.W.
1930 Joplin, Miss Germaine Anne, B.Sc., Ph.D., "Huyton", 18 Wentworth Street, Eastwood, N.S.W.
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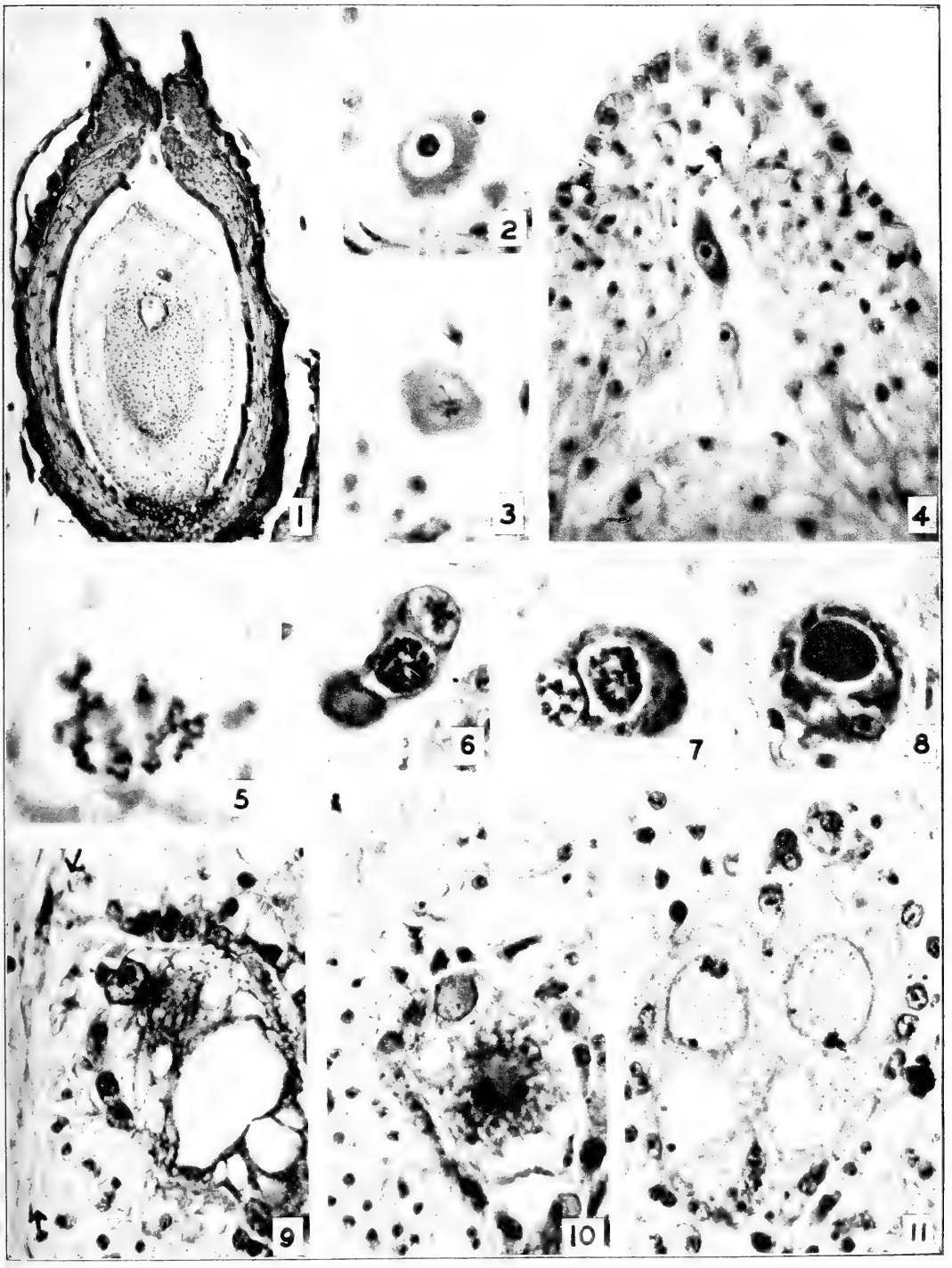
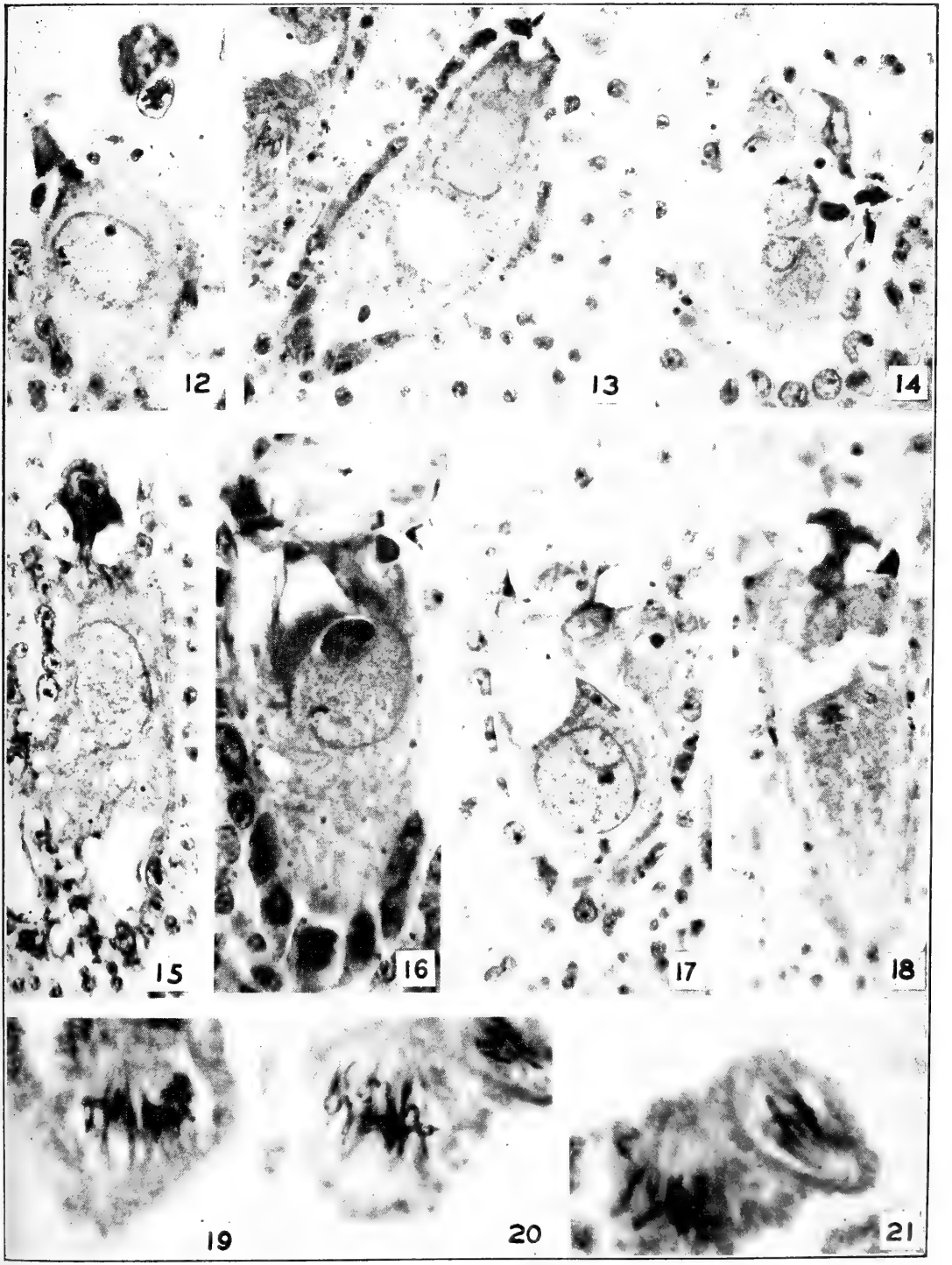


Pherosphaera hookeriana.



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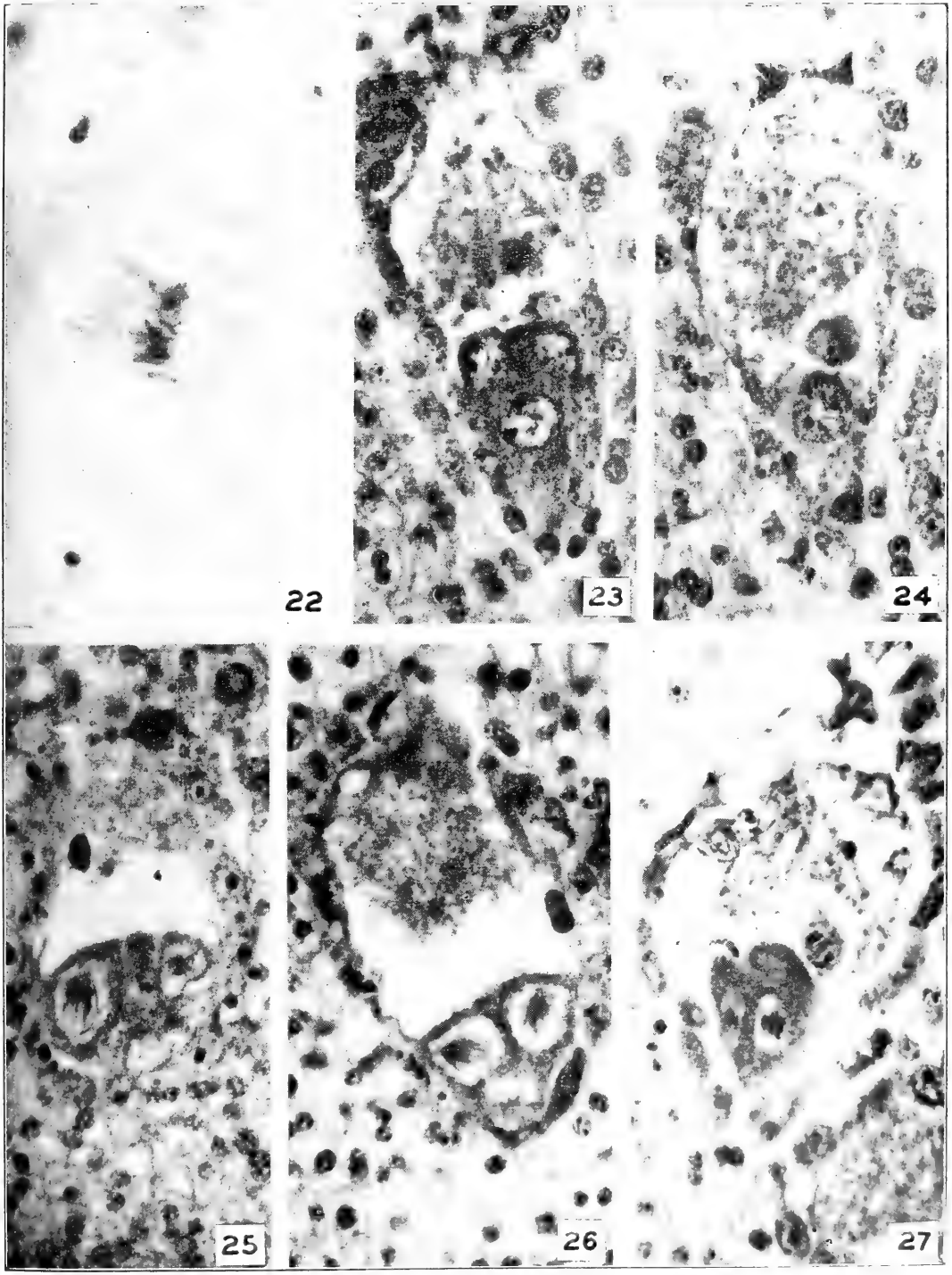

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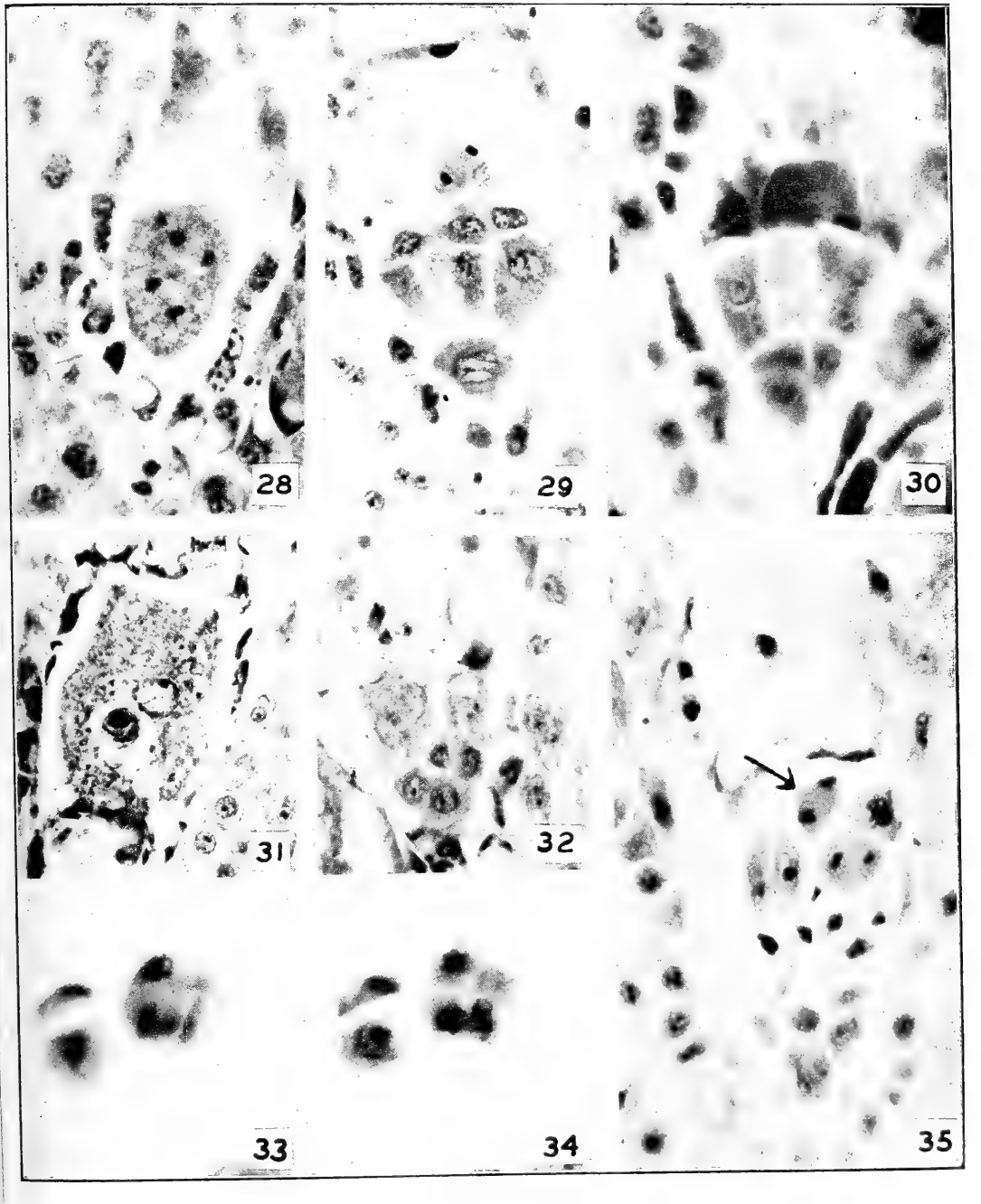

Pherosphaera hookeriana.

(*Issued 6th June, 1950.*)

Vol. LXXV. **Nos. 347-348.**
Parts 1-2.

THE

PROCEEDINGS

OF THE

LINNEAN SOCIETY

OF

NEW SOUTH WALES

FOR THE YEAR

1950.

Parts 1–2 (*i–xxiv, 1–98*).

CONTAINING THE PROCEEDINGS OF THE ANNUAL GENERAL MEETING AND PAPERS READ IN MARCH-APRIL.

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[Plates i-iv.]

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1875–76	I*	3 0	3 0	5 0	7 6	1899	XXIV	12 6	12 0	10 0	10 6
1877	II	4 0	4 0	4 0	—	1900	XXV	8 0	10 6	10 6	17 6
1878	III	5 0	5 0	6 0	7 6	1901	XXVI	10 0	9 0	5 0	17 6
1879	IV	6 0	6 0	8 0	6 6	1902	XXVII	7 0	7 6	7 6[3]	15 0
1880	V	6 6	—	7 6	7 6	1903	XXVIII	9 0	12 6	14 0	15 0
1881	VI	6 0	10 0	10 0	—	1904	XXIX	10 0	7 6	9 0	10 0
1882	VII	7 6	10 0	5 0	10 0	1905	XXX	6 0[4]	10 0	12 6	8 0[5]
1883	VIII	10 0	5 0	7 0	8 0	1906	XXXI	12 6	12 6	12 6	15 0
1884	IX	8 0	12 0	25 0	25 0	1907	XXXII	8 0	8 6	15 0	18 0
1885	X	12 0	7 6	15 0	17 6	1908	XXXIII	7 0	9 0	14 0	12 6
1886	I†	10 6	12 0	13 0	12 6	1909	XXXIV	12 0	17 0	14 0	16 6
1887	II	7 0	8 0	12 0	27 0	1910	XXXV	11 0	11 0	7 0	12 6
1888	III	15 0	24 0	20 0	18 0	1911	XXXVI	9 6	9 6	9 6	10 0
1889	IV	11 0	16 0	19 0	11 0	1912	XXXVII	8 6	25 0	12 6	15 0
1890	V	11 0	9 0	9 0	9 0	1913	XXXVIII	14 0	7 6	6 0	13 0
1891	VI	10 0	9 6	17 0	7 6	1914	XXXIX	13 0	17 0	25 0	19 0
1892	VII	6 6	4 6	8 0	8 0	1915	XL	17 0	12 6	10 0	11 0
1893	VIII	5 0	11 0	6 0	9 0	1916	XLI	10 0	12 0	15 0	19 0
1894	IX	12 0	12 0	13 0	8 0	1917	XLII	14 0	9 0	12 6	16 6
1895	X	15 0	8 6	10 0[1]	12 0	1918	XLIII	20 0	14 0	21 0	19 0
1896	XXI	9 0[2]	6 6	7 6	27 6	1919	XLIV	12 6	11 6	17 6	13 0
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1898	XXIII	3 0	6 0	12 0	14 0	1921	XLVI	9 0	8 0	7 6	9 6

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1923* ..	XLVIII	2 6	12 0	16 0	13 6	2 0	—
1924* ..	XLIX	2 0	13 6	12 6	10 0	2 0	—
1925* ..	L	2 0	12 0	8 9	14 6	2 0	—
1926* ..	LI	2 0	13 6	9 6	15 0	2 0	—
1927* ..	LII	2 6	10 6	14 0	12 3	2 0	—
1928 ..	LIII	2 0	8 6	8 9	10 0	9 0	2 0
1929 ..	LIV	2 0	6 3	8 6	11 0	12 6	2 0
1930 ..	LV	2 0	10 0	8 9	8 6	16 3	2 0
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1933 ..	LVIII	8 0	11 9	8 6	1947 ..	LXXII	8 3	12 0	18 6
1934 ..	LIX	6 6	8 6	10 9	1948 ..	LXXIII	13 0	20 0	25 0
1935 ..	LX	7 9	10 3	11 3	1949 ..	LXXIV	23 6	23 6	23 6
1936 ..	LXI	6 6	7 0	9 9	1950 ..	LXXV	23 6		
1937 ..	LXII	6 0	9 3	8 9					
1938 ..	LXIII	6 3	11 6	9 9					
1939 ..	LXIV	12 3	12 3	8 9					
1940 ..	LXV	11 9	10 6	8 0					
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1942 ..	LXVII	9 0	12 3	6 9					
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1944 ..	LXIX	6 6	9 0	8 0					
1945 ..	LXX	6 0	8 9	13 6					

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(*Issued 22nd September, 1950.*)

Vol. LXXV.
Parts 3-4.

Nos. 349-350.

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OF

NEW SOUTH WALES

FOR THE YEAR

1950.

Parts 3–4 (99–249).

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[Plates v-viii.]

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1881	VI	6 0	10 0	10 0	—	1904	XXIX	10 0	7 6	9 0	10 0
1882	VII	7 6	10 0	5 0	10 0	1905	XXX	6 0[4]	10 0	12 6	8 0[5]
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1890	V	11 0	9 0	9 0	9 0	1913	XXXVIII	14 0	7 6	6 0	13 0
1891	VI	10 0	9 6	17 0	7 6	1914	XXXIX	13 0	17 0	25 0	19 0
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1893	VIII	5 0	11 0	6 0	9 0	1916	XLI	10 0	12 0	15 0	19 0
1894	IX	12 0	12 0	13 0	8 0	1917	XLII	14 0	9 0	12 6	16 6
1895	X	15 0	8 6	10 0[1]	12 0	1918	XLIII	20 0	14 0	21 0	19 0
1896	XXI	9 0[2]	6 6	7 6	27 6	1919	XLIV	12 6	11 6	17 6	13 0
1897	XXII	10 0	8 6	9 0	12 6	1920	XLV	10 9	7 6	9 0	11 0
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1924* ..	XLIX	2 0	13 6	12 6	10 0	2 0	—
1925* ..	L	2 0	12 0	8 9	14 6	2 0	—
1926* ..	LI	2 0	13 6	9 6	15 0	2 0	—
1927* ..	LII	2 6	10 6	14 0	12 3	2 0	—
1928 ..	LIII	2 0	8 6	8 9	10 0	9 0	2 0
1929 ..	LIV	2 0	6 3	8 6	11 0	12 6	2 0
1930 ..	LV	2 0	10 0	8 9	8 6	16 3	2 0
1931 ..	LVI	2 0	8 0	7 0	7 3	8 9	2 0

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1934 ..	LIX	6 6	8 6	10 9	1948 ..	LXXIII	13 0	20 0	25 0
1935 ..	LX	7 9	10 3	11 3	1949 ..	LXXIV	23 6	23 6	23 6
1936 ..	LXI	6 6	7 0	9 9	1950 ..	LXXV	23 6	23 6	
1937 ..	LXII	6 0	9 3	8 9					
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1939 ..	LXIV	12 3	12 3	8 9					
1940 ..	LXV	11 9	10 6	8 0					
1941 ..	LXVI	6 6	9 3	9 6					
1942 ..	LXVII	9 0	12 3	6 9					
1943 ..	LXVIII	5 6	6 9	8 3					
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