BULLETIN

OF THE

MUSEUM OF COMPARATIVE ZOÖLOGY

AT

HARVARD COLLEGE, IN CAMBRIDGE.

VOL. XIX.

CAMBRIDGE, MASS., U. S. A.
1890.

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KRAUS REPRINT CORPORATION
New York
1967

Printed in U.S.A.

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No. 1. — *Studies on Lepidosteus.* Part I. By E. L. Mark.[1]

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I. Introduction.

I became deeply interested in the embryology of Lepidosteus through reading the paper on that subject published by Mr. Agassiz in 1878, and determined to avail myself of the first opportunity of following up the study. I desired particularly to pursue the development of the early stages. A little later I was further incited to this by the brief account of it which Balfour gave in the second volume of his Comparative Embryology (1881).

I had already formed plans for going to Black Lake, in the vicinity of Ogdensburg, N. Y., in the spring of 1882, for the purpose of getting material for the contemplated study, when I learned from Mr. Agassiz that Balfour had in hand an extensive paper on the subject. Mr. Agassiz also informed me that he still had left a part of the material from which Balfour had been supplied, and he kindly placed this at my

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[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoology at Harvard College, No. XV.

disposal. I thought it would be desirable, nevertheless, to procure an additional supply of eggs and embryos, and especially to endeavor to rear the young beyond the stages already in my possession.

On account of my duties in College it was impossible for me to leave Cambridge until nearly the middle of June, — almost a month after the usual time of spawning. Nevertheless, owing to the extreme backwardness of the season, I hoped that I might be able to procure some material, and was confirmed in this by correspondence with Mr. J. H. Perry, through whom I learned that up to a day or two before the time I had fixed upon for setting out the gar-pike had not spawned.

I arrived at Black Lake on the evening of June 13th. The weather had meantime grown warm, and the fish had already spawned, but I was able to secure some eggs which were not very far advanced in development. By a number of processes I killed and preserved at short intervals sets of embryos which presumably belonged to the same lot of spawn. The eggs which were collected from different localities were kept in separate earthen-ware dishes and supplied with fresh water every twelve hours. In this way the embryos were easily kept alive until they hatched. Then they soon attached themselves by means of their peculiar maxillary disks to the sides of the dishes, and near the surface of the water, where they clung with a tenacity truly surprising.

A number of eggs were preserved — principally by means of Kleinenberg's picro-sulphuric mixture — at intervals of a few hours, beginning on the afternoon of June 14th and extending through several succeeding days. This method was controlled at intervals by preservations made in alcohol, in chromic acid, in osmic acid followed by potassic bichromate, and in the last named reagent alone.

Besides the large number of embryos which were preserved at Black Lake, I took away with me (June 20) many more — upwards of a hundred — which had recently hatched. These living fishes were carried in a narrow-necked tin pail, to the sides of which they adhered very firmly.

Instead of returning directly to Cambridge I took the gar-pike with me to my summer residence on the south shore of Lake Erie, about forty miles southwest of Buffalo, N. Y. Although this journey extended over more than three hundred miles the fishes survived it well. The greater part of it, however, was rendered comparatively easy, since it was made by steamboat instead of railway. It is fortunate for such an undertaking that these fishes have so large a yolk-sac, since it obviates the necessity of procuring food for many days after hatching.

The question of being able to raise them beyond the stages already secured by Mr. Agassiz so evidently depended on finding a suitable food for them, that I spared no pains to accomplish this end. Many kinds of meat and fish were minced and fed to them, but none of these was acceptable. The minced liver, which Mr. Agassiz used with success at first, was likewise refused. Fragments of meat were suspended in the water by fine threads, but neither when moved about nor when left perfectly quiet did they seem to attract attention. Great numbers of water-fleas (Cladocera) were put in the water with the young fishes, but the latter made no attempts to catch them. It was not until after many fruitless trials that organisms were found which were seized with such eagerness, and so persistently, as to leave no doubt that they were the natural food of the young gar-pike. These were *the larvæ of the common mosquito.* They constituted the exclusive diet of the young fishes until the latter became large enough to catch and swallow minute "mud minnows" (Fundulus), on which they subsequently fed as long as they were kept alive.

When it was once ascertained that the young fishes would take mosquito larvæ, there was no longer any serious question about the feasibility of rearing them, nor was it doubtful that these larvæ formed their natural food, for the shallow and quiet waters at the margins of Black Lake and along the creeks which feed it abound in mosquitos. It was by means of this diet that the fishes were kept in a thriving condition during the stages immediately following the absorption of the yolk.

From the 20th of June until the 1st of July specimens were, with a few exceptions, killed every twenty-four hours; and from the 1st of July until the beginning of August, usually at intervals of about forty-eight hours.

By the 3d of August there remained besides those which had not been preserved only about a dozen living fishes. On that date I started for Newport, R. I., travelling by rail to New York. These remaining fishes were carried by hand in a tin pail suspended by a spring; but owing to the difficulty of carrying in the pail a sufficient number of mosquito larvæ, and more particularly to the impossibility of properly renewing the water, about half of them succumbed to the unfavorable conditions of railway travel, and were put into alcohol. One more died on the way from New York to Newport; but the remaining ones, having been fed on larvæ after my arrival at Newport, appeared to thrive. At the end of a month they were taken to Cambridge, where they were put into a large glass jar and supplied with running hydrant water.

Here they were also kept on the diet of mosquito larvæ until from one cause or another they had all died.

My failure to secure early stages of the eggs in the spring of 1882 made me desirous of repeating the attempt at a more seasonable time the following year. With this object in view I left Cambridge for Ogdensburg, May 18, 1883.

Judging from my previous experience that it would be difficult to procure fertilized eggs in sufficient quantities without great labor, if they were to be individually detached from the rocks, I procured several yards of thin muslin of a color resembling the stones in the lake. I planned sinking this and loading it with small stones in the water on some of the "points" most frequented by the gar-pikes at spawning time. I hoped in that way to secure a large number of eggs firmly attached to the cloth, which I could then remove to a box suitably provided with wire nettings to allow the necessary circulation of the water. Had it proved successful, this device would have enabled me to have under control the eggs thus acquired, and would have allowed me a degree of certainty as to the age of the preserved material not otherwise easily attainable.

Unfortunately for my plans, the weather proved to be in several ways very unpropitious. A long period of cold and rain delayed the spawning to a time much later than common, and when at length, a few days after the 1st of June, the weather and water became warm enough to impel to the act of spawning, such high winds prevailed that it was impossible to watch the movements of the fishes, and the most of them had spawned before the water became quiet enough to allow one to discover their places of rendezvous. Some of the localities which they had visited with the greatest constancy during the past years were apparently deserted. Moreover, the cloths, which had of necessity been anchored near the shore, were either set free by the dashing of the waves, or rolled into ropes which presented a comparatively small surface for the reception of ova.

The limited time in the latter part of May left at my command, after completing some work which I was compelled to take with me, was utilized in studying the ovarian ova of females captured before the spawning period arrived, and in some attempts at artificial fertilization. I then succeeded in getting a fairly satisfactory knowledge of the interesting structure of the egg membranes and of the micropyle, but I did not learn the peculiar relation of the latter to the granulosa until a few months later, when I had made microscopic sections of the whole ovarian egg, including membrane and granulosa.

As the outcome of this journey I secured a number of series of eggs, beginning with the early stages of segmentation, from which I prepared at intervals and by various methods a considerable quantity of material. I was also able to bring to Cambridge about two hundred young fishes just hatched. Some of these were kept alive until September 26, 1886, — nearly three and a half years.

The fishes brought to Cambridge were put into running hydrant water and fed on mosquito larvæ for several weeks, — until about the 1st of August, — when they were large enough to swallow small "mud minnows" of nearly their own size. These were gradually substituted for the larvæ, and those fishes which were large enough to avail themselves of this kind of diet grew much more rapidly than their mates.

I have also received some eggs from Mr. Perry since my last trip to Black Lake, and although his attempts at fertilization did not prove to be more successful than my own, I still hope to secure before long the early stages which are needed to fill the gaps in my material.

II. Habits of the Young Fishes.

The habits of young gar-pike have already been quite fully described by A. Agassiz ('78^a) and Wilder ('76, '77), so that I shall not have much to add to what has been previously published.

When first hatched the fish is so small in comparison with the size of the yolk-sac that it swims only with the greatest difficulty, and its movements are anything but graceful. It is so disinclined to swim, that, were it left alone in water sufficiently pure to meet its requirements, I have no doubt it would not move from the point of its first attachment for many hours, or even days. When hatched in confinement the young fishes always swim nearly up to the surface of the water and attach themselves to the sides of the dish. When there are a large number of them they may attach themselves to floating objects. Frequently the superficial film of the water — aided possibly by secretions from the oral disk — serves to support an individual in the middle of the dish. Sometimes half a dozen or more individuals form in a cluster, and appear to hang suspended simply from the surface of the water. It is evident that they are not merely floating in the vertical position, because in such cases the surface of the water in their vicinity is always more or less depressed, and upon the slightest touch the fishes begin at once to sink slowly; if the water has not been too much disturbed, they make no motion while sinking until they have nearly reached the bottom. Before they actually touch the bottom of the dish they appear to recognize their proximity to it, and then begin to make vigorous efforts to swim up to the surface again. This is apparently a very laborious undertaking, and, if they fail to attach themselves at once, they again begin to sink slowly; they seldom attach themselves at the bottom, — especially if the water has remained for some time unchanged, — but always as near the surface of the water as possible. If there are too many to be accommodated in a single row, those last to come crowd in between the tails of those already attached, thus forming a second row; but if there are still others, they usually attach themselves to other fishes rather than take a lower position on the sides of the dish. During the period of yolk absorption they hang pendent and nearly motionless, except for the respiratory movements; those which hang from the surface of the water are vertical, and any sudden motion in the water shows that their bodies are quite limp. When the absorption of the yolk is well advanced, the flexibility of the body is shown in a striking way by the snake-like motions which the animal slowly executes while remaining attached.

The disinclination to swim lasts about as long as the yolk-sac persists. With the gradual disappearance of the latter, the fishes show an increasing tendency to swim about. When at length the sac is nearly absorbed they rest in quite another way. They float near the surface, taking a horizontal position, and remaining perfectly straight and motionless until disturbed; whereupon, by vigorous strokes of the tail, they swim away with remarkable celerity. In transferring the fishes from one dish to another I was accustomed to use a small spoon, but after the absorption of the yolk-sac I found it exceedingly difficult to capture them in that way, so rapid were their movements. The stage at which the fishes begin to swim and float is reached in eight or ten days after hatching. Even at this early age locomotion is accomplished by two distinct methods. The rapid motions are executed by vigorous strokes of the whole caudal region. A slower, gliding motion is maintained by means of the very rapid vibrations of the extreme end of the tail, which are so characteristic of the caudal filament at a later stage, and by the still more rapid motions of the pectoral fins. Not only do the pectorals vibrate when the tip of the tail is motionless, and *vice versa*, but either of the pectorals may be in rapid motion while the other is at rest. This second method of locomotion is apparently very serviceable to the fish, in allowing it to approach its prey unobserved.

At a later stage of development, when the upper lobe of the tail is reduced to a caudal filament, this gliding motion is accomplished, principally at least, by the action of the pectorals. When the fish is advancing, these fins are directed obliquely backward; but when, as often happens, the motion becomes retrogressive, they are directed more nearly at right angles to the body. The motion of the fins is so rapid, that I have been unable to determine by observation if, as is probable, the direction of the *stroke* is reversed in the two cases. Not only the direction of the long axis of the fin, but also the inclination of its transverse axis to the horizon, is conspicuously changed at such times.

The vibrating movement of the caudal filament perhaps assists the forward motion of the fish, but it cannot be considered essential to it, since the filament often remains motionless while the animal is gliding by means of the pectorals. The amplitude of the vibrations made by the filament is not great in any case, — about 15°, — and the terminal half alone is vibratory. When in motion the direction of its axis is usually continuous with that of the spinal column, although it *may* droop more or less while in motion, and is quite liable to do so when at rest; it then presents an even curvature, as seen from the side, and often inclines a little either to the right or left.

When the fish is stemming a current, or, in swimming, is directing the head downward, the caudal filament is kept in rapid vibration; it then takes a dorsal turn, and the curvature is rather abrupt at its base. The whole curvature may amount to enough to make the extreme tip of the filament perpendicular to the axis of the body, but usually it is much less.

During the night of August 6–7, 1883, one of the individuals hatched in June of that year escaped from the tank, and was found in the morning lying in only sufficient water to keep the body moist. Upon being returned to the tank, though still able to swim, it showed evident signs of weakness. The body was considerably arched, just as it has been figured for somewhat younger fishes by A. Agassiz ('78^a, Plate IV. Fig. 39, and Plate V.). I think this case suggests an explanation of the peculiar curved shape exhibited by the fishes reared by Mr. Agassiz. I had already in the previous year imagined that the arched condition was not common, for all my fishes were quite straight, at least so long as they were well nourished. The curved condition of the escaped fish was apparently due to muscular weakness; the curvature was also accompanied by a slight distortion from the sagittal plane. Inasmuch as it subsequently regained its normal condition and became straight, I have no doubt that the curved condition is abnormal, and its appearance in the fishes raised by Mr. Agassiz was probably owing to insufficient nutrition. The control of the pectorals in the case of this escaped fish was not lost, although somewhat impaired. The fish was also able at once to execute vigorous though ill-planned motions with the tail, and was therefore able to swim rapidly, but in a reckless manner. The control of the caudal filament, however, was entirely lost; there was not the least trace of the rapid vibratory movement, even when the pectorals were in active motion. By the following day the use of the caudal filament had been partially regained; but its vibrations were rather feeble, and were resumed only after long intervals of repose.

The temperature of the water greatly affects the power of locomotion, and very cold water may even produce fatal results in a comparatively short time. In the summer of 1882 I placed a dozen or more fishes in the cold water of a spring; within twelve hours half of them were dead. The first signs of an abnormal condition are shown by uncorrelated movements, reckless swimming, and the inability to keep the dorso-ventral axis vertical. The appearance is as though the centre of gravity were located *above* the centre of volume, and the fish gradually became incapable of remaining in its normal position of unstable equilibrium. In swimming the body rolls from side to side. After a time the fish sinks to the bottom of the vessel, and can regain the surface only with considerable exertion; it cannot remain at the surface in a motionless condition. At length the body usually becomes curved sidewise. In this condition the fish may remain for many hours, or even several days. Restoration to fresh water of the ordinary temperature seems to have only a temporary effect, or none. The only way to afford relief is to place the fishes in direct sunlight until the water becomes warm, when, if not already too much affected, they will gradually recover.

The movements of the eyes are principally in the horizontal plane. When one eye is directed obliquely forward, the other looks obliquely backward at about the same angle, so that the axes of the two eyes are kept approximately parallel.

The manner in which young gar-pike capture and swallow their prey is interesting, and serves to show why it is so difficult to get them to feed on anything except living objects. When very young, as previously stated, they will not feed on anything but mosquito larvæ. The fish always approaches the larva by a slow, even motion, resulting from the vibration of the pectorals and the tip of the tail, until the prey is about opposite the middle of the "bill"; then with a quick lateral motion of the head the larva is snapped up. Occasionally it is bitten through, and, whether struggling or not, is allowed to escape; but the reason of this I did not discover. Usually the larva is not snapped at until by some slight movement it shows signs of being alive, and then only a single snap is made. If unsuccessful, the fish remains quite motionless, or glides slowly away by means of the same motion of the pectoral fins. Fishes, especially when less than an inch in length and when well fed, rarely make a lunge forward toward their prey when they snap at it; the motion is usually only a sudden sidewise bend in the neck region. So cautious are the fishes not to snap at dead larvæ, that, after they have advanced so that the insect is opposite the middle of the bill, if it does not move, they begin to swim obliquely forward, pushing the larva before them without allowing it to glide backward along the bill. In this way a larva may be pushed about several inches before it makes the fatal movement which betrays its condition; or it may, if it remain entirely passive, escape altogether, since the fish, failing to discover evidences of life, leaves it and glides off in search of other food.

The number of insects caught by a single gar-pike is undoubtedly large. I was accustomed to feed the fishes twice a day. In July, 1883, I had the curiosity to watch the largest of them, then a month old and about 2½ inches (62 mm.) long, during its feeding. In the course of ten minutes it caught twenty-one large mosquito larvæ and made nine ineffectual attempts at seizure. But the voracity of young fishes was still more forcibly exhibited when, early in August, they were given small minnows (Fundulus) for food; for then they did not hesitate to catch the minnows even when the latter were considerably larger than themselves; but they succeeded in swallowing only those which were of their own diameter or smaller. Since the minnows have much thicker heads than the gar-pikes, the total weight of the former was doubtless always somewhat less than that of the latter.

The minnows were caught in the same way as the mosquito larvæ, by a sudden sidewise motion of the head; but being too large to be swallowed at a single gulp, they were at first impaled on the sharp teeth, and then by a series of deliberate movements put in a position to be swallowed. Almost without exception, the minnows were swallowed head first, irrespective of the region of the body first seized. If caught near its tail, as usually happens, then the prey is moved between the jaws — to which it lies crosswise — by successive shiftings, until it is held near the base of its head. A few movements usually suffice to make it take a direction parallel to the jaws, and the head end of the prey is thus brought to lie in the gar-pike's throat, which is often greatly distended by it. The movements by which the shifting of the minnow is accomplished are rather complicated, and require a nice correlation to be successful. During the process of transferring it crosswise between the jaws, the latter have to be opened quickly, and this motion is instantly followed by a quick lateral motion of the whole head in the proper direction. This lateral motion is accompanied by two others; one a forward thrust of the whole body, and the other a depression of the floor of the mouth. To accomplish the first movement there is a preparatory curving of the post-anal portion of the body, the sudden straightening of which at the instant the jaws are loosened gives the necessary forward impetus, and helps to prevent the escape of the prey; this is further guarded against by the second motion, — the depression of the floor of the mouth. The gill covers being in contact with the sides of the body, this latter motion produces a tendency to a vacuum in the mouth, which can be satisfied only by a sudden influx of water between the jaws. The current thus produced of course has the effect of carrying with it any movable object in the mouth or its immediate vicinity, and of impeding the escape of the prey until the jaws are again closed upon it. While the hold upon the prey is gradually shifted from its tail region to its head region, the part of the jaws which holds it is also not the same as at first. By the time the fish has been fully shifted laterally, it will be very near the base of the jaws, for at each loosening of the latter they have been thrust forward a little by the motion of the whole body. But that is not all, for when the prey has been shifted back to near the base of the jaws, — as one can see better in the case of the larger gar-pikes, — each subsequent movement of the latter causes the prey to take a slightly different direction, so that it is finally swung around until it is *parallel* with the jaws instead of crosswise to them. I am not entirely certain how this is effected; but it is all accomplished when the fish is held in the jaws near their base. It is possible that the teeth of one ramus of the jaw are not loosened quite as promptly as those of the other, and that as a consequence they act for an instant as a sort of pivot for the rotation of the prey. But that both sides of the jaw are ultimately set free at each motion is probable, from the great liability of the prey to escape at this very critical step in the process. Or it may be that the lower jaw is moved slightly toward one side as the jaws are being opened, thus giving a swing to the prey which changes its axis slightly before it is again caught by the closing jaws. Whatever the means by which it is effected, it is certain that this changing of the direction of the prey is all accomplished at the time of the renewals of the grasp.

After the fish has been thus nearly oriented, its head end is introduced into the throat by a single forward lunge on the part of the gar-pike. These forward movements are continued for some time, the jaws being slightly opened at each lunge. When the head is well introduced into the gullet, the jaws are no longer suddenly opened and closed, but remain more or less gaping, while the prey slowly disappears, doubtless being drawn on by peristaltic motions of the gullet. There are usually slight motions of the jaws during this latter process, — a slow opening and partial closing of them. The movement of the lower jaw is somewhat unsteady, almost tremulous, a peculiarity which is also seen, although not so distinctly, at other times than when feeding.

The advantage of a great divaricability of the rami of the lower jaw and of the fulness of the skin connecting them is at once apparent when a comparatively large fish is half swallowed, for then the thin membranous floor of the mouth is greatly distended and the rami pushed far apart. A side view of the head of the fish then resembles somewhat the appearance of the throat of a feeding pelican.

Gar-pikes sometimes take food which they apparently discover to be objectionable only after they have partially swallowed it. In such cases, it is ejected from the throat with a sudden jet of water.

They do not hesitate to snap at each other when kept in confinement, as I have many times observed, and as the mutilated condition of the fins, and especially of the tail, makes very evident. I think it may be inferred that they are not altogether free from danger from their own kind when living in their natural haunts, for they always show a remarkable sensitiveness to being touched in the region of the tail. The caudal filament especially is so sensitive that the slightest touch from a foreign body causes the fish to dart away with utmost speed, whereas one may touch any other part of the body with comparatively little effect. The young fishes become easily accustomed to the touch of the hands, and may even be lifted altogether out of the water without offering any resistance, provided it be done gently and without any quick motion. But none of them ever become so tame as to allow the slightest contact with the caudal filament without immediate efforts to escape. It is almost invariably the tail end which is snapped at by their mates, though I have a few times seen two individuals with interlocked jaws carry on a short contest without fatal results to either. I have known of only one case in which a gar-pike swallowed one of its mates. Near the end of July, 1883, I found an individual in the process of swallowing a somewhat smaller mate. The bill of the victim and part of its head were still protruding from the distended jaws of the captor; so that this individual was swallowed tail first, contrary to the more usual method.

The movements of the gill covers vary in rapidity at different times, but they are always executed with considerable promptness. Their adduction is quickly followed by their abduction, but the interval that follows before another adduction is usually rather prolonged; it is the more variable element. It may be so short as to make the abduction and adduction separated by equal intervals, or it may be prolonged to several seconds. These respiratory movements seldom exceed sixty per minute, and may diminish during the torpor of winter to scarcely more than one a minute.

The emission of bubbles of gas, which begins soon after the young fishes detach themselves from their fixed supports, at first takes place through the gills of one side. It is usually preceded by a forward lurch of the body, accompanied by a slight rolling to one side and the elevation of the gill covers. The bubble usually emerges before the fish regains its normal position, and consequently comes through the gill slits of the side which happens to be uppermost. Occasionally two smaller bubbles escape from beneath the gill covers, one from each side. When the fishes have become much older, the amount of gas is so great that the bubbles often escape not only from beneath both gill covers, but also through the mouth opening, and the rolling of the body does not always accompany the escape of gas.

In the earlier stages of their growth the gar-pikes remain most of the time very near the surface of the water in a horizontal position. In such cases the only premonitory symptoms of the escape of gas are the motions just described; but as they grow older they gradually habituate themselves to lying in deeper water, and then they almost invariably ascend to the surface before emitting gas. The ascent is nearly always accomplished by a slow forward and obliquely upward motion, the body being at an angle of about 45° with the horizon. The motion is usually deliberate, and at a uniform rate. After reaching the surface the body is allowed to assume the horizontal position before any effort is made to expel the gas. This motion of ascent is so characteristic, that after studying their habits one may predict with tolerable certainty whether a given fish is about to emit gas.

I believe the slight rolling of the body to one side is for the purpose of bringing the slit in the roof of the throat, through which the gas must be forced, into a position more favorable for its escape. If the opening through which the gas is obliged to pass is directed downward, its expulsion will require greater effort than if the opening is directed sidewise. An advantage depending on the same physical properties of the gas may perhaps also explain the universal habit of coming to the surface of the water to disengage the bubble. At least, the pressure of the water to be overcome in forcing out a bubble when the fish is at a considerable depth must be greater than when it is near the surface.

The escape of gas, which may be several times repeated during the process of swallowing a large fish, shows clearly enough that the bubbles are not simply air taken in at the mouth to be immediately discharged through the gill openings. The repeated emission of gas from the same fish, without the possibility of any fresh air having been taken in through the mouth, even led me to conjecture at one time that air was never taken in through the mouth. At least, it was certain that the young fishes often discharged gas without lifting any portion of the body out of the water.

The rate at which gas escapes from the gill openings is extremely variable, depending on the temperature of the water, the recency of feeding, etc. Perhaps the following observations give a fair idea of the rate during a warm summer day. In the course of ten minutes (August 6) eight fishes together emitted forty bubbles, or an average of one in two minutes for each fish. On August 17, a single fish, 62 mm. long, came to the surface six times in ten minutes, and caused bubbles to escape from the gills.

In the case of older fishes, snapping at the prey is frequently accompanied by an escape of gas, which is apparently involuntary, the sudden motion of the head and the opening of the jaws being sufficient to cause the escape of a few bubbles.

The nature of the gas will be considered in the following section.

III. The Respiratory Function of the Air-Bladder.

Lepidosteus, as well as some other fishes, has the habit of coming to the surface of the water and emitting through the gill slits or the oral opening bubbles of gas. This habit has attracted the attention of all who have had the opportunity of examining the fish while living.

Poey ('55, p. 136) observed that, when placed in a basin of water, "every five or eight minutes he [Lepidosteus] would come to the surface to swallow a mouthful of air, returning downwards immediately. One second after, half a dozen air-bubbles, some quite large, escaped by the opening of the branchiæ. The air," he adds, "remains in the bladder one second, sometimes one and a half, and this time is probably sufficient for the absorption, digestion, and expulsion of the inspired air. Besides, it is certain that, the animal not attempting to swim, the bladder was not used in augmenting or diminishing the density of the body, as most fish do, in order to ascend and descend in water." Poey further strengthened his opinion that "some sort of pulmonary respiration existed in the Lepidosteus" by dissections and injections of the aorta, which showed the great vascularity of the bladder (pp. 134–136).

Louis Agassiz ('57), in exhibiting before the Boston Society of Natural History some young living gar-pikes, called attention to the fact that this fish is "remarkable for the large quantity of air which escapes from its mouth. The source of this Prof. Agassiz had not been able satisfactorily to determine. At certain times it approaches the surface of the water, and seems to take in air, but he could not think that so large a quantity as is seen adhering in the form of bubbles to the sides of the gills could have been swallowed, nor could he suppose that it could be secreted from the gills themselves."

More recently Wilder ('76 and '77) also has studied the young of this fish. He says ('76, pp. 151–153): "Very often these young individuals of *L. osseus*, and more frequently the adults of the smaller species (*L. platystomus*), would protrude the snouts from the water in the respiratory act; but the length of the jaws made it impossible to determine whether this was intentional, and for the purpose of inhaling as well as of exhaling the air." He inclined to the opinion that air is taken in as well as given out, because the fishes uniformly approached the surface, whereas, if exhalation were alone sought, that "could be as well accomplished at any depth." As the result of some experiments in restraining the motions of *Amia*, he says: "There seems no doubt that with *Amia* there is a true inspiration as well as expiration of the air. The same may be considered probable, though not yet proved, with *Lepidosteus*."

So far as I know, these are the only published accounts of this peculiar habit in the case of Lepidosteus. While Agassiz maintained a conservative attitude regarding the question of the source and nature of the bubbles, Poey and Wilder expressed the conviction that it was air which had served the ordinary purposes of respiration, and both of them supported their belief with arguments.

Close attention to the movements of young fishes kept in confinement led me at first to doubt the accuracy of this conclusion. The result claimed by Moreau ('63 and '63^a, p. 820) — that, after the artificial removal of gas from the air-bladder of fishes generally, the gas is regularly renewed, *even when they are restrained from coming to the surface* — also served to confirm my doubts upon this point. Usually it was only the tip of the bill which protruded from the water, and it seemed incredible that such a movement could be sufficient for the acquisition of even a limited volume of air. In addition, it has often occurred that several times in succession a fish has been observed to come near the surface and emit bubbles of gas *without any portion of the head region breaking the surface of the water.*

This seemed conclusive upon at least one point: coming near the surface could not be *solely* for the purpose of acquiring air. I have given elsewhere (pp. 12, 13) what appeared to me to be the probable explanation of the upward movement of the fish previous to emitting gas.

The possibility that fresh air was not always — perhaps not generally — acquired, led me to reflect further about the possible functions of the air-bladder. Starting with the assumption, that no organ arises absolutely *de novo*, and that the air-bladder is the result of a differentiation in the alimentary tube, what, in a phylogenetic sense, was likely to have been its first function? Did it arise as a hydrostatic apparatus to be ultimately diverted to the service of respiration for the higher vertebrates, or was the hydrostatic function already a superimposed one? Might it not be that the original function was purely one of *excretion?* Perhaps in so primitive a fish as Lepidosteus this original function was still the dominant one.

These reflections led me to think it more than ever desirable to subject the gas emitted by the gar-pike to chemical analysis, — really the only method of arriving at definite and satisfactory conclusions. I therefore determined to undertake an analysis as soon as the fishes had attained a sufficient size to make the amount of gas given off in the course of a few hours voluminous enough for easy experimentation.

Accordingly, in December, 1883, when the fishes remaining were six or eight inches in length, some preliminary experiments were begun. The analysis was attempted by using a bent tube of about 10 mm. calibre, roughly graduated to 5 mm.; but the result showed that satisfactory conclusions were attainable only by employing more suitable apparatus. Through the kindness of Professor Cooke the mercurial bath and other apparatus for gas analysis at the Chemical Laboratory of Harvard College were placed at my disposal, and all the subsequent work of analysis was carried on in that Laboratory. I am also indebted to Professor Cooke for valuable suggestions during the progress of the work. To Prof. A. V. E. Young, then a private student in the Chemical Laboratory, I am under especial obligation, since he carried on the manipulations and measurements of the gases. From his skill and previous experience in gas analysis the results are entitled to more consideration than if the measurements had been conducted by one less familiar with such manipulations.

It was my aim in these experiments to ascertain, without sacrificing the fishes, the composition of the gas in the air-bladder, or at least of that which was emitted in the form of bubbles, presumably from the air-bladder. No attempt was made to ascertain the results of branchial respiration.

The collection of the gas, even from fishes 20 cm. long, is a tedious process. An inverted glass funnel large enough to allow the fish to swim freely inside, was first employed; but the inclined sides of the funnel not allowing the fish to assume a horizontal position except when deep in the water, appeared to interfere with the emission of the gas. After ascending to the apex of the funnel, the fish would make violent efforts, apparently for the purpose of attaining the surface of the water, but only occasionally would it emit gas under these circumstances, the emission usually taking place only after it had begun to descend. If freed from the restraint of the funnel, the fish invariably came at once to the surface outside the funnel, and emitted the customary bubbles.

Afterwards a nearly flat-bottomed glass dish, only a trifle smaller than the one containing the fish, was inverted over the latter (see Figure 1); but this apparatus was only slightly more successful than the one at first employed. The fishes came to the under surface of the inverted vessel, and after long intervals some of them emitted small portions of gas, but this was too small an amount to be satisfactorily analyzed. Another method — the one finally employed, although a tedious process — will be mentioned further on.

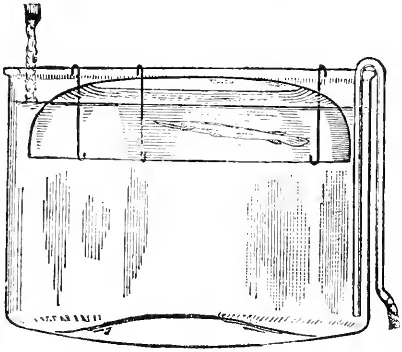

FIGURE 1.

In my preliminary analyses I had found that a very thin stratum of air under the inverted vessel was sufficient to allow the ordinary emission of gas. The first experiment in which the gas was accurately analyzed was therefore conducted upon fishes confined under the inverted dish, beneath which was a bubble of air of known volume, which had been *deprived of all its carbon dioxide.* The details of the experiment were as follows.

Experiment 1. — Dec. 13, 1883. At 11:45 A. M. six gar-pikes, varying in length from 13 to 21 cm., were removed from running water (+ 12° C.) to the experimental jar, containing recently distilled aerated water at about the temperature of the room (+ 20° C.), over which the glass vessel, with a bubble of air (deprived of carbon dioxide) measuring 66.71 c.c.[1] had been previously inverted. The experiment was continued until 3:15 P. M. ($3\frac{1}{2}$ hours) without renewing the water. The fishes were then taken out and the gas collected. In the final transfer over the mercurial bath a portion of the gas was lost, so that its total volume could not be ascertained.[2] The portion remaining was collected over mercury, and found to measure 19.27 c.c. (reduced). Treated with potassic hydrate, the volume was diminished to 19.07 c.c. Absorbed, .20 c.c. = 1.04% = carbon dioxide. This volume (19.07) transferred to water and treated with pyrogallate of potash measured over water 16.27 c.c. Absorbed, 2.80 c.c. = 14.5% = oxygen.

Experiment 2. — Dec. 14, 12:30 P. M. Six gar-pikes (the same as in Experiment 1) were removed from water (+ 11° C.) to the experimental jar containing water at + 21° C. The inverted vessel contained 65.20 c.c. of air deprived of its carbon dioxide. The experiment was continued until 4 P. M. ($3\frac{1}{2}$ hours). During the last hour all the fishes remained at the top of the water, and became somewhat swollen in appearance. One of the six did not recover from the effects of the abnormal conditions. The gas, re-collected and measured, was found to have

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[1] The eudiometer employed was graduated to fifths of a cubic centimeter. All the volumes given are the volumes reduced to 0° C. and 1 m. pressure.

[2] In a preliminary experiment it was found that under nearly the same conditions there had been a slight *increase* in volume. The conditions were as follows. Dec. 3, 1883, 12:30 P. M. Four fishes (17–20 cm.) were transferred from cold water into an experimental jar (Fig. 1, p. 16) containing water at +20° C. and a bubble of ordinary air measuring 53 c.c. At 2:30 P. M. five other somewhat smaller fishes were added. At 4:30 P. M. the experiment was discontinued, and it was found that the bubble had increased to about 56.5 c.c., the increase being nearly 3.5 c.c.

diminished nearly 18.3% in volume; it measured only 53.29 c.c. This diminution, however, is evidently not to be accounted for as the result of actual absorption by the fishes, but rather as the result of their over-distention with gas swallowed, — probably in the vain endeavor to compensate themselves for the altered condition of the atmosphere. The recovered gas (53.29 c.c.) was divided into two portions (A, B), and treated separately.

A = 43.20 c.c. (reduced) measured over water. This was treated with potassic hydrate solution, but the volume remained unchanged. Therefore carbon dioxide = zero. This volume (43.20 c.c.) was then treated with pyrogallate, which reduced it to 38.88 c.c. The diminution, 4.32 cc., = 10% = oxygen.

B. The average of three readings made this volume = 10.07 c.c. Treated with pyrogallate, it was reduced to 9.12 c.c., the reduction in volume, 0.95 c.c., being equivalent to 9.4+ % = oxygen.

Experiment 3. — The gas for this experiment was secured by collecting the bubbles as they were given off by the fishes. A number of the gar-pikes were placed in an uncovered experimental jar containing recently distilled water at + 20° C. It was found that by using a small glass funnel, — the one employed was about 65 mm. in diameter, — held near the surface of the water, the fishes were not prevented from the ordinary movements accompanying the emission of bubbles, as they were when confined under a larger funnel. The funnel was held so that the tip of the snout, but not the gill region, projected beyond its edge. The otherwise very slow process of collection is somewhat hastened by employing a number of fishes in the same jar. As they rise one after another, at comparatively short intervals, the collector, anticipating their movements, holds the funnel in the proper position, and seldom fails to secure the desired bubbles. But even in this manner the amount to be collected in the course of a few hours is not large. In this experiment the (reduced) volume of gas collected in about two hours was 9.54 c.c. Treated with potassic hydrate, the volume was reduced to 9.38 c.c. The difference = 0.16 c.c. = 1.7–% = carbon dioxide. Treated with pyrogallate, the volume was further reduced to 8.20 c.c. The diminution, 1.18 c.c., = 12.4–% = oxygen.

For convenience of comparison the results of measurements may be tabulated as follows: —

	Volumes in c.c. reduced to 0° C. and 1 m. pressure.			Per cent Carbon Dioxide	Per cent Oxygen.
	Original.	After Potassic Hydrate.	After Pyrogallate.		
I. Mixed air,	19 27	19.07	16.27	1.04	14.5+
II. " "	53.29				
A,	43.20	43.20	38.88	0.	10.
B,	10 07	——	9.12	0.	9.4
III. Respired air,	9.54	9.38	8.20	1.7−	12.4−

In considering the significance of these results, it is to be kept in mind that there are two principal problems involved. I have not attempted to find out whether *nitrogen* is consumed or produced in this process; but simply to ascertain the changes effected in the atmosphere respired as far as regards (1) the oxygen and (2) the carbon dioxide. The difficulty in drawing at once a satisfactory conclusion from the analyses rests primarily on the fact that the emitted gas could not be collected under conditions which allowed it to be assumed that at the time of analysis it was in the same condition which it presents in the air-bladder of the fish. For previous to analysis the gas had bubbled through the water in which the fishes were living, and had remained exposed to a limited portion of its surface. The reduction of the proportion of oxygen contained in the atmospheric air is so great, — between one third and one half, — that the influence of the water upon the composition of the emitted gas in this respect would certainly have been too insignificant to modify essentially the result. With the carbon dioxide, however, the case is different. The coefficient of absorption in water for the latter is immensely greater than for oxygen, and the total amount of carbon dioxide in comparison with the volume of the water to which it was exposed is so small (never having exceeded 1.7% of the volume of gas collected) as to make immediate deductions from the analyses of little value.

In a series of very carefully conducted experiments upon the intestinal respiration of *Cobitis fossilis*, Baumert ('53) arrived at the conclusion that the gas from the intestine, in bubbling through the water and during its subsequent exposure to that liquid, was *not essentially altered* either by the water or the gases contained in it (p. 48). The conditions under which his collections of gas were made[1] were so like

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[1] Baumert ('53, p. 39) employed five or six fishes (Cobitis fossilis) of medium size which were kept in a vessel containing about twelve litres of water from the river Oder. The gas was collected by means of a large inverted funnel terminating in a small-necked receiver, and usually about two hours were required for its accumulation. Three analyses, made at intervals of three days without any

those which I employed, that it seems justifiable to make use of his conclusions in the present case. The ingenious device employed by Baumert ('53, p. 46), in verifying the accuracy of his previous results, to suppress the branchial respiration, and thus secure the effects of intestinal respiration alone, could not, from the nature of the difference between the modes of respiration, be made available in the case of Lepidosteus.

The means (viz. protracted boiling) which Baumert employed for extracting the absorbed gases contained in the water of experimentation were as complete as the chemical methods at his time permitted. Since then, however, Gréhant ('69) has shown that simply boiling gas-impregnated water, although sufficient to eliminate all the oxygen and nitrogen, does *not* remove all the carbon dioxide, but that a combination of the mercurial pump with the method of boiling is capable of accomplishing this result. By this process he has demonstrated that

renewal of the water, gave in 100 volumes of gas respectively 1.77, 0.47, and 0.13 volumes of carbon dioxide, and 10.46, 13.71, and 11.92 of oxygen. It will be seen, therefore, that not only the conditions under which the collections were made have been in both cases very similar, but also that the composition of the gas as determined by analysis was nearly the same in Lepidosteus that it was in Cobitis. Since the ratio of the gases in the two cases was practically the same, there is no reason to suppose that the water absorbed a greater proportion of gas in one instance than in the other.

As regards the *temperature* of the water at which the experiments were made, although not definitely stated by Baumert, it is safe to infer, from the temperature at which his numerous experiments on *branchial* respiration were made, that it was considerably *lower* than the temperature of the water in which the Lepidostei were placed, so that the tendency to an absorption of carbon dioxide in the latter case would certainly have been *less* than in Baumert's experiments.

One of the plans adopted by Baumert to test the influence of the water and its contained gases upon the emitted gas — whether the latter were either deprived of any of its oxygen or carbon dioxide, or received accessions while exposed to the water — was (1) to prepare *artificial* mixtures of atmospheric air and carbon dioxide in different proportions (4 : 1 and 6 : 1) and cause them to bubble through water in which fishes were living, and after collection to allow these gases to remain exposed to the water, as in the experiment with the *natural* gas; (2) in a similar way, to cause atmospheric air to pass through water in which the fishes had been living for several days. In the second of these experiments, the atmosphere remained absolutely unaffected, showing not only that it did not lose oxygen, but that it also did not acquire carbon dioxide. In the first of these methods, however, while the proportion of nitrogen and oxygen remained practically the same, the carbon dioxide was diminished by absorption to the extent of about one half its original volume. To this evidence of considerable absorption, it seems to me, Baumert does not allow proper weight, when, by a more satisfactory method, he subsequently finds what he believes to be the true composition of the emitted gas.

the water of the river Seine contains *oxygen* and *nitrogen* in the proportions determined by Humboldt et Provençal ('09), but that, owing to the defective means of extracting the carbon dioxide employed by them, they secured only one fortieth of the volume of that gas which was actually present (Gréhant, '69, p. 379).

Such being the case, it is evident that reliance cannot be placed on Baumert's conclusions, for his opinion — that there was no perceptible absorption of gases under the conditions of experimentation previously detailed — rested ultimately upon his experiment with intestinal respiration in distilled water, and his ability subsequently to extract *the whole* of the gas *absorbed by the water* during the experimentation.

Being unwilling to sacrifice the limited number of young fishes in my possession for the purpose of securing the contents of the air-bladder, and not having the opportunity of getting the gas from the air-bladder of adults, I determined to employ a method which seemed likely to furnish positive evidence of the presence of carbon dioxide, if any were really eliminated, even though it would not give a rigid quantitative test.

The plan was, to place the fishes in a vessel of water having a limited air-chamber, which could be rapidly swept of its contents by a continuous flow of pure air through it, and to lead the air, thus drawn from the experimental jar, through baryta water, which would indicate the presence of even a small amount of carbon dioxide by the formation of a precipitate.

Accordingly, a glass jar (Figure 2, J) of about 20 litres' capacity, provided with a thick ground-glass cover having a central neck and orifice for a stopper, was selected. The cover was larded to secure a close joint, and in addition the edge was sealed with melted paraffine, and the jar nearly filled with recently boiled distilled water. This was siphoned into the jar with as much precaution as possible in order to prevent exposure to the atmosphere. To make the water habitable for the fishes, it was of course necessary to aerate it, and it was at the same time important not to introduce in this process any carbon dioxide. To accomplish this successfully was found to be a task much more laborious than was at first anticipated. The apparatus shown in Figure 2 was that which finally proved satisfactory.

A Bunsen pump (P) attached to the hydrant provided the suction required, and the system of tubes and chambers through which the air was drawn was arranged as follows. Four glass combustion tubes (K) each about 2 meters long and 2 cm. in diameter were loosely filled with fragments of potassic hydrate, and joined by short pieces of rubber

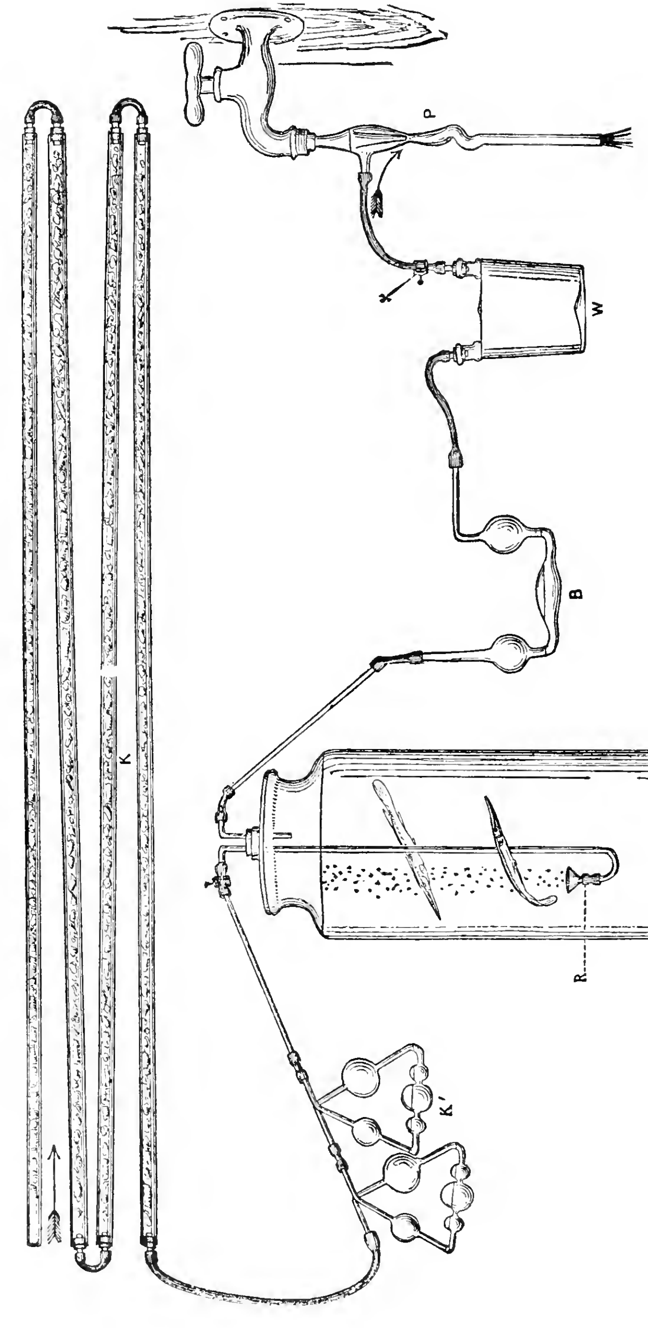

FIGURE 2.

tubing, as indicated in the figure. After traversing these tubes, the air was compelled to bubble through two sets of potash bulbs (K′), and thence by a glass tube was led through the stopper of the experimental jar (J). The glass tube was carried to within about 10 cm. of the bottom of the jar, and, after describing a U-shaped bend, terminated in a platinum "rose" (R). Thence bubbling through the water, the air was led from the air-chamber of the jar by a glass tube to the baryta bulb (B). To guard against the effect of an accidental diminution of pressure in the hydrant water, and a consequent reflow of water into the baryta bulb, the latter was not directly connected with the pump, but an ordinary Wolff's bottle (W) was interposed.

The preliminary test imposed upon the apparatus was, that it should run twenty-four hours with no fishes in the water, but otherwise under the same conditions that would be required when the fishes were introduced, without giving a perceptible precipitate in the baryta bulb. It required some time and considerable attention to details before this was attained. All the connections were made by means of close-fitting rubber tubing, which was made as limited as possible, narrow glass tubing being used wherever practicable, and all the joints were in addition sealed with melted paraffine. While the pump was in operation, the water in the jar was of course under diminished pressure,[1] the diminution being equivalent to a column of water equalling the distance between the surface of the water in the jar and the lower end of the bent glass tube to which the "rose" was attached. Evidently the removal of the stopper from the neck of the jar (through which it was proposed to introduce the fishes) under such circumstances would allow the entrance of considerable impure air, which might give a precipitate as soon as the pumping was resumed. To obviate this source of error, the tubing of the apparatus was clamped at X, and by means of a hand bellows air was injected through the potash system until equilibrium was restored in the chamber of the jar, this being indicated when the water rose inside the bent tube to the same height as outside. In the preliminary test, this was done in the same manner as subsequently when the fishes were introduced, and the stopper was also removed for the length of time which experience had shown would be necessary for the introduction of the fishes. Thus, as nearly as possible the same conditions were observed in the preliminary and the final experiments.

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[1] It is to be observed that, so far as this diminution of pressure affects the absorption of gases by the water, it can only act favorably in preventing such absorption.

When the preliminary experiment had proved that the air which for twenty-four hours was being pumped through the apparatus did not contain enough carbon dioxide to cause any trace of a precipitate in the baryta bulb, then the tubing was clamped at x. Equilibrium was restored by forcing in air as before, four fishes were introduced through the neck in the cover to the jar, and the pumping was resumed. It was kept up for six hours, at the end of which time *not the slightest trace of a precipitate had been formed,* although the fishes had been emitting bubbles at the surface of the water in the ordinary manner during the whole time. The baryta water was subsequently tested to make sure that no error had been made in the matter of its sensitiveness to carbon dioxide.

The immediate inference from this experiment is, that the gas eliminated by the fishes contained no trace of carbon dioxide; but here, again, the possibility of an absorption of eliminated carbon dioxide by the water cannot be rigidly excluded.[1] It seems highly improbable, however, that with an air-chamber which required at most only a few minutes to be swept of its contents, there could have been a sufficiently prolonged exposure of the gas to allow the absorption of its carbon dioxide. It might be urged that this, being but a single experiment, can be entitled to little weight, — that, even if in this one instance there was no elimination of carbon dioxide, it is to be considered simply as an exceptional individual case. It is, however, to be borne in mind that there were *four* fishes under experimentation, and that in the case of the *analysis* which failed to show the presence of carbon dioxide there were *six.*

As the result, then, of these various experiments to determine whether carbon dioxide is eliminated, it must be concluded for the present, until opportunity is presented for a careful analysis of the gas taken from the bladder without exposure to water, that *the gas of the air-bladder of Lepidosteus is not likely to contain more than two per cent of carbon dioxide at the most, with a strong probability that in many cases it does not contain an appreciable amount of that gas.*[2]

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[1] If this experiment were ever repeated, it is perhaps desirable that *control experiments* should be carried on at the same time. In the control experiments atmospheric air mixed with known volumes of carbon dioxide, varying from zero to 2% of the total volume, should be made to bubble near the surface of the water. By observing the frequency and estimating the volume of the gas emitted by the fishes in a given time during the actual experiment, practically the same conditions could be observed in the control experiments. The introduction of the carbon dioxide mixture could easily be accomplished by means of a third glass tube, piercing the rubber stopper of the jar and dipping just below the surface of the water.

[2] The recent analyses made by Jobert ('77 and '78ª) on the gases taken from

But if little or no carbon dioxide is eliminated by the air-bladder, what is the purpose of this aerial respiration? The answer must be evident from the amount of oxygen which invariably disappears. The emitted gas contains only from two thirds to one half the amount of oxygen found in an equal volume of atmospheric air. I believe it is therefore definitely and satisfactorily settled by these experiments, that *the air-bladder respiration in Lepidosteus is subservient to the oxygenation of the blood.*

There arise two more questions, which I believe to be intimately related to each other. If the air-bladder does not provide for the elimination of a larger amount of carbon dioxide than is stated, how is this product disposed of? And, secondly, What is the relation between branchial and aerial respiration in Lepidosteus, and how has it been brought about?

The most probable method of elimination of the carbon dioxide is by means of the gills. To determine the question definitely would require the complete separation of the effects of branchial respiration from those accomplished by other means; but it seems to me at present extremely doubtful if any method can be devised which will enable one to do this in the case of Lepidosteus. With Cobitis, or any other fish with intestinal respiration in which the gas is emitted through the anus, the solution of the problem would be comparatively easy.

But there is already some evidence that the gills in such cases effect an increased amount of elimination. At least the comparisons which Baumert instituted between Tinca and Cyprinus on the one hand, and Cobitis on the other, indicate clearly that there is a more rapid elimination of carbon dioxide through the gills in the latter case than in the former.

If, then, it can ultimately be shown that in cases of aerial respiration the gills accomplish most of the elimination of carbon dioxide, and that the air-bladder is serviceable principally as a means of securing additional oxygen, something will have been accomplished toward appreciating the natural conditions which must have led to the imposition of this

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the air-bladder and from the intestines of several Brazilian fishes gave the following results: —

I. Fishes with intestinal respiration,
  a, emitting gas from the anus, *Callichthys asper* C. & Val. : 1.5–3.8% carb. diox.
  b, emitting gas from the mouth and gill slits, *Hypostomos* : 1.5–2.8% carb. diox.

II. Fishes with air-bladder respiration, *Erythrinus tæniatus* Spix, or *Erythrinus brasiliensis* Spix, } : 1.3–2.4% carb. diox.

function upon the alimentary canal, or one of its dependencies. The water in which such fishes lived may have been at times incapable of furnishing the necessary amount of oxygen, but sufficiently serviceable as a means of removing, through the gills, the carbon dioxide. The solubility of carbon dioxide in water, as compared with that of oxygen, favors such an explanation.

I have not succeeded in finding many analyses of the gases held in solution by water which seem capable of throwing light on this question.

A. Morren ('41, pp. 471, 478–480, and '44, p. 12), who conducted numerous experiments to ascertain the effect of light and of green organisms on the composition of the gases dissolved in water, has recorded some interesting facts which seem to me to bear upon the problem. He ascertained that when the per cent of oxygen in the gas extracted (by boiling) from the water fell below 18, 19, or even 20, the fish contained in his experimental reservoir began to languish, and many of them died. He also deduced from his experiments these conclusions: that water which [is stagnant or] flows slowly over a slimy bottom is subject to conditions which serve to explain why it may be habitually less oxygenated than water which runs rapidly over a sandy bottom, and why it undergoes greater variations in the composition of its dissolved gases.

Morren also found that the oxygen in the gas contained in the waters of the river Marne fell as low as 18 per cent on the 18th of June, 1835, when there was a remarkable mortality among the fishes in the river, and he ascribed this mortality to the want of oxygen in the water.

If such gas is incapable of supporting the life of fishes, it might occur under certain circumstances that the proportion of oxygen would be considerably below the normal 32 per cent, and still be far from producing asphyxia. Under such conditions, which presumably happen more often in the stagnant water of shaded swamps than elsewhere, fishes which could avail themselves of the oxygen in the atmosphere would be able to survive when others could not. They might still employ their gills for the elimination of carbon dioxide into the water, — 100 volumes of which can absorb about 120 volumes of the gas, — but would have to depend largely upon the atmosphere for their supply of oxygen.

One might conclude, then, that in the transfer of the respiratory function from the gills to the prospective lungs the two components of the respiratory process were separated from each other, and that the oxygenating function was the one first to be transferred; that, so long as the animal lived in the water, the gills were, under all ordinary circumstances, the customary channel for the elimination of carbon dioxide; and that finally, during the passage from water to land, this function was also imposed on the vesicular organ of the intestinal tract, which thus became a lung in the fullest sense of the word.

IV. Embryology.

The interesting accounts of the ontogeny of Lepidosteus given by A. Agassiz and by Balfour and Parker have put us in possession of important data concerning vertebrate development. It is hoped that a more extended study in this field will serve to answer some of the questions left unsettled by them, and will afford additional results of general value in discussions concerning the phylogeny of vertebrates.

There would be some advantages in beginning the subject of the development of Lepidosteus with an account of the formation and growth of the ova. Although I have considerable material for a study of oögenesis, it has seemed to me better, on the whole, to defer a consideration of the topic until the close of that part of these studies which deals with organogeny, and to begin the description with the ovarian ovum as it exists at the time of oviposition. But I have deviated from the plan in the first part of the subject, — the egg membranes, — in order to give a more complete exposition of the structures which envelope the mature ovum.

1. Egg Membranes.

The only account of the structure of the egg membranes of Lepidosteus is that given by Balfour and Parker ('81, p. 112, and '82, p. 362 and foot-note).[1] It is as follows: "They [the ova of Lepi-

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[1] Ryder ('85, p. 146) has since given the following brief account: "In the ova of Ganoids, *Amia* and *Lepidosteus*, the *zona* is composed, in the first instance at least, of short, parallel, elastic fibers disposed in a plane vertical to that of the membrane, these fibers being fused at their ends or just below the inner and outer surfaces of the membrane. Sections through the egg membrane of *Lepidosteus* seem to indicate the same condition of things as in *Amia*, in fact Dr. E. L. Mark of Cambridge, Mass. has kindly shown me drawings which show the fibers of the zona of the former isolated in the same condition as I have been able to separate those forming the egg membrane of the latter."

It will be seen by the following description that I do not agree with all that dosteus] have a double investment consisting (1) of an outer covering formed of elongated, highly refractive bodies, somewhat pyriform at their outer ends (Plate 21, fig. 17, *f. e.*), which are probably metamorphosed follicular cells, and (2) of an inner membrane, divided into two zones, viz.: an outer and thicker zone, which is radially striated, and constitutes the *zona radiata* (*z. r.*), and an inner and narrow homogeneous zone (*z. r.'*)." In a foot-note the authors state, in addition, that "the ripe ova in the ovary have an investment of pyriform bodies similar to those of the just laid ova," but that their attempts to ascertain the nature of these peculiar pyriform bodies proved futile on account of the bad state of preservation of the material at their command.

A. Observations.

The observations which I have made on the egg membranes of Lepidosteus will be followed by an historico-critical account of what is known about the structure of egg membranes in other fishes.

I have examined the membranes in fresh eggs, as well as in those which have been treated with various reagents, and have been able to carry my investigation somewhat further than Balfour and Parker. It will appear in the course of the following account to what extent my results agree with theirs, and in what they differ.

a. *Zona Radiata and Villous Layer.*

Omitting for the present the modifications in the micropylar region of the membranes, I will consider first the structure of the envelopes in the recently laid egg which has not been subjected to the action of reagents, and subsequently will describe what is to be gained by the study of sections made from eggs that have been hardened and stained. The differences between the membranes of recently laid eggs and those of mature ovarian eggs are so unimportant that it will not be necessary to give a separate account of each. The nature of the differences, when such exist, will be pointed out in the course of the description. When the ripe eggs are artificially removed from the female by "stripping," they have at first irregular, more or less polyhedral forms, due to mutual pressure in the ovary, and the membrane investing each is in a pliable condition. This state is retained for a long time, provided the eggs do

Ryder here states. He seems to have entirely overlooked the existence of two membranes, and gives such an account of his "zona" as to make me believe that he has had in view what I have described as the villous layer.

not come in contact with water; but when immersed in water they soon exchange the flaccid for a more rigid condition, like the eggs of many other fishes. Whether the egg is in water or in air, its surface is excessively sticky, as Mr. S. Garman[1] has already accurately observed. The eggs adhere equally well to polished and to roughened surfaces. When let fall directly from the female into 95 per cent alcohol, with a view to ascertaining if there was any special superimposed layer of viscid substance such as that described by Kupffer ('78ª, p. 178) for the herring, the eggs have furnished no evidence of the existence of any such continuous film, nor of any covering additional to that which is distinguishable in the mature ovarian ovum, except small amber-colored bodies mentioned later.

When laid the egg of Lepidosteus is enclosed in a single membranous envelope about 50–60 μ thick (Plate I. Figs. 5, 11). This membrane is, however, composed of two distinct but firmly united layers. The outer layer, which embraces from one fourth to one third of the total thickness of the membrane,[2] I shall call the *villous layer* (*st. vil.*); the inner, the *zona radiata* (*z. r.*). The former is the outer covering of "elongated, highly refractive bodies," described by Balfour and Parker; the latter undoubtedly embraces both the zones — *z. r.* and *z. r.*′ — described by those authors. It will be convenient to consider the two layers together rather than separately.

Examined in the fresh condition, the outer surface of the egg envelope is of a faintly yellowish or brownish tint, which is in part due to the presence of small ovoid bodies of variable size, of an amber color and a waxy appearance (Plate VIII. Fig. 5), which are scattered over the surface. I am unable to say how or where these bodies are formed, but possibly they result from the disintegration of the granulosa. Aside from these bodies the surface presents a roughened or shagreen-like appearance, which is found upon microscopic study to be due to slightly rounded prominences of nearly uniform diameter which are separated from each other by regular nearly straight lines, so that a view perpendicularly upon the surface (Plate I. Fig. 1) presents a field divided by these lines into small polygonal (four- to six-sided) nearly equal areas. The average size of the areas increases slightly as one approaches the vegetative pole of the egg. When the envelope has been removed and torn

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[1] See A. Agassiz, '78ª, p. 66.

[2] Measurements of the fresh membrane of an ovarian egg left twelve hours in glycerine gave a total thickness of 68 μ, of which 50 μ represented the zona and 18 μ the villous layer. After the addition of weak hydrochloric acid the latter increased to twice its original thickness (36 μ).

into pieces, it is readily seen to be composed of the two layers mentioned, which for the most part remain firmly united. Along the torn edges, however, it often happens that the lines of rupture in the two layers do not coincide, so that for a considerable area there is a separation of one layer from the other (Plate I. Fig. 5). Such regions are the most satisfactory ones for the separate study of the two structures.

Both layers are translucent; to the outer belongs the brownish tint seen in surface views, while the inner is slightly opalescent. When seen from the edge or in optical section, or, better still, when cut with a razor into thin radial sections, both exhibit radial striations, which are much closer to each other in the inner layer than in the outer (Plate I. Figs. 4, 5, 11).

Aside from certain exceptions which will be considered later, the fine radiate markings of the inner layer, or zona, appear as nearly straight parallel lines, which are traceable through the whole thickness of the layer, but which become gradually less prominent toward the deep surface.

The markings of the outer or *villous layer*, on the contrary, are less uniform; they traverse the whole thickness of the outer layer, but are most clearly defined near the periphery, their deeper portions being more irregular and confused, and often exhibiting a tendency to a zigzag course. They indicate the boundaries of highly refractive prismatic bodies, of which the layer is composed, and which seen endwise produce the appearance of polygonal areas already alluded to. When viewed from its deep surface (compare Plate I. Figs. 2, 7), the villous layer has a somewhat ragged appearance; it also exhibits polygonal areas, but they are less regular and less clearly defined than those seen from the external surface. When the egg membrane has been left for some time in water, or, better still, in a mixture of water and glycerine to which a trace of hydrochloric acid has been added, the prismatic elements which compose the villous layer undergo a remarkable change, during which the cause of the peculiar zigzag appearance of their boundaries is made evident. After a time the free rounded ends of some of the prisms appear to protrude above the neighboring ones (Plate I. Fig. 4), thus giving the surface a less even contour than it had at first. On comparing the conditions before and after the application of acid, it at once becomes apparent that the layer has increased in thickness. At its free edges the prisms become more or less detached from each other, and it then is possible to appreciate their real form.

There are recognizable at least three distinct regions in each prismatic villus, and each may be roughly compared to a stalk of grain, with its head, shaft, and root. These names may be applied not inappropriately to the three regions of a villus. The peripheral portion or head (*cap.*), embracing one fourth, or sometimes as much as one third, of the original thickness of the layer, is distinctly prismatic and highly refractive ; its sides are parallel, and it is little affected by the acid, so that, although it increases very slightly in size, it still retains to some extent its angular form. Its free end is always more or less rounded (Plate I. Figs. 4, 9, *i*). Following this terminal head, and marked off from it by a slight constriction, comes the stalk (Fig. 9, *i*, *pd.*), a long, also prismatic fibre, which is less highly refractive than the head, and is so crowded as to be folded back and forth, thus giving to it the appearance of a spiral spring. In fact, many of the fibres are coiled into a tolerably regular spiral, but the majority are simply folded irregularly, evidently being accommodated to the space most available.

Through the action of acid these stalks begin to swell, and some of them — since they are affected more promptly than others — cause an earlier protrusion of the corresponding prismatic heads (Plate I. Fig. 4). The increase in the thickness of the layer — which soon reaches twice its original dimensions — is due almost entirely to the swelling of these deeper portions of the villi. When isolated, they may in some cases be elongated to ten or twelve times the length of the coils which they at first formed (*a*, *b*, Fig. 9, Plate I.). A portion of this elongation is due simply to the unfolding of the compressed stalks ; but ultimately in proportion as it elongates the stalk becomes more attenuated. It often happens in this process that different portions of the stalk are at first unequally affected. Usually it is the deeper portion which is first to uncoil and to become attenuated (*n*, Fig. 9). When fully extended the stalk is slightly tapering, being narrowest at a little distance from its basal or root end, and although generally quite uniform in calibre, it occasionally exhibits varicosities. In many cases the isolated villi (Fig. 9, *g*, *h*) appear as though temporarily prevented from straightening out because of delicate longitudinal structures of a band-like appearance. The aspect of the stalk is then remarkably similar to the pouched condition of the mammalian *colon*, to the longitudinal muscles of which these band-like structures correspond. The apparent "bagging" is usually all in one direction, namely, toward the attached end of the villus (*h*, Fig. 9). The basal end or root (*rx.*) appears to terminate regularly in a number (3–9) of tapering root-like diverging prolongations (Fig. 9, *f*, *g*, *i*, *k*), which are often apparently connected with each other by membranous expansions of the basal portion of the villus.

These roots serve to fasten the villous layer very firmly to the zona radiata, in a manner to be explained in connection with the account of that layer.

The much finer radial markings of the *zona radiata* (*z. r.*) are entirely different in character from those of the villous layer; seen from either surface with a moderately high power they appear as punctations, dark or light according to the focusing (Figs. 3, 8, Plate I.), evenly scattered over the surface, and yet so arranged as to give the whole area a very characteristic appearance. Although rather evenly distributed, they are arranged in groups or systems. One may trace over a considerable area a series of dots placed at the intersections of a system of imaginary equidistant lines crossing each other at right angles; near by may be other series, in which the systems of imaginary lines cross at angles varying widely from that of 90°; in still other series, the lines are arcs of circles; the circles may vary somewhat in size, but the arcs are never to be traced for more than a few degrees. These different systems abut upon each other in the most fortuitous manner, and the intervening spaces are filled with dots so evenly arranged as not to interfere with a fairly uniform distribution over the whole surface (compare Plate III. Fig. 5). Higher powers show that the punctations are circular in outline, of very nearly equal diameters (0.5 μ at their outer ends), and placed at intervals averaging about 1.5 μ. My notes of May 24 and 25, 1883, make the intervals between the pore-canals, as determined by measurements on the shell of an ovarian egg that had lain in glycerine over night, only $\frac{2}{3}$ μ, less than half the value given above; but I believe that the larger distance fairly represents the average condition.

Thin tangential sections show by focusing that these markings are due to minute canals (*pore-canals*), which are ordinarily hollow, or at least contain a substance that is less refractive than the common homogeneous mass of the matrix which they traverse. I have not seen any evidence of a differentiation in the optical properties of the walls of these pore canals which would allow one to speak of them as *tubules*.

Weak hydrochloric acid causes the zona to swell slightly, and ultimately renders the pore-canals less conspicuous or entirely invisible. There are certain of them which do not fade away, however, even after treatment with acid, and which at length become the only visible structures in what otherwise appears as a homogeneous layer. (Fig. 10. Compare also Figs. 4, 6.) These canals very generally have a spiral course, and are noticeably broader at the outer surface of the zona than elsewhere; they taper gradually toward the inner surface of the layer, but seldom reach it; most of them are traceable only a short distance from the outer surface. They owe their prominence to the fact that they are filled with a highly refractive substance having the form of a corkscrew. When the villous layer has been torn from the zona, this substance appears to terminate exteriorly in a ragged, broken end, which in some instances is drawn out into a tapering appendage (Fig. 10, α). There can be no doubt — according to evidence to be gained from ovarian eggs — that this substance is continuous with that of the prismatic columns of the villous layer, of which they are in reality the roots. The roughened appearance of the inner surface of the separated villous layer is largely due both to the lacerated ends of these roots and to the fact that many of them are wholly withdrawn from the pore-canals when the two layers are torn asunder. This relationship of the layers also explains why it is so difficult to separate them over even a limited area.

Sections of stained eggs, both radial and tangential, give instructive views of the egg membranes. In radial sections the difference between the villous layer and the zona becomes at once apparent from the deeper stain which the former takes on. The pore-canals are also usually more distinct than in the fresh egg, although the effect of certain acid preservative reagents (Perenyi's fluid, picrosulphuric mixture) is such as to obscure the radial markings of the zona. In the villous layer a still more striking contrast is produced between the heads and the stalks of the villi, since the former almost invariably take a much deeper stain than the latter. Especially is this true when stained in picrocarmine, by which the heads are colored a deep carmine while the stalks and roots remain unstained or take a greenish-yellow hue from the action of the picric acid (compare Plate IV. Fig. 1). In borax carmine both portions are usually stained, and almost invariably the head much deeper than the rest of the villus; but it has occasionally happened that the heads were less deeply colored, and presented a slightly yellowish tint (Plate IX. Fig. 2). I am unable to account for the difference, unless possibly a prolonged decoloration in hydrochloric acid is the cause of the feeble stain of the head ends. In all these stained radial sections it is to be seen that the transition from the head to the stalk, although not marked by a sharply defined line, is nevertheless abrupt. Owing to this, and the fact that the stalks, as well as the heads, are of nearly uniform lengths, radial sections of well stained specimens always exhibit the stalks in the form of a broad band or zone, sharply marked upon both edges, — more deeply stained than the zona radiata, but less deeply than the narrower well defined band which is made up of the heads of the villi. Each of the heads has its external free surface more or less rounded and not quite smooth, its sides nearly parallel and straight, and its ill-defined deep face also tolerably straight. Along the last it is distinguishable from the stalk, with which it is continuous, by its greater refractive power as well as deeper color, and by a slight diminution in the size of the stalk. The last distinction becomes more conspicuous the more the stalk is elongated. The differences between head and stalk are emphasized by the fact that the villi have a greater tendency to rupture along this line of union than elsewhere (Plate II. Fig. 1, and Plate III. Fig. 1). The outlines of the free end and the sides of the head are sharp, and in thin sections, especially such as cut the heads crosswise, the margins seem to be limited by a narrow double-bordered dark band (compare Plate III. Fig. 3), as though the head were invested in a thick deeply staining membrane. Since I have never been able to find evidence of the separation of any membranous structure from the surface of the head, I am disposed to believe that the appearance simply results from a differentiation of the cortical portion of the head, which otherwise appears perfectly homogeneous. In some cases this cap-like cortical part seems to exert a restraining influence on the swelling of the central portion ; at least I interpret in that sense certain conditions of the heads frequently met with. In such cases their sides are not strictly parallel, especially when the villi stand in an isolated position (Plate II. Fig. 1). The head, instead of being marked off from the stalk by a constriction or shoulder of the ordinary form, has its outline gradually broadened or flaring as it approaches the peripheral end of the stalk, and its cap-like sheath appears to end abruptly with edges which are slightly everted ; the connecting portion of the stalk is as broad as, or even broader than, the basal end of the head, so that the direction of the resulting shoulder is just the reverse of that commonly seen. The most natural explanation of this appearance which occurs to me is, that the free edge of the cap-like sheath is distended, and even sometimes everted, by the swelling which takes place in the region where head and stalk are continuous, and that the sheath in all probability acts as a restraining investment in preventing any great distention in the rest of the head.

The stalks, in radial sections of eggs which have been subjected for some time to the action of water before hardening, have the appearance of comparatively slender columns, which are often slightly sinuous, but in general nearly parallel. They taper at first quite rapidly for a short distance from the head, and then only very gradually toward the basal or root end. The spaces between the stalks are much greater than those between the heads; while the latter sometimes remain — even after the prolonged action of water — in a continuous layer, the stalks often appear to stand individually isolated. It is more common, however, to find, as the result of the swelling, that both the heads and the stalks are arranged in groups or patches, — better shown in tangential sections. Even when the heads are not thus separated, the stalks may be gathered into clusters which leave in radial sections broad lenticular spaces between them (Plate III. Fig. 1).

The stalk gradually diminishes in size to near its zonal end, where it enlarges rather promptly into a sort of conical foot, which exhibits dark longitudinal or radiating markings continuous with the dark outer ends of corresponding pore-canals in the zona. In some cases the foot is split into two or three strands, between which there is then left a space that in radial sections is triangular, with its base resting on the zona and its more acute angle rising into the stalk. The roots proper embrace only the portions of the villi still occupying the pore-canals of the zona. In some cases they are to be recognized as occupying every pore-canal, in others some of the canals appear to be destitute of villous contents. The roots are highly refractive, like the stalk, and seem to stain even more deeply than the latter. They are always broadest at the outer end, and taper until they are exceedingly fine threads. They seldom reach more than a tenth or an eighth of the way through the zona, although longer and larger roots are met with at intervals. They always appear more tortuous — zigzag, or spiral — than the pore-canals which do not contain roots, and are at times so irregular in form as to have caused great distortions in the canals (Plate IX. Fig. 2). Their finest tips, however, always appear continuous with the much more faintly marked pore-canals. I cannot doubt, therefore, that they are accommodated by simple enlargements of the pore-canals. The great regularity in their distribution, too, allows no other interpretation than that the position of the roots is practically determined by that of the pore-canals.

Tangential sections of stained eggs (Plate III. Figs. 2–5) afford the most satisfactory evidence of the shape and grouping of both heads and stalks, and is the only safe means of controlling the views of the foot region gained by radial sections. The heads are at first close set, leaving only the finest narrow lines, with here and there an irregular opening where the prisms incompletely match (Plate III. Fig. 2); their cross sections are angular and range from variously proportioned triangles to six- or sevensided polygons. After the prolonged action of water they become less angular, and begin to separate along irregular lines, so as to leave the heads arranged — as already indicated — in patches, which vary considerably in size but are for the most part of a characteristic polygonal outline, with borders which are necessarily jagged owing to the nature of the lines of separation, for the latter never split a prism, but simply separate adjacent ones. The heads may vary in diameter in the ratio of one to two.

The dark border already alluded to is best seen in thin tangential sections (Plate III. Fig. 3), and is readily distinguishable on all the heads when well stained and cut sufficiently thin. The line of separation between prisms is not always distinguishable, but whether this is due to actual contact or not it is difficult to say, since the least obliquity in the section is sufficient to obscure so faint a marking.

The stalks also are found upon cross section to be prismatic, even after the process of swelling has completely isolated them (Plate III. Fig. 4). They are also arranged in groups which correspond fairly to those of the heads, but the spaces between them are much greater. Occasionally sections of stalks are to be seen, even from the middle of the stalk-zone, the central part of which has not been stained (Plate III. Fig. 4, α, α). Careful examination shows that such stalks are really *hollow*, the boundary of the colorless area being sharply defined. I have never seen vacuoles in the middle region of any of the stalks examined in radial section ; besides, these cavities can often be traced continuously on successive tangential sections toward the foot. They are, moreover, increasingly frequent as one approaches the zonal attachment of the stalks. The consideration of all these facts makes me quite sure that many of the stalks, at least in their basal halves, are really hollow prisms, although I have never been perfectly certain that I have seen this condition in radial sections. One may, however, as before stated, readily see on radial sections that the expanded foot of the stalk is often apparently split into diverging roots, and that there is an intervening unstained region. The prolongation of this space, which is triangular in side view, forms, I believe, the cavity of the stalks in question. Although the prisms appear sharply marked in cross sections, there is very generally a trace of a filmy substance projecting here and there from their edges in the form of faintly marked threads, which sometimes end indefinitely in the inter-prismatic spaces, but at other times appear loosely to connect neighboring stalks (Plate III. Fig. 4, β). This substance seems to stain less deeply than the stalks themselves, but it is exceedingly difficult to decide whether the faintness of color is due to a specific difference of substance, or is simply the result of the tenuity of the film itself. In the former case, one would perhaps be justified in concluding that there was an *inter-prismatic substance* which served the purpose of a cement to hold the stalks together. The peculiar longitudinal band-like structures noticed during the elongation of the stalks (Plate I. Fig. 9, *g*, *h*, *l*) are possibly to be referred to the same substance.[1] But on the second assumption these shreds of faintly stained substance could be hardly more than the lacerated edges of the stalks themselves. I consider the latter the more probable explanation.

Owing to the spherical form of the egg, tangential sections are circular in outline, and in a given section the centre represents the deepest part. When the centre of such a section is occupied by the superficial part of the zona radiata, the periphery is formed by a circular band of the villous layer, the deeper portions of which are nearer the centre of the section.

A segment from that portion of the band which cuts through the bases of the villi, their roots, and the superficial portion of the zona, is shown in Plate III. Fig. 5. Proceeding from the outer (in the figure upper) toward the central portion of the section, one observes that the cross sections of the villi increase somewhat in size, that the stalks which embrace cavities become more numerous, and that the outlines of the stalks become more and more star-shaped, and then irregular, and that finally they break up into detached spots, which a little farther along become smaller and smaller until they cannot be distinguished in size from the pore-canals.

Since the sections of the membrane are successively increasing in diameter, the deep face of each will pass through a broader portion of the zona than the upper face will. If for the purpose of examination the section be inverted, so that the deep face is uppermost, the relation of parts can be much more easily and satisfactorily studied than if it be viewed from the upper face only, because the zona offers less impediment to vision than the thick-set columns of the villous layer. Attentive focusing shows conclusively on such preparations that the rays or branching roots of the prismatic columns lead each to a pore-canal, and it becomes possible in many cases to note the exact number of pore-

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[1] Dilute hydrochloric acid causes the distance between the heads of the villi to increase. This would be readily explainable as the result of the swelling of an inter-villous substance, could the existence of such a substance be satisfactorily established.

canals in which a given stalk takes root. The substance of the roots and of their rib-like extensions up the stalk appears to be more deeply stained than that of the expanded foot of the stalk; but this is perhaps only an appearance due to the fact that they are considerably thicker than the membranous portion which connects them.

As the successive sections pass through deeper and deeper portions of the zona radiata, the calibre of the pore-canals grows very gradually finer, and those which are plugged with deeply stained villous roots become less numerous, but otherwise there is no essential difference in the appearance of the sections. The characteristic arrangement of the pore-canals previously described is visible here, and may be made out more easily than on the fresh egg-shell, provided the sections are made perpendicular to the canals and are sufficiently thin.

In radial sections from eggs that have been hardened and stained, the zona is usually of a uniform faint tint (Plate III. Fig. 1), but often there is a very gradual deepening in the intensity of the color in passing from the outer to the inner boundary of the layer (Plate II. Fig. 1). In a few instances this deeper stain seems to extend toward the outer surface of the zona in flame-like jets (Plate IV. Fig. 1). The outer boundary of the zona, although appearing slightly irregular, owing to the variable lengths of the root-like prolongations of the villous layer, is in reality fairly even and sharply marked (Plate II. Figs. 1, 7, 8, Plate III. Fig. 1, and Plate IV. Fig. 1). The inner boundary is still more precisely defined, and appears as a fine continuous line, which sharply separates the zona from the peripheral layer of the yolk. Nowhere is there any evidence of a gradual transition from the yolk to the membrane. Occasionally, when the section is not exactly perpendicular to the inner surface of the zona, this boundary appears double, but careful focusing in such cases always shows this to be an optical illusion. In a few instances I have seen a similar appearance which was not thus explainable. For a considerable distance a layer of nearly uniform thickness appeared to intervene between the zona radiata and the yolk (Plate II. Fig. 7). But the line which separated this from the rest of the zona was never to be made out for more than a small portion of the circumference of a section, for it either terminated abruptly, or, gradually approaching the inner boundary of the zona, became confluent with it. Its inconstancy and its want of continuity are together sufficient to show that the layer in question is not entitled to be considered a distinct membrane, nor even a differentiated portion of the zona radiata. I may add, that I have never seen a section of this kind in which it was not possible to discover in some part of the layer evidences of pore-canals continuous with those of the remaining portion of the zona radiata. I am therefore convinced that *the zona radiata is a single homogeneous layer which is in direct contact with the surface of the yolk, and is traversed by pore-canals which reach from the yolk to its outer surface.*

When radial sections of the zona are broken, they occasionally show a tendency to rupture in lines concentric with the surface of the egg, but this is so rarely the case as hardly to be characteristic. The fracture is usually irregular, and not dependent on any structural feature; even the pore-canals do not appear to have much influence on the direction of the line of separation. The nature of these canals can be more readily studied on sections of hardened specimens than on the fresh shell. Their proximity to each other is not so readily determined from radial sections as by means of the tangential sections already described. The same general features which were mentioned in describing their appearance on the fresh egg are usually visible with even greater clearness on those which have been hardened. The distinctness of the pore-canals varies, however, considerably in different specimens, depending undoubtedly upon the refractive power of the mounting medium, which penetrates the canals, as compared with that of the matrix of the zona itself. Upon the most favorable preparations the canals can be easily traced from end to end, so straight is their general course. At the periphery of the zona they are uniformly somewhat broader than at its deep surface; but they taper so gradually as to make the difference in calibre, even at their two ends, trifling. In the case of almost every canal a slightly spiral course is noticeable near the outer end, whether it be plugged with the root of a villus or not; and throughout the whole length there is usually the faintest trace of a wavy or zigzag course. Aside from this, however, the canals are remarkably straight and parallel. There are no enlargements or irregularities in the calibre, save those which appear to result from the distention of the canal with the substance of the villous roots already described.

There still remain to be considered some peculiarities of the villous layer, which either result from particular methods of treatment, or have not been observed sufficiently often to allow one to consider them characteristic features.

Of those dyes which I have used, acetic acid carmine gives the sharpest differential staining for the heads of the villi. While the stalks and roots remain comparatively pale, the heads (Plate II. Fig. 2) take a deep rose tint, and the transition from the substance of the head to that of the stalk is rather abrupt. It happened that many of the villi from the shell of a mature egg, that was let fall into ninety per cent alcohol without contact with water, and was afterward stained for twelve hours in acetic acid carmine, exhibited a very peculiar appearance at the free surface of their heads. At or very near the middle of this surface the dark border, so characteristic of the heads of the villi, seems to be interrupted, and there projects from the free end of the head a short conical or longer finger-like process. This issues from the head, apparently through a circumscribed opening in the cortical layer, and may assume a variety of forms, several of which are shown in Plate II. Fig. 2, *a–m*. This peculiarity is interesting, as showing that there is a region of least resistance in the cortical layer near the apex of each head, which allows the protrusion of a part of the substance of the head when it is subjected to the swelling influence of the acetic acid; but whether this fact is capable of throwing any light on the source of this villous layer, or the method of its formation, I greatly doubt.

There is often to be seen in radial sections of the villous layer a strong tendency for the villi to fuse (Plate II. Fig. 2, *l*). This is especially true of the region of the stalks, although it is also to be observed among the heads. Since this tendency seems to be much greater in some cases than in others, I am induced to believe that it is due to the influence of the reagent with which the egg was hardened, and sometimes perhaps is dependent on the length of time the egg has been in the water before hardening.

In a few cases — especially in certain nearly mature ovarian eggs which were hardened in chromic acid — I have seen peculiar markings in the villi, which at first led me to think they might be traversed by spaces analogous to the pore-canals of the zona. They were first noticed on tangential sections, and appeared there like minute circular holes in the segments of the prismatic villi (Plate II. Figs. 4, 5). Focusing showed that their contents were much less refractive than the substance of the villi, and they were consequently very sharply defined. But the notion that they were optical sections of tubes, like pore-canals, was at once corrected upon finding isolated villi, which had fallen out of the layer and were seen sidewise. When the thickness of the section is about equal to the diameter of the villi, it is difficult, if not impossible, to decide whether the isolated angular blocks are seen endwise or sidewise; but by selecting the thicker sections, where the length of the villous segments is greater than their diameter, the difficulty is avoided.

and it at once becomes evident that the spaces supposed to be canals are for the most part minute spheroidal cavities or vacuoles. Usually there is only a single vacuole in a villus, although occasionally two are to be seen in the same cross section (Plate II. Fig. 4). Not all of the villi contain these cavities. Taking into the account their abundance on successive sections from the villous layer, I should estimate that not more than one half or three quarters of them present this feature. The proportion to be seen upon a single section is, of course, much less than this. They are most abundant in the stalks, but occasionally one is also seen in the head. Upon the egg, where they were found most abundantly, they were rather more numerous in the micropylar region than at the opposite pole. In the latter region there were, however, sometimes as many as three or four in one villus, although the size (0.5 to 1 μ) was the same as at the micropylar pole. I have in a few cases observed that the vacuoles were elongated, and then they were always of uniform calibre and were curved. Occasionally (Plate II. Fig. 4) such a tubular vacuole appears to communicate at one end with the intervillous spaces. Concerning the nature of the contents of these vacuoles, I can only say that they do not stain, and do not appear differently from what one would expect if they were cavities simply filled with the mounting medium.

The differences between the membranes in mature ovarian eggs and those recently deposited are principally the result of the swelling of the layers by the water, and do not require any further explanation.

The foregoing account of the zona radiata in Lepidosteus contains descriptions of two features which appear to me to bear directly on the condition of the zona radiata of fishes in general.

First. The *proof* that the striate appearance of the zona is due to *pore-canals*, although very generally assented to by the most competent observers, especially in recent years, has nevertheless hitherto rested upon comparatively slight evidence. That this evidence has been meagre depends upon the excessive minuteness of the structures in question. It is not to be overlooked, in the first place, that the tubular nature of the pore-canals in the case of the perch, as originally described by Johannes Müller ('54) for what he called the "Eikapsel," has not the slightest bearing upon the nature of the pore-canals of the zona radiata, since the egg capsule of Müller is a structure entirely different from the zona. I cannot, however, avoid the conviction that his opinion as to the tubulated condition of that capsule has had considerable influence in effecting the general acceptance of similar conclusions as to the nature of the radiate markings of the zona. Neither the evidence produced by Müller, — the possibility of pressing yolk globules through the "pore-canals" of the capsule, — nor the vacuolated condition described by Ransom ('68, p. 455), can have any direct bearing on this question.

Leuckart ('55, p. 258) appears to have been the first to assert with the utmost positiveness that the radial striations of the *zona* were due to pore-canals; and although he nowhere states the exact nature of the evidence which convinced him, we are doubtless at liberty to infer that it was, in part at least, the kind of evidence which he elsewhere ('55, p. 106, foot-note) makes use of; namely, the now well understood differences in optical effects produced by elevations and by depressions of surfaces. Kölliker ('58, p. 83) soon furnished additional evidence, derived partly from the study of *thin sections* of the zona in the trout, but more especially, as it appears to me, from the fact that maceration in fresh water causes the middle region of these supposed pore-canals to be converted into *vacuoles.* Aside from the arrangement of the dot-like appearances as seen from the surface of the zona, which has been very generally recognized, and the features emphasized by Leuckart and Kölliker, I am not aware that any additional evidence in proof of the nature of the pore-canals has yet been produced. If, then, the facts warrant the description I have given of the zona in Lepidosteus, the evidence that it is a canaliculation which produces the radial striate markings in the zona radiata of fishes' eggs has received an additional confirmation.

Secondly. Although Müller (as well as more recent observers) has shown that the pore-canals in the *outer* envelope, or capsule, in the case of the perch may have a *spiral* course, no one has hitherto observed a similar feature in the case of the canals of the true zona radiata. The natural injection of these canals in Lepidosteus with a substance continuous with that which constitutes the villous layer, renders it comparatively easy to establish the spiral course of the canals in that fish; and this makes probable the inference, that certain irregularities in the direction of these canals, shown by other observers to exist in the case of other fishes, may in reality be referable to the same spiral condition, which, from the minuteness of the canals, has not been recognized.

b. *Micropyle.*

The micropyle was apparently overlooked by Balfour and Parker, since it is not mentioned by them; nor has it been mentioned, I believe, by any one else, although it occupies a region which is so conspicuously marked that, having once seen it, one could readily find it with the aid of a simple lens. Except in eggs that have lain for some time in water, the region of the micropyle appears, when seen under a hand lens, like a minute hole in the shell; in surface views with a higher power it looks like a deep circular pit (Plate IV. Figs. 3, 4) sunk in the egg membrane. Its diameter is five or six hundredths of a millimeter. Its outline is nearly always circular, and it has a clearly cut edge. In a few cases a cross section of the pit has proved to be oval instead of circular, occasionally with one diameter of the oval more than twice as long as the other (Plate VII. Fig. 4). A similar appearance, though not so marked, is often produced, even when the pit is really circular, if the plane of the section is oblique to its axis. Sometimes the pit is partly filled by a whitish, apparently spheroidal body (Plate IV. Fig. 4). When the egg is so viewed that this depression lies in the equator, the profile of the egg in its vicinity may be slightly modified, and show a low conical elevation, at the apex of which the pit is located. This is not commonly the case, however, for usually there is nothing in the profile to denote the position of the pit. In eggs nearly mature, and in those which have been recently laid, its place can be easily found by its relation to the lighter colored animal pole of the egg. It is invariably located over some part of the germinal area, and usually precisely over its centre (Plate IV. Fig. 3).

The real nature of this pit and its relations to the two layers of the egg membrane and to the yolk can be studied on optical, but still better on actual sections. For a general survey radial sections are most instructive, but for the elucidation of some questions sections tangential to the egg at the animal pole are more valuable.

In strictly radial sections through the region of the micropyle, it is to be seen that the surface of the egg is deeply depressed. The form of the depression varies somewhat in different eggs, from that of a funnel, i. e. with sloping walls (Plate IV. Figs. 1, 5), to that in which the walls are for some distance almost parallel (Plate V. Fig. 2). This depression results from an infolding of both layers of the egg membrane; it forms, however, only an approach to the true micropyle, or *micropylar canal,* the latter being a minute passage through both layers which begins at the bottom of the depression.

The *funnel*, as I shall call that portion of the egg membrane which forms the walls of the depression, involves a modification of both the zona radiata and the villous layer. Both are affected in two ways, in thickness and in direction.

The villous layer begins to grow thinner at some distance from the edge of the funnel. Sometimes it retains its normal thickness to within a distance equal to the diameter of the funnel; at other times it begins to grow thinner at three or four times that distance from the pit. Its diminution in thickness is quite gradual and very nearly uniform until it reaches a minimum at the micropylar canal. The stalks of the villi are shortened more than the heads, in comparison with their appearance on other parts of the capsule, and the boundaries between them gradually become less distinct. The diameter of the villi also decreases considerably. Near the bottom of the funnel they become very short, but frequently it is evident that, instead of constantly diminishing in diameter, they may even increase as compared with other regions of the funnel (Plate VI. Figs. 5–8).

In all parts of the funnel the villi retain a direction perpendicular to the outer surface of the zona. In the lateral wall, and especially near the bottom of it, they are slightly wedge-shaped or conical, the head ends being narrower than the root ends. They thus accommodate themselves to the diminished space at their disposal (Plate VI. Figs. 6–8).

The zona radiata (Plate IV. Fig. 1) likewise begins to diminish in thickness at some distance from the micropylar canal, and continues to do so until it reaches the canal; but it does not, like the villous layer, grow thinner at a uniform rate. Its thickness decreases very slowly to within a short distance of the region where the membranes begin to bend inward to form the funnel, and then it suddenly narrows to one third its normal dimension, after which it again decreases more slowly until it reaches the micropylar canal. The pore-canals are not perceptibly finer nor more closely set in the vicinity of the micropyle than elsewhere. They retain in most regions a rectilinear course perpendicular to the surface of the zona, but at the region of most rapid reduction in the thickness of the latter, and for a little distance on either side of it, their course is curved, the concave side facing the micropylar canal.

The change in the direction of the two layers of the egg-shell results in the formation of an external depression, which is considerably deeper than the total thickness of the shell, so that, even with a great diminution in the thickness of the latter, its inner surface projects into the yolk as a conical elevation, which is nearly as high as the thickness of the shell. In this deflection of the membranes, the zona radiata seems to bend more abruptly than the villous layer; this, however, is due principally to the fact that the region of greatest curvature is also the region of most rapid change in the thickness of the zona. From this it results that the inner contour of the zona is much more abruptly curved than the outer, in some cases appearing almost angular. As a further consequence of this, the conical elevation appears to arise abruptly from the inner surface of the membrane; its apex is rounded, and in the ovarian egg its surface is everywhere in contact with the yolk. An inquiry as to whether this infolding is the result of a process of absorption, or is due to a peculiar local modification of the activities which produce the membrane, will best be deferred until I have given a description of the layer of cells which immediately invests the ovarian ovum.

The micropyle proper, or the *micropylar canal* (Plate I. Fig. 11^a, Plate IV. Fig. 1, Plate VI. Figs. 3, 4), is straight and of uniform calibre. It begins at the centre of the bottom of the funnel, and passes through both villous and zonal layers of the egg membrane; it is about 8 μ long. Its cross section is circular and about 2 μ in diameter. There is no flare to the canal, either at the external or internal end, so far as I have been able to observe. I am unable to say whether the diameter which I have given is that which the micropyle possesses at the moment the egg is laid. From measurements of spermatozoa allowed to dry upon the slide (Plate VII. Fig. 3), one would imagine that the calibre of the micropylar canal must be at least 3 μ, that being the diameter of the heads of spermatozoa thus treated; but according to measurements made upon living spermatic cells the heads are only about 1.8 μ in diameter, so that I think 2 μ is probably the normal average calibre of the canal. Still, I have sections in which its diameter is 2.5 μ, and in the case of some fresh membranes it was only 1.5 μ in diameter. The narrowness in the latter case I attribute to the swelling of the zona when exposed to water and glycerine, in which the membranes were examined.

c. *Granulosa.*

Nearly mature ovarian eggs are closely enveloped by an uninterrupted cell layer, which is everywhere in contact with the outer surface of the villi. Over the greater part of the egg this layer — the follicular epithelium or *granulosa* — is composed of thin, flat polygonal cells, arranged in a sheet only one cell thick. In surface views the granulosa cells (Plate V. Fig. 4) appear of fairly uniform size, — 15–20 μ in diameter, — are slightly granular, stain feebly, and exhibit each a single large (5–10 μ) nucleus, with an even outline and a circular or oval form. When seen in profile, — as in radial sections of the egg with its membrane and granulosa (Plate V. Fig. 3), — a majority of the cells are observed to be very thin, and their nuclei flattened; but there is occasionally a cell whose nucleus is not so much flattened, and which therefore protrudes beyond the general surface of the granulosa. Radial sections of the ovum with its granulosa are further instructive in showing the relations of the cells to the heads of the villi. *Each granulosa cell corresponds in size to from four to eight villi, but there is no constancy in the position of the cells or their nuclei in reference to the underlying villi.* Nothing intervenes, however, between the cells and the villi except occasional artificial spaces. Externally the granulosa is limited by a thin, homogeneous delicate membrane, the *membrana propria* (*th. fol.*) of the *theca folliculi.*

This is the condition which obtains over all parts of the egg except in the vicinity of the micropylar funnel. Elsewhere the granulosa retains great uniformity of thickness. At a considerable distance from the micropyle its cells begin to elongate so that the granulosa grows thicker; as the cells approach more and more the condition of columnar epithelium they become inclined, their outer ends being directed toward the axis of the micopyle (Plate VII. Fig. 1). They still continue to form a layer only a single cell deep until they reach the vicinity of the rapid declivity in the wall of the funnel. Here the cells, having now attained an elongated columnar form, become superposed, and *fill completely the micropylar funnel.* With a single exception the cells composing this mass are fairly similar to each other. They are considerably elongated, irregularly columnar or spindle-shaped, and contain each a single oval nucleus about 10 μ by 8 or 9 μ in diameter. The cells themselves vary from 15 μ to 40 μ in length, and are about 10 μ in diameter. When the hardened egg, with its membranes, is removed from the follicle, it often happens that this conical plug of granulosa cells is left with the .rest of the granulosa in the follicle. But even when the majority of the granulosa cells of the plug are thus removed from the funnel, there is usually left behind a single one which is unlike the others. It occupies the bottom of the funnel, which it completely fills, and is much larger than any other of the granulosa cells (*m py. cl.* Plate IV. Figs. 1, 4, 5, Plate V. Fig. 2, Plate VII. Fig. 2).

When I first became aware of the existence of such a cell it was from the study of radial sections of a recently deposited egg in which a "maturation spindle" was visible near the micropylar pole (Plate IV. Fig. 1). As there were no other granulosa cells left attached to the egg, the first impulse was to regard this as one of the "polar cells" formed by the ovum during maturation. This seemed the more probable on account of the undoubted existence of a maturation spindle. A serious obstacle to this view was the great size of the cell as compared with the narrow mycropylar canal. Even the elongated condition of the cell would hardly warrant the assumption that it had passed through so narrow an orifice. The examination of suitable sections from ovarian ova (Plate IV. Fig. 5, Plate V. Fig. 2, Plate VII. Fig. 2) soon showed that this interpretation was inadmissible, and made it as certain as one could expect, without having traced it from its origin, that the cell in question was a specially modified granulosa cell. It may be appropriately called the *micropylar cell*, for, whatever may be its function, the morphological fact remains that it occupies the micropylar funnel, and lies directly over the micropylar canal. I have not been able to discover that its substance extends into the canal, but the number of favorable cases which I have examined is not enough to allow me to say that such a condition is improbable. So far as I know, nothing of this kind has been found in the case of any of the osseous fishes, unless the figure given by Hoffmann ('81, Taf. I. Fig. 20) for Leuciscus is capable of being thus interpreted.[1] Hoffmann himself has evidently not considered the condition of the granulosa in the region of the micropyle sufficiently important to give it any attention in the text, but there is not the least doubt in my mind that the accumulation of granulosa cells which he has figured is the equivalent of the granulosa plug in Lepidosteus. I am inclined to believe, moreover, that Hoffmann has overlooked a real difference between the cells in this region, and that an equivalent of the micropylar cell of Lepidosteus will be found in Leuciscus, and perhaps in many other of the osseous fishes, especially in those where there is a large micropylar funnel. In fact the three cells which in Hoffmann's figure (Plate I. Fig. 20) seem to occupy the funnel, are all slightly larger than the remaining granulosa cells, and one of them — the deepest — fairly represents in its position the micropylar cell. Since all the cells have a somewhat diagrammatic appearance, it is not too much to expect that a more careful examination would show a difference between them.

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[1] Since this account was written, Owsjannikow and Cunningham have both found similar conditions in other fishes. A review of their articles will be found at the end of the historical section of the present paper, pp. 104–110.

d. *Origin of the Zona Radiata and the Villous Layer.*

The youngest ovarian eggs in which either of the egg membranes has been observed were about 430 μ in diameter, and the ovaries to which they belonged were preserved just before the period of spawning began. Sections of such an egg are shown in Plate VIII. Figs. 1 and 2. Tangential sections (Fig. 2) show that the egg is enveloped in a layer of polygonal granulosa cells whose boundaries are exceedingly faint, and whose nuclei have very irregular outlines, being lobed or deeply incised, in some cases almost to complete division. The nuclei contain one, and frequently two small nucleoli, but otherwise appear homogeneous, and are uniformly stained. Upon focusing just below this layer of granulosa cells, one sees the surface of the yolk covered with innumerable fine, close-set points, which are evenly distributed.[1]

Radial sections (Plate VIII. Fig. 1) supplement the surface views, and show that the granulosa cells are relatively thin, and easily separable from the underlying structures. Their protoplasm is finely granular, and their boundaries are not distinguishable; neither do their deep surfaces appear to be defined by any membrane. Their nuclei are considerably flattened, and irregular in outline.

Immediately beneath the granulosa the surface of the yolk exhibits fine radial, nearly parallel markings, which are close together and very short. They are so intimately joined to the yolk that they seem to form an integral part of it, and nowhere show the least tendency to become detached from it. With high powers one can recognize a very thin cortical portion of the yolk (membrane ?), with which they seem to be continuous. It is very difficult to ascertain the distance between the markings, but about 21 of them may be counted in the space of 17 μ, so that the average distance is not far from 0.8 μ. The length of each is about 0.5 μ.

It would not be easy to determine from this stage alone whether the markings indicate the beginnings of the formation of the zona radiata or the villous layer. But even in this early condition the punctate markings of tangential sections appear brighter rather than darker when one focuses high, so that the inference must be that they are due to minute bodies which are *more highly refractive* than the surrounding substance.

This conclusion is abundantly confirmed by the study of somewhat larger ova. These bodies seem to increase in length with considerable

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[1] In Fig. 2 (Plate VIII.) these punctations appear much too scattered in the middle of the area which shows them. They are better represented toward the margin of the area.

rapidity, for when the egg has attained a diameter of about 600 μ (Plate VIII. Fig. 3, Plate IX. Figs. 4, 5) they may have reached the length of 3–3.5 μ. In this stage the layer when seen from the surface presents an appearance (lower half of Fig. 3, Plate VIII.) which so closely resembles that of the zona radiata in the mature egg, that one is involuntarily led to believe that it is the zona. Even the peculiar arrangement of the markings in curved lines recalls the appearance of the zona when seen in a similar position. Notwithstanding the striking resemblance, there cannot be the slightest doubt that this layer is not the zona radiata. In radial sections it is difficult to distinguish between a layer composed of a homogeneous matrix pierced with minute parallel *canals,* and one composed of parallel *rod-like* structures, but in surface views this is much easier. Careful focusing shows the same optical properties as were observed in the earlier stage, and with much greater distinctness. The staining, too, is such as is to be observed in the villous layer rather than in the zona; for the highly refractive bodies take the deeper stain, the intervening substance having the paler color of the yolk. But the last possibility of doubt concerning the nature of this layer is dispelled by the appearance presented when the elements which compose it are separated from each other. It frequently happens in mounting thin sections that portions of the layer are detached, and even resolved into their constituent elements. In such cases clusters of two or three rod-like bodies, and even single ones, can be found in such proximity to the layer as to leave no doubt that they are elements detached from it. They have the same length and thickness as the markings of the layer; they are highly refractive and deeply stained. They can in no way correspond to anything that is observed in the zona radiata. but do resemble in several particulars the villi of older eggs.

From all this evidence I am certain that the layer which is first to make its appearance between the yolk and the follicular epithelium is the villous layer.

In this stage, too, the union of the layer with the yolk is much more intimate than its relation to the granulosa. The latter is often separated from the layer, the yolk never.

The cells of the follicular epithelium (Plate VIII. Fig. 3) have become somewhat smaller than in the previous stage, but their nuclei retain the same dimensions and the same lobed appearance which they had during the earlier stage. As a consequence, the nuclei are closer together. It will be seen that in the stage figured on Plate VIII. Fig. 3, the diameter of a single average-sized granulosa cell corresponds to the distance occupied by about a dozen of the villi. This fact will be of some interest later, when a comparison is made with the conditions in the mature egg.

In the sections figured on Plate IX. the villous layer has become still thicker and the villi are correspondingly elongated; they are also somewhat farther apart, as well as thicker.[1] The thickness of the individual villi is really greater than that of the spaces intervening between them, but the appearance as seen under the microscope is represented with tolerable accuracy in the figures. The villi of the egg shown in Fig. 3 (Plate IX.) have attained a little greater length than those of the other eggs figured, but the egg itself was probably somewhat smaller than the one shown in Plate IX. Figs. 1 and 5. I am not entirely certain of this, because the egg was incomplete, the yolk having all disappeared except a portion directly underneath the villous layer.

A more advanced condition in the development of the ovum and its membranes is to be seen in Plate VII. Fig. 5. The evidence that this egg is more advanced than those last described is found in its slightly greater size (nearly 0.7 mm.), and also in the increased size and elongated condition of the yolk bodies which already occupy all parts of the egg except a peripheral layer. The villous layer has here attained a thickness of 5.5 μ, or about one third its thickness in the mature egg, but the individual villi have not changed perceptibly from the condition in the previous stage, except in regard to length.

I have no stages between this condition and that which the eggs present at maturity, but already enough of the egg membranes has been formed to allow several conclusions as to the method of their production.

It is to be observed, that immediately before spawning there is no structure, even in the latest of the stages here described, which can be considered the zona radiata; neither are there at this time any stages older than the one last described, except the mature ova. It seems to me, therefore, perfectly safe to infer that *the* ZONA RADIATA *is developed after a large part, if not the whole, of the villous layer has been produced, and that it is wholly formed during the twelve months immediately preceding the spawning.* From its late production and its position inside the villous layer, as well as its intimate relations to the yolk, it is further to

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[1] In Fig. 5 (Plate IX.) they are represented a little too far apart and not quite thick enough, whereas in Fig. 3 (Plate IX.) they have been represented too close together. The granulosa cells in Fig. 5 are too sharply defined, especially on the side toward the villous layer.

be inferred that *the zona radiata is exclusively the product of the yolk.* It is also probable, from the evidence of stratification sometimes seen in the completed structure, that the zona is produced in successive layers. If such is the case, it follows that portions of the zona nearer the yolk are formed after those which have a more peripheral position.

The question as to the source of the villous layer is not so easily answered. The fundamental difference between it and the zona radiata at once suggests for it a different origin. If the latter arises from the yolk, the former might be produced by the follicular epithelium. This view would seem to receive confirmation from the peculiar way in which the roots of the villi in the mature egg penetrate the pore-canals of the zona radiata. I have no doubt that this condition would be regarded by many observers as a welcome confirmation of the theory that the pore-canals are primarily for the purpose of transmitting nutritive material to the growing egg. Such observers might look upon the villi as secretions from the granulosa, which, owing to slight physical and chemical changes, had not passed through the pore-canals as nutriment, but remained partly outside the zona to subserve other functions. This view might be further supported by the fact that during the formation of the villi the inner surfaces of the granulosa cells are not sharply marked off by membranes from the underlying structures.

Nevertheless, it seems to me that the arguments which may be adduced to support the opposite view, — that the villous layer is the product of the secretive activity of the ovum itself, — greatly outweigh these considerations.

During the early stages of their formation the villi are so intimately related to the ovum that they appear to be rods imbedded in its substance, and at no time during its formation is the villous layer separable from the yolk. If the latter is by any means removed from the membrane, there is always a superficial portion of the ovum which remains attached to its inner surface. The separation of the granulosa cells from the membrane during this period, on the contrary, is quite common. What might otherwise be a serious obstacle to considering the villi the product of the ovum, — the presence of a zona between the two, — is entirely nullified by the fact, previously established, that the villous layer is produced *before* the zona radiata.

Whatever renders improbable the formation of the villi from the follicular epithelium is, of course, favorable to the opposite view. If the villi were products of the epithelium, one would expect some constancy in the numerical relations between the two, but this is certainly wanting.

I have made some measurements and comparisons between eggs half a millimeter in diameter and those having a diameter of about two millimeters, which indicate that the *number* of the villi remains constant during the period of growth from the smaller to the larger size.

In an egg 0.5 mm. in diameter there occur about 30 villi in a space of 35 μ; i. e. the villi are about 1.15 μ from centre to centre. In an egg 2 mm. in diameter from the same ovary, treated in the same manner and cut at the same time, the villi are 4.5 μ from centre to centre (compare Plate V. Figs. 3, 4). Allowing for the growth of the smaller egg, which at the larger size would have a diameter four times as great as at first, it is evident that the interval for a villus would be four times 1.15 μ, or 4.6 μ, which agrees fairly well with the space (4.5 μ) actually occupied by a villus in the larger egg measured. There are also other reasons for believing that the villi do not increase in number after the egg has reached a diameter of half a millimeter. If new villi were interpolated, one would reasonably expect to find the younger ones shorter than the older ones; but at no stage which I have seen is there any marked difference in their lengths.[1]

In the larger eggs measured (2 mm.), the nuclei of the granulosa were on the average about 14 μ apart, from centre to centre; i. e. there were about three villi to the diameter of each cell. But in eggs about half a millimeter in diameter (compare Plate VIII. Fig. 3, and Plate IX. Fig. 5) it is to be seen that from six to fourteen villi correspond to the diameter of a single granulosa cell. If there has been no change in the number of villi, it follows that the granulosa cells must have increased in number *at least* fourfold between the half-millimeter stage and the two-millimeter stage. It is for this reason I contend that there is no constancy in the numerical relations of villi and granulosa cells, and that consequently it is improbable that the former are the product of the latter.

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[1] It is evident that there has been a corresponding increase in the *diameter* of the individual villi during the growth of the ovum, for in the mature condition they form a continuous layer, with little or no intervening substance.

Ransom ('67) has claimed that the pore-canals of the zona radiata increase in number during the growth of that membrane. If one were to disagree with me, and to regard the markings which first appear at the surface of the ovum as the incipient zona instead of the villous layer, he would be compelled to adopt Ransom's view, for the intervals between the markings on eggs half a millimeter in diameter (1.15 μ) would become, unless there were interpolations, 4.6 μ apart when the eggs had increased to two millimeters in diameter. In order to reduce the intervals to the condition actually found in the zona of the mature egg (1.4 μ), the number of pore-canals would have to be increased more than threefold!

At first thought one might regard the modifications of the villous layer in the micropylar region as the direct result of an alteration in the secretive powers of the granulosa cells situated at that place; but it seems to me that the thickness of the layer ought, on this assumption, to be greater than elsewhere, since the granulosa cells are here more numerous and larger. Besides, the corresponding diminution in the thickness of zona radiata could not be thus accounted for, but must be assumed to be the result of diminished secreting activity on the part of the ovum in this region. Hence the same explanation would certainly be more reasonable in the case of the villous layer. This is a point which seems to me of considerable importance; the diminished activity of this region which is shown during the formation of the zona was already manifest during the formation of the villi.[1]

From these several considerations, I believe there can be little question that *the* VILLOUS LAYER *of the egg membranes in Lepidosteus is also the product of the ovum itself rather than of the follicular epithelium surrounding it.*

If this conclusion is established, it follows that the parts of the villi first to be produced are those which are most superficial. I believe that this is confirmed by the fact that the forming villi are readily stained in carmine. It is probable that, even in the latest stage of the immature eggs (0.7 mm.) which I have seen, not much, if anything, more than the heads of the villi have been produced. The length and the highly refractive condition of the villi at this stage, and the fact that they are not at all folded, all point to this conclusion.

There still remains much to be done in following out the exact course of the development of the membranes in Lepidosteus, — especially in determining when the formation of the zona begins in relation to the completion of the villous layer, — but I think that the main features of the process as outlined above will not be disproved by subsequent study.

I have no explanation to offer of the apparently sudden change in the nature of the secretions from the ovum which is registered in the production of structures so dissimilar as the zona and villous layer are; but it is possible that some light may be thrown on this question when the period of the transition has been carefully worked out.

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[1] This is an evidence of the polar differentiation of ova (which exhibits itself in many other phenomena) to which attention has not hitherto been called.

B. Historical and Critical Review of the Literature on the Primary Egg Membranes[1] and the Micropyle in Fishes.[2]

It is possible that the eggs of fishes may present as many as *four* essentially distinct kinds of enveloping membranes before separation from the ovary. The innermost of these, if it exists, may be considered a true *vitelline membrane*, the equivalent of the cell membrane in general. I have made no observations concerning it, and shall have little to say regarding the conflicting testimony as to its existence. The second, proceeding from the yolk outward, is radially striate, and I shall call it, as in the preceding description, *zona radiata*. Although this is totally different in structure from the next membrane, there are several reasons why it will be best to consider both at the same time. This third membrane I shall call, as previously, the *villous layer*. The fourth and outermost, when it exists, is formed exclusively from the granulosa cells, and may be called by the name first given to it by Johannes Müller, — *capsular membrane*.[3]

a. *Cyclostomata.*

The eggs of the myxinoids are enveloped in a "horny capsule," which was first described by Thomson ('59, pp. 50, 51) for *Myxine glutinosa*. He evidently considered it the equivalent of the egg cases of selachians. Since the latter are formed in the oviduct, they cannot be considered

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[1] I use the expression *primary egg membranes* in the sense in which it has been employed by Ludwig ('74 p. 197), i. e. for all membranes which are the product of either the ovum itself or the follicular epithelium surrounding it.

[2] Owing to delays in publishing my studies I have been able to extend this review, and to bring it down so as to include papers which have appeared since my own account was written.

[3] I have the less hesitancy in adopting this name because Müller ('54, p. 189) — notwithstanding some misconceptions as to its real nature in the perch — gave the following concise, and, in my opinion, still perfectly applicable definition: "Eine von dem Eifollikel, Ovisac eines Wirbelthiers erzeugte Eihülle scheint von der Eischale anderer Eier unterschieden werden zu müssen als *capsulare Eihülle*, oder Eicapsel." When subsequent observers, — as for example His ('73), — ignoring the true explanation of Müller's investigations given by Leuckart ('55, pp. 257–260), transfer the name Eicapsel to the zona radiata, one is compelled to protest that that was not the structure described by Müller under the name of "Eicapsel," and that no one has yet brought forward satisfactory evidence that the zona is "produced by the egg follicle," as Müller's definition demands. It therefore seems to me that it is better, for the sake of avoiding confusion, to drop entirely the name capsule — whether egg capsule or "cartilage capsule" (His) — as a designation for the zona radiata.

as primary egg-membranes; but Steenstrup ('63) subsequently showed that the egg of Myxine glutinosa possesses this covering before it leaves the ovary, from which it follows that the "horny capsule" is really a primary membrane.

THOMSON'S ('59) account is brief: "I have found that in the Myxine glutinosa the globular yolk is enclosed in a horny capsule of similar consistence and structure [to that of the oviparous cartilaginous fishes], but of a simple elongated ellipsoidal shape, and in place of four terminal angular tubes, a number of trumpet-shaped tubular processes projecting from the middle of the two ends, which probably serve the same purposes as the differently shaped appendages of the ova of the shark and skate."

STEENSTRUP ('63, pp. 233–238, Figs. *a–h*) also saw the horny egg-shell and the peculiar projections from its ends. He says (p. 236): "In the last received individuals the eggs now had not only the same considerable size [as some large eggs previously described] and more oval-elliptical form, but besides they were surrounded with a somewhat firmer, almost horn-like egg-shell, which was furnished at the ends with a large number of slightly curved or S-shaped horn-threads. Each horn-thread ends in a head-shaped portion with three or four projecting spines or hooks, and has thereby some resemblance to a ship's anchor. The threads recall — even though somewhat remotely — the horn-threads projecting from the eggs of the rays and sharks, much as the shell itself recalls the firm capsule of these cartilaginous fishes. The accompanying figures exhibit both the appearance of the capsules (*f*, *g*) and the manner in which they hang in the mesovarium (*h*** and *h****), together with eggs of the same appearance as *c*, *d*, *e* (Fig. *h**), and with a large number of only slightly developed eggs (*o*, *o*, in Fig. *h*)."

In the two eggs with horny shell figured by Steenstrup, the shell has been represented as though it were composed of two parts separated by a sharp continuous line; the egg appears cut through near one pole by a plane perpendicular to its long axis. The appearance recalls that seen in the egg-shells of certain trematodes, where one end serves as a lid which opens to allow the larva to escape; but whether the author regarded this as a similar provision for the escape of the young hag, or as an accidental condition, is not stated in the text.

WILHELM MÜLLER ('75, pp. 114–117, Taf. V. Figs. 14, 15) appears to regard the "Testa" of Myxine glutinosa — which I suppose to be the same as the "horny capsule" of Allen Thomson — as resulting from the secretions [metamorphosis?] of a layer of [granulosa] cells, which immediately invest the ovum. He does not expressly state this, but it seems to me he leaves one to draw such an inference. He says that the ovarian egg when 0.6 mm. in diameter is surrounded by a single layer of very flat polygonal cells, outside of which is a thick layer of fibrous connective tissue, and that when the eggs have attained a length of 18 mm. and a thickness of 6 mm. there are two connective-tissue envelopes; an outer thinner, a continuation of the mesovarium, and an inner, which at the ends of the egg is thickened (0.4 mm.) and vascular. At its inner surface the inner membrane is condensed into a lustrous membrana propria 2 μ thick, and is firmly attached to the underlying "Testa." In contact with the inner surface of this membrana propria is a layer of cells. In the middle of the egg the cells are cubical, but they become more and more cylindrical towards its poles, where the layer becomes three or four cells deep.

I believe there can be no question that this layer of cells inside the membrana propria represents the granulosa; but it seems as though Müller must have overlooked the egg membrane, if one existed at that stage, and must have taken the granulosa to be in some way the equivalent of it. Perhaps, assuming that the granulosa cells secreted the membrane, his idea was that the granulosa ought itself to be considered as a part of the "Testa," for he afterwards (p. 126) mentioned, in the case of *Petromyzon Planeri*, "a very thin folded egg membrane which exhibited a polygonal pattern when seen from the surface." Moreover, he says, with regard to two *deposited* eggs of Myxine which he examined, that there was no trace of either inner or outer connective-tissue envelope, and from this fact concludes that they must have undergone complete regressive metamorphosis, similar to that which the enamel organ of the teeth suffers after the completion of the enamel.

W. Müller is the only person who has seen anything of a *micropylar apparatus* in the myxinoids. "Exactly in the middle of the white pole of the egg," he says (p. 115), "this cell layer [granulosa] exhibits a conical infolding 0.1 mm. deep and 0.06 mm. broad, which contains a funnel-shaped opening, the micropyle, which is directed straight toward the underlying nucleus and the protoplasm surrounding it." This is the whole of his description; and from it I infer that he has seen that portion of the granulosa which occupies the micropylar *funnel*, but that the micropylar *canal* — which is *a passage through a membrane, not an involution of a cell layer* — has not been seen by him. If the condition in Myxine is at all comparable with that in Lepidosteus, it is certain that Müller has seen the equivalent of what I have called the micropylar plug of granulosa cells, and it is therefore probable that he was the first person to observe that peculiar structure in any fish-like animal. If he were less positive in his assertion that the infolding contained an opening, I should question if the cells took the form of a hollow funnel; even as it is, I doubt if the membrana propria is infolded.[1]

The first account of the membranes in *Petromyzon Planeri* was by MAX SCHULTZE ('56, pp. 1–5). When taken from the body, the eggs had besides the yolk membrane a firm "Eischalenhaut," or "chorion," which was surrounded with a scarcely discernible thin layer of gelatinous substance, which was quickly swollen, when it came in contact with water, to a thickness of not more than a quarter of a line. It was delicate and fugitive, and was easily removable from the firm underlying membrane. In the course of eight days it mostly disappeared, being dissolved in the water; it was not an "albuminous layer," but was rather to be compared to the gelatinous mass uniting frogs' eggs; its chemical composition was not known.

The firm "Eischalenhaut," which closely enveloped the egg, was a clear membrane about 0.0015‴ (probably should be 0.015‴, or about 0.03 mm.) thick, which had a tendency after being torn to roll in at the edges. It appeared very finely punctate when viewed from either the inner or the outer surface. Schultze was inclined to regard the punctations as due to very fine canals traversing the membrane, but on account of the delicacy of the object he could not reach a perfectly satisfactory conclusion on this point. For this finely punctate membrane and that found in bony fishes, the author would use the name *chorion* rather than vitelline membrane, for a true vitelline membrane (or egg-cell membrane) exists inside the punctate structure.

OWSJANNIKOW ('70^a, p. 184) says that the gelatinous layer of the outer egg membrane is very little developed, so that the fertilized eggs are only feebly attached to the objects on which they fall, the least current carrying them away.

CALBERLA'S ('78, pp. 438–441) account is in some particulars more extended than that of Schultze. The eggs, he says, instead of being round, are slightly ellipsoidal. The membrane (zona) consists of two layers, which are not, however, sharply separated from each other. The outer is highly refractive, rough externally owing to all sorts of elevations and tooth-like structures (Zacken); the inner is much thinner

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[1] For a review of more recent work on Myxine, see pp. 91–93, 107–110.

and translucent. With low powers the outer appears as though made up of concentric layers, but with higher powers it is seen to be a homogeneous substance traversed by fine radial canals which are continuous with those passing through the inner layer. At the outer surface each of these canals opens out at the base of one of the elevations (Zacken). Calberla regards this whole layer as a secretion from the peripheral layer of the yolk. The proof of it he finds in the conditions of the membranes in nearly ripe and in over ripe eggs. On the former, the boundary between the two layers is sharper and the inner layer is much thicker than on mature eggs; whereas on the latter all distinction between inner and outer layer has disappeared.

As soon as the egg comes in contact with the water, the tooth-like projections on the surface of the egg membrane (zona) quickly swell, in consequence of which the whole egg appears as if surrounded with a delicate area of hyaline substance. This may well be the cause, he adds, of the stickiness of the surface of the egg.

It seems to me that there is considerable reason for believing that these external projections described by Calberla correspond to the villi of Lepidosteus, both in function and in position. An examination of his figures (Taf. XXVII.) lends support to this view. I believe also that, when the genesis of the membrane has been studied, it will be found that these "Zacken" are formed before the zona itself. It is true that

Kupffer und Benecke ('78, pp. 9, 10) find the conditions somewhat different from those recorded by Calberla. They claim that the envelope of the egg consists in both P. Planeri and P. fluviatilis of a double membrane (Eihaut), and of a continuous covering of gelatinous material which is replaced at the watch-glass-like elevation of the membrane by a structure known as A. Müller's "Flocke." The inner membrane — which they figure as being much thicker than the outer — contains closely set pore-canals, but these they assert positively are not continued into the outer layer. The difference in structure between the two membranes is demonstrable by means of 0.5 per cent hydrochloric acid. The outer membrane swells more in water than the inner, but not quite uniformly. It appears here and there as though it were restrained by a filament of less easily-swelling substance. And this, they say, is probably the cause of "Calberla's unzutreffende Angabe, dass diese Rindenschicht mit allerlei Erhebungen und Zacken besetzt sei, an deren Basis Porencanäle mündeten."

But even if Calberla's description is not quite satisfactory, it is evident that this outer envelope is not homogeneous, and that the toothed appearance which he has figured must have had a basis in optically different portions of that envelope. According as the imbibition of water has proceeded less or more, this marking might be more or less conspicuous. From a comparison of the figures by Calberla with those by the last mentioned authors, I should think that Calberla's outer layer of the zona by no means corresponded with the outer layer of Kupffer und Benecke, and that the latter, being very thin, had been overlooked by Calberla.

The *micropyle* of Petromyzon, though sought for by Schultze ('56) and A. Müller ('64) was not found by them.

Owsjannikow ('70^a, p. 184), who discovered it, says that it is very small, but that it remains visible for several days after fertilization. In mature eggs it occupies a position over the eccentric nucleus.

Calberla ('78, pp. 439, 440) has given a careful description of the micropyle, which, he says, agrees in all essential particulars with that of osseous fishes. His account is substantially as follows. At one pole of the elongated egg its membrane is thickened, and bulges out, much as though a shallow watch-glass — with shorter radius of curvature than the rest of the egg membrane — had been set into one end of the membrane. Radial sections which pass through the centre of the elevated portion of the membrane show that in the middle of it there is a very flat saucer-shaped depression, the centre of which is further depressed into a funnel. From the narrow end of the funnel a canal is continued through the membrane, and opens on its inner surface with a slight flaring. A little below its middle the canal exhibits a spindle-shaped enlargement, which is shown in Calberla's Taf. XXVII. Figs. 2 and 3.

The views held by Kupffer und Benecke ('78, pp. 9–15) regarding the nature of the micropyle are not easily summarized. They are based on close observations of the deportment of the egg and spermatozoa at the time of fertilization, but do not appear to have been corroborated by sections of the egg membranes.

In the region of the watch-glass segment of the membrane described by Calberla, the mucilaginous envelope outside the membranes is wanting, and in its place is a hyaline dome (A. Müller's "Flocke") composed of a substance which, unlike the mucilaginous layer, is permeable for spermatozoa. Usually only one spermatozoön passes through the inner and outer egg membranes and reaches the yolk; but the place of its passage is by no means always the centre of the watch-glass area. It was such only six times out of fifty. The passage may occur even near the margin of this area. Neither is it always the spermatozoön that first reaches the outer membrane, after having traversed the "Flocke," which passes through.

The statement that the egg membrane is not alone permeable at a single spot would lead one to suppose that the authors were ready to deny the existence of a micropyle. They do not, however, directly assert its absence, although they were unable to find anything of it on the unfertilized egg. But as soon as the spermatozoön has passed through the membrane, a small circular spot may be seen from the surface; this is due to a shallow depression in the surface of the *inner* layer of the membrane, the outer layer never showing any passage through it. The authors hint at the possibility of a chemical action on the part of the spermatozoön resulting in a loosening of the two layers and a partial solution of them, and endeavor to make that view harmonize with the conclusion that the micropyle "is the remnant of an opening in the inner layer of the egg membrane, which exists during the stay of the egg in the follicle, corresponding to the condition which Herr von Jhering recently established in the case of the eggs of the mussels." The outer layer would be formed, they imagine, afterwards, and would cover over this opening, leaving a remnant of it recognizable on the inner membrane. "The micropyle, therefore, is not an open passage, as it appeared from Calberla's description and drawings, but only a permeable place."

b. *Selachii.*

What Ludwig wrote in 1874 concerning oögenesis in the selachians, that it had been studied by only a very few investigators, was equally true of the primary egg membranes of the group. LUDWIG ('74, p. 145) himself, although he studied the development of the ova, had nothing to add to what was already known about the egg membranes, and since him there have been only two writers who have dealt with the subject, Schultz and Balfour.

LEYDIG ('52, pp. 87, 88) speaks incidentally of a vitelline membrane, and a thin albuminous layer surrounding it, in the case of Raja batis. The latter probably corresponds to one of the membranes seen by later observers.

GEGENBAUR ('61, p. 518) recognized the existence of a homogeneous egg membrane on eggs of Raja from 1‴ to 2‴ (2–4 mm.) in diameter; its external contour was delicate, but internally it was sharply limited. In the case of Acanthias there was only this one membrane to be observed; it attained a thickness of 0.08‴ (175 μ) on eggs 4‴–5‴ (9–11 mm.) in diameter.

Gegenbaur considers it probable that this membrane is produced by the follicular epithelium, but is evidently not certain of it. He says: "Es liegen hier wohl bei den Selachiern andere Verhältnisse vor als bei den Vögeln und Reptilien, und eine Dotterhaut, wie sie dort von Seite des Dotters durch Umwandlung seiner peripherischen Schichte zu Stande kam, kommt hier wohl nicht vor, sondern der Dotter bleibt auf dem früheren Stadium der Differenzirung bestehen, dagegen bildet sich eine Hülle von aussen her, wozu wahrscheinlich die Zellen des Follikelepithels das Material abscheiden, wenn man den Vorgang der Bildung jener Membran nicht auf die Oberfläche des Dotters selbst verlegen will."

Schultz and Balfour disagree in their conclusions as to the origin of the fugitive membranes which envelop the ovarian eggs of selachians. Schultz ascribes their formation to the follicular cells; Balfour, to the ovum itself.

SCHULTZ ('75, pp. 574–576) claims that in Torpedo oculata the follicular epithelium is composed of two kinds of cells: genuine *granulosa cells*, derived from the germinal epithelium of the ovary, and, alternating with them, *lymphoid cells*, which are derived from the stroma of the sexual organ. "The cells of this follicular epithelium, especially the lymphoid cells, are merged at their deeper ends into a homogeneous cuticular layer (Fig. 8), and there form a structure having the morphological value of a chorion." This homogeneous layer at no time has a morphological relation to the egg protoplasm, but retains the closest connection with the follicular cells. On objects subjected to pressure the outer margin of the homogeneous layer appears jagged like a wood-saw,[1] the remnants of the lymphoid cells corresponding to the teeth, in the intervals between which the granulosa cells are lodged. The latter are also attached, he says, to the homogeneous layer by means of protoplasmic processes, and even appear to fuse with it, but do not show any differentiation within the substance of the layer. It is not possible even with the highest powers to demonstrate any such structural peculiarities (radial striation, pore-like perforations) as are met with in the egg membranes of most classes of animals, even in Raja batis itself.

"Finally, when the egg cell has reached maturity and the follicle approaches the stage of rupturing, the lymphoid cells together with the homogeneous layer are converted into connective issue, in the interstices of which the granulosa cells persist, although the latter finally undergo fatty degeneration. Only at a single place, corresponding to the whole extent of the germinal disk, do the follicular cells and the

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[1] "Gleichsam hohlsägeformig [hohlzsägeformig?] gezackt."

homogeneous layer persist unchanged up to the bursting of the follicle. It is from this part that those granulosa cells come which are occasionally encountered on the escaped [egg] and within the empty follicle."

On eggs of Acanthias, Scymnus, and Mustelus, Schultz found a homogeneous layer joined with the follicular layer, and inside the latter a zona radiata, the inner margin of which was sharply defined against the yolk. "The pores of this cuticular zona were traversed by protoplasmic processes, which stretched from the homogeneous layer to the egg protoplasm and fused with the latter."

The author concludes that, so far as his own observations reach, there are to be distinguished in selachians the four "following conditions of the follicular epithelium": (*a*) simple epithelium (embryonic stage of selachians); (*b*) epithelium with homogeneous basal margin (Torpedo); (*c*) epithelium with homogeneous perforate basal margin (Raja); (*d*) epithelium with broad homogeneous and narrower perforate basal margin (Squalidæ).

BALFOUR ('78^b, pp. 402, 403) has confirmed the existence and subsequent disappearance of two membranes — an outer homogeneous, and an inner striate — in one of the Squalidæ, Scyllium; but he believes that they are produced by the ovum, not by the follicular epithelium, and that they are absorbed, not converted into connective tissue. Two similar membranes are also found in Raja, and are believed by Balfour to be common probably to all sharks. The homogeneous membrane is formed before the striate one. In Scyllium "the [homogeneous] membrane would seem indeed to be formed in some instances even before the ovum has a definite investment of follicular cells." Consequently it is called a *vitelline* membrane. In ova 0.12 mm. in diameter it is not thick enough to be accurately measured; in those of 0.5 mm. diameter it has a thickness of 2 μ, and there may also be observed inside it faint indications of the differentiation of the outermost layer of the vitellus into the perforate or radially striate membrane of Schultz. The latter Balfour does not hesitate to call a zona radiata.

In ova 1 mm. in diameter the zona has increased in thickness (to 4 μ) and "is always very sharply separated from the vitelline membrane, but appears to be more or less continuous on its inner border with the body of the ovum, at the expense of which it no doubt grows in thickness." In larger eggs both membranes increase in thickness, especially the zona, which now becomes marked off from the yolk. "In many specimens it appears to be formed of a number of small columns as described by Gegenbaur [for the alligator] and others."

The size of the ova at the time of the maximum development of the membranes is not stated; but after this stage is reached both membranes gradually atrophy. "The zona is first to disappear, and the vitelline membrane next becomes gradually thinner. Finally, when the egg is nearly ripe, the follicular epithelium is separated from the yolk by an immeasurably thin membrane, — the remnant of the vitelline membrane," which is no longer visible when the egg becomes detached from the ovary. Both vitelline membrane and zona are found in Raja, but in a much less developed condition than in Scyllium, and the zona is developed at a much later period than in that species.

If the account given by Balfour is correct, — and his description seems to be both more complete and more accurate than that of Schultz, — then there is an interesting parallelism between the primary egg membranes in selachians and those of Lepidosteus and the bony fishes. Not that the villous layer in Lepidosteus is *structurally* comparable with that which Balfour calls in sharks a vitelline membrane, but *genetically* they are alike. *They are the membranes which are first to be produced,* — i. e. before the zona radiata, — *and they are in both instances the product of the ovum, not of the follicular epithelium.* I shall take occasion later to refer to the theoretical importance of this discovery by Balfour.

The ultimate disappearance of both membranes renders the formation of a micropyle superfluous. It would be interesting to learn, however, whether there is at any time a trace of such a structure.

c. *Ganoidei.*

The only ganoids besides Lepidosteus whose egg membranes have been described are Amia and Acipenser. I have elsewhere (p. 27) quoted Ryder's account of the membranes in the case of Amia.

From the accounts given by Kölliker, by Kowalevsky, Owsjannikow und Wagner, and by Salensky, I believe there must be considerable similarity between the conditions of the egg membranes in Acipenser and Lepidosteus.

Kölliker ('57, p. 197) says that the porous membranes in the case of the sturgeon form "three layers; two inner, darker, thinner, closely porous, and an outer pale, thicker layer, apparently with fewer pores. This outer layer, which is also softer than the others, shows its outer surface divided into small polygonal areas, which appear to correspond to the epithelial cells of the egg capsule [follicle]. These cells are extremely delicate and pale, but yet seen from the surface they show a fine punctation. It as the appearance, therefore, as though these cells secreted the porous layers; however, concerning this, as well as concerning the corresponding parts of the eggs of other animals, only careful studies made on eggs of all ages can give an answer, wherefore I abstain for the present from any opinion."

According to KOWALEVSKY, OWSJANNIKOW UND WAGNER ('70^a, p. 172), the outer membrane in Acipenser is thick, shagreen-like, and possesses numerous very fine canals. When the ripe eggs fall from the oviduct, this membrane sticks to objects; with a certain amount of skill it may be rather easily detached from the egg. The inner membrane is much finer [has finer canals?], transparent, and very firm.

SALENSKY ('81, pp. 234–236) applies the name chorion to the outer of the two layers composing the "thick capsule" which envelopes the ripe egg of Acipenser; the inner he calls vitelline membrane. At first the two are so intimately joined to each other that it is difficult to separate them; but after the egg has been deposited for some time, the chorion is easily detached from the vitelline membrane, and may be removed from the whole egg. From the study of sections of stained eggs the author determined that the stickiness and the roughness of the surface were due, not to the chorion, but to two special cell layers which invest it.

"As to the origin of these two membranes, there is no doubt that they are derived from the two layers of cells which constitute the epithelial wall of the ovarian follicle."

"The chorion," he adds, "is probably a product of the secretion of the membrana granulosa of the follicle; when the latter ruptures, the epithelial cells remain adherent to the chorion and are expelled with the egg, and are again met with slightly modified at the surface of the deposited egg."

"The examination of microscopic sections of the egg shows that this envelope, which, as has been said, is divided into two after deposit, — into chorion and vitelline membrane, — presents three distinct layers. The external and the internal have about the same thickness; the middle one is thinner. In separating the chorion from the vitelline membrane one may convince himself of the fact that the chorion is composed of two layers, the vitelline membrane of only one."

From Mayzel's abstract (Salensky, '79, p. 220) I learn further, that the outer layer is stained deeply by hæmatoxylin, the two remaining layers not at all, and also that all three layers are radially and finely striate. In the figure of the membranes given by Salensky ('78^b,

Tab. I. Fig. 8 B) the inner membrane is only slightly thicker than the middle one, and both present a lighter appearance than the outer one.

I believe it is probable that the outer layer will be found to correspond closely to the villous layer of the gar-pike. There can be no doubt that the inner layer is the zona radiata, and I am inclined to regard the middle layer simply as the differentiated outer half of the zona; but the question can be answered satisfactorily only after renewed investigations which give more particular attention to this point. The principal reasons for my conclusion regarding the middle layer are, that it evidently resembles the middle layer more than the outer, especially in its capability of being stained, and that differences between the inner and outer portions of the zona have been observed in the case of other fishes. I know of no case, I admit, in which the outer half of the zona may be easily removed with the villous layer, so that it still is possible that the middle layer in Acipenser corresponds to the stalk region of the villi in Lepidosteus.

I do not clearly understand what the author means by saying that the two membranes are derived from the two layers of cells which constitute the epithelial wall of the follicle. It is true he claims that there are two distinct cell layers, which, according to his figures of *early stages* ('78ᵇ, Tab. I. Figs. 5–7), are "granulosa" — next to the yolk — and "follicular epithelium" — immediately outside the latter; but he gives no figure showing both these at advanced stages of development. I doubt their existence. But even if there were two separate epithelial layers, I fail to understand how *both* could share in the production of membranes which lie wholly inside the inner cell layer.

The eggs of Acipenser are altogether unique so far as regards the condition of the membrane in the micropylar region.

The earliest observer of the micropyle was Kölliker ('57, p. 197), who incidentally remarks that there is a *single* micropyle in the sturgeon's egg; but subsequent observers have claimed that there is a *group of several micropyles.*

Kowalevsky, Owsjannikow und Wagner ('70ᵃ, p. 172) state that at one pole of the egg there are seven micropylar openings, — one in the centre, with the other six arranged in a circle around the first.

Salensky ('81, pp. 235, 236, Planche XV. Fig. 1 A) gives a more complete account of the micropylar region, which is illustrated by figures. He says: "At the germinative pole of the egg there is found a micropyle.

The orifices of the micropyle are so small on hardened eggs that it is probable that they are narrowed by the action of the reagents, which also cause a retraction of the capsule of the egg. For this reason it is very difficult to find these orifices."

The micropylar apparatus is composed of several (5 to 13) orifices, and Salensky states that, although he has examined a great number of eggs, he has never found two which were identical either in the number or distribution of the orifices. "Each orifice consists of a quite small pit (fossette) having the form of a crater; it is surrounded by small, very slender cylinders."

Salensky has given only surface views of the micropylar region, but it has occurred to me that his "very slender cylinders" may be villi which surround the crater-like depression. They would appear to have a radial arrangement about the crater as a centre, if the villous layer were seen in optical section at a plane a little below the outer margin of the crater.

d. *Dipnoi.*

The conclusions reached by BEDDARD ('86[a]) relative to the ova of *Lepidosiren* possibly rest on too limited material to receive immediate acceptance. So far as regards the egg membranes, the story is certainly far from being satisfactorily completed. In the youngest eggs there was no trace of any membrane; but as development proceeded, a delicate homogeneous membrane encircled the ovum. This was from analogy thought to be the product of the egg protoplasm, even though it was more firmly adherent to the follicular epithelium than to the ovum, and it is called vitelline membrane. In more mature ova there was underneath this a much thicker radially striate membrane, probably corresponding to the zona radiata of other vertebrates, which in places seemed to pass gradually into the substance of the protoplasm. This membrane (zona radiata) began to disappear with the first steps in the formation of yolk. During the period of yolk formation the vitelline membrane became thicker, and also radially and coarsely striate. The author believes that there was a stage which succeeded this, during which there was no membrane of any kind, and that at this time an immense number of follicular cells migrated into the yolk. But in addition there followed still another stage, — when the ovum was entirely occupied by yolk, — in which the epithelium was separated from the contents of the ovum by an extremely delicate homogeneous membrane, which either corresponded (in some cases) to the persistent vitelline membrane, or (in other cases) was a new formation; but even in the latter case the author maintains that it is homologous with the vitelline membrane!

The fact that the layer called by Beddard *vitelline membrane* becomes radially and *coarsely* striate, suggests comparison with the villous layer of Lepidosteus. As in the ganoid, so in Lepidosiren this is the layer which is produced first.

e. *Teleostei.*

The numerous descriptions which have been given of the egg membranes in different *osseous fishes* show that there is not uniformity either in their number or structure. Besides the wall of the follicle with its epithelium, the granulosa, there is perhaps only one investment of the egg which is universally present, the zona radiata, and even this may be wanting, or at least may wholly disappear in the case of certain viviparous fishes. I believe it is certain that the homologue of the zona radiata is invariably present in oviparous fishes, and I am likewise of opinion, notwithstanding the inability of some observers to discover the presence of pore-canals in the eggs of certain fishes, that the canals are also always present.

In many cases there is a membrane intervening between the zona and the granulosa, sometimes thin and homogeneous, at other times of a more complicated nature. It may even (villous layer) resemble somewhat the zona itself, although in reality very different from it in structure. Whether the thin homogeneous layer is homologous with the more complicated villous layer cannot as yet be definitely answered, but Balfour's account of the origin of a similar structureless membrane in Elasmobranchs makes me incline to the belief that it is.

In addition, the cells of the granulosa undergo in some instances a remarkable metamorphosis, accompanied probably by a process of secretion, and thus furnish still another primary envelope to the ovum.

As to the existence of membranous structures *inside* the zona radiata, there is much less certainty. The presence of a structureless *vitelline membrane* has been maintained with more or less confidence by Vogt, Aubert, Thomson, Lereboullet, Kölliker, Eimer, and others, and more recently by Owsjannikow and Scharff. Its existence has also been denied by eminent authority.

The question as to the presence and nature of a so called *zonoid layer* seems, especially in the light of Brock's recent contributions, to demand a more extended and thorough investigation. In the case of some of the eggs studied by Eigenmann ('90), structural conditions have been observed which can hardly bear any other interpretation than that of a striate zone of substance inside the zona radiata proper; but in other cases (Esox, Amiurus) a somewhat similar though perhaps not identical appearance is due to a retraction of the vitellus from the zona, which leaves strands of vitelline substance stretching across the space thus produced.

1. *Zona Radiata and Villous Layer.*

JOHANNES MÜLLER ('54) has often been credited with having discovered the fine radial canals which traverse the zona radiata, and give to it its most characteristic appearance. This is a mistake which has arisen from Müller's misunderstanding the relation of the peculiar membranes of the perch to those of other fishes. What he described as "pore-canals" in Perca belonged to a much thicker membrane than the zona. This membrane lies outside the latter, and is a result of the activity and metamorphosis of the granulosa cells. Müller, it is true, supposed this to be the equivalent of the "shell membrane" previously described by Vogt, and therefore imagined that he had been able to demonstrate on a more favorable object what Vogt had claimed on grounds of analogy rather than on satisfactory proof.

In VOGT'S studies on *Coregonus palæa* he ('42, pp. 1, 8–10, 27, 28) claimed the presence of two membranes. The inner one — being thin, transparent, and without apparent texture — he called *vitelline membrane;* the outer one he called a *shell membrane,* and homologized it with the "membrane coquillière" of birds' eggs. This outer membrane presented the appearance of shagreen, which seemed to result from a quantity of small opaque points uniformly distributed over its surface. Treated with hydrochloric acid, the points became more transparent, and then resembled minute warts. Valentine called Vogt's attention to the resemblance between this structure and that of the carapace of the cray-fish, where he had found that a similar effect was due to perpendicular tubes, filled with lime, traversing a membrane composed of regularly polyhedral cells. Vogt, admitting that the "shell membrane" was too thin to allow the attainment of exact results relative to the nature of the "points," nevertheless claimed that the position, behavior, and reticulate appearance of the latter warranted one in supposing that the structure was analogous to that of the carapace of the cray-fish. Thus it appears, he continues, that the shell membrane is formed by the union of flattened cells, which are arranged around the primitive egg only toward the epoch of its maturity; the presence of these minute tubes, which traverse the membrane, would in his opinion sufficiently explain the absorption of the water into the interior of the shell membrane. (Compare also Vogt, '42, pp. 27, 28.)

From this summary it is evident that Vogt had under consideration appearances which were due to the pore-canals of the zona radiata, and that he moreover believed in the presence of canals, but it cannot be claimed that he demonstrated their existence. Unlike his predecessors, he rightly claims that the shell membrane (in Salmo umbla) originates in the ovary.

In a paper written in 1845, but not published till many years later, VOGT ET PAPPENHEIM ('59, pp. 357, 361, 362) also maintain that the shell membrane of the eggs of fishes, which is uniform and elastic, is constituted by the fusion of a cell layer formed *in the ovary*, and therefore not to be compared with shell membranes which are produced in the oviduct. They made the mistake of insisting that "this cell layer is not to be confounded with another epithelial layer which one finds in the ovisacs of the youngest ovules, and which is composed of large extremely pale cells which subsequently disappear and give place to this second layer."

MECKEL VON HEMSBACH ('52, p. 421, Taf. XV. Fig. 1) saw and figured a *radial structure* of the "zona pellucida" in the case of Cyprinus auratus, after treating the membrane with acetic acid and crushing it, but he expresses no opinion as to the real nature of the striation.

LEUCKART ('53, pp. 796, 797), who probably had not yet seen Meckel's paper, was evidently not impressed with the explanation which Vogt adopted to explain the appearance of the outer membrane in Coregonus; for he says simply, "That which characterizes the eggs of teleosts is the possession of a special firm egg-shell (*chorion*), which is already formed in the follicle around the primitive yolk membrane, and generally presents a delicate marking resulting from regularly grouped granules or points."

During the five years beginning with 1853 there appeared a large number of papers dealing with the egg membranes of fishes, and the subject was brought to a temporary conclusion by the thorough work of Kölliker.

AUBERT ('53, pp. 94, 95, Taf. VI. Fig. 1) was the first to figure well the appearances due to the pore-canals of the zona radiata. In addition to "a very finely granular, but otherwise structureless skin, which envelops the yolk," and which I believe must have been in his opinion the vitelline membrane.[1] Aubert describes the "shell" of the egg of Esox as a transparent thin membrane, furnished with fine points, which closely envelops the yolk. These points exhibit a great regularity of arrangement, being placed at the intersection of symmetrical curved lines. When the shell has lain some time in water, it separates in many places into two membranes, of which the outer is very thin, finely granular, and irregularly elevated, while the inner is thicker, uniform, and upon sections exhibits fine radially placed streaks.

I believe it is certain that the radial streaks described were due to the pore-canals, although the author does not fully commit himself to that view. "The spermatozoa are so large," he says, "that it would be difficult for them to pass through the 'points' of the shell, in case the latter are regarded as the lumina of fine canals."

Also LEREBOULLET ('54, pp. 240, 242, 245, 249) wrote concerning the pike: "The ripe egg is surrounded by two membranes: the external is pierced by microscopic tubes, which serve for the absorption of water, and consequently for the respiration of the egg; the internal, applied to the vitellus, is a simple, extremely thin and amorphous protecting envelope." He also saw the pore-canals in the perch, and argued that the expulsion of albuminous globules from the fertilized egg proved the absence of a vitelline membrane at that time, and that it went to confirm the opinion of those who regarded the chorion as produced by the primitive vitelline membrane, which was itself detached from the vitellus.

Although J. MÜLLER ('54) contributed much to the knowledge of the egg membranes, especially of the perch, and was also the first to appreciate the importance of the difference in origin between the egg-shell in birds and what he called the "egg capsule" in fishes, he did not fully comprehend the structure of what he called the "Dotterhaut" (zona).

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[1] I cannot agree with His ('73, p. 2, foot-note, compare also p. 14) in his criticism of Aubert when he says: "*Der Name Dotterhaut*, welchen die früheren Schriftsteller für eine besondere den Dotter unmittelbar umhüllende structurlose Membran gebraucht haben, *wird von H. Aubert* (Beiträge, etc., 94) *auf die Eikapsel angewendet.* Er spricht nämlich bein Hecht-Ei von einer Trennung der Dotterhaut [!] in zwei Schichten, eine äussere dünne, fein granulirte und eine innere, dicke, mit radiären Streifen. Eine Begründung seiner abweichenden Bezeichnungsweise giebt er nicht."

Aubert says distinctly enough that it is the "*Schale*" which is divided into two membranes; and although he *nowhere employs the word "Dotterhaut*," there seems to me no doubt that he has Dotterhaut in mind when he says: "Der Dotter wird von einer sehr fein körnigen, sonst structurlosen Haut überzogen." His may have been misled by the statement that "Die Schale etc. den Dotter *eng* umgibt."

He claimed for Cyprinus erythrophthalmus, Perca fluviatilis, and Acerina vulgaris a velvety appearance of the external surface of this membrane "as if beset with tufts" (Zotten[1]), which he ascribed to "very small cylindrical projections or rods with rounded ends," — prolongations of the vitelline membrane itself. Here again Müller unfortunately confounded the unlike conditions of different eggs. While his conclusions have been confirmed in the case of Cyprinus erythrophthalmus, I know of only one author whose observations give any evidence of the presence of such a "pile" or velvety structure outside the zona in either of the other fishes mentioned. Hoffmann ('81, pp. 19, 20, Taf. I. Figs. 9, 10), it is true, not only figures an external layer of the zona in a nearly ripe egg of Perca, which is thinner and *much more sparsely striate* than its inner layer, but he also describes and figures an October egg as possessing outside the still thin zona a layer of *minute, close-set tubercular projections* which "fully correspond to the Zöttchen of the Cyprinoids." I believe that Hoffmann has in some unaccountable way fallen into an error in this matter. At least, no other observer has seen any trace of the structure which he describes, and an examination of the eggs of our American perch in October reveals nothing of the kind. I think the condition in Acerina is probably similar to that in Perca, — certainly no one has shown the presence of a "Zöttchen" layer. That being the case, what was it that Müller mistook in Perca and Acerina for "Zöttchen"? A statement by Owsjannikow ('85, p. 18) makes me believe that Müller may have had under view the branching deep ends of the tubular structures which according to Owsjannikow traverse the gelatinous envelope, and which are left sticking in the pore-canals of the zona when the two layers are artificially separated. It may be, however, that Müller saw the more conspicuously — *but not*, as Hoffmann makes it, *more sparsely* — striated outer portion of the zona itself, in which event it might be

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[1] Muller was not the first to see the appearance presented by these "Zotten," or even to suggest the name. Von Baer ('35, p. 7) had, twenty years before, seen the same thing in species of carp. "Die äussere Eihaut ist aber nicht ganz formlos und gleichartig in sich. Sie enthält in den Karpfenarten, die ich zu untersuchen Gelegenheit hatte, keine [should be *kleine*] *dunkleren Vorragungen*, die ihr bei starker Vergrösserung ein *zottiges* Ansehen gaben." It is evident that Von Baer confounded the radial "tubes" in the outer envelope of the perch egg with these villi of the carps, for he adds: "Im Barsche ist diese Hülle noch sehr viel dicker und man sieht, dass die *dunklern Flecken*, die hier lang und schmal sind, in der äussersten Schicht sich befinden." One will find little occasion for surprise at this parallelism, in view of the fact that some of the most recent observers, with the best modern appliances at command, have arrived at a similar, though I believe erroneous conclusion.

fairly claimed that he had seen the pore-canals; but even in that case it remains perfectly evident that he did not understand at all the structure of the zona radiata.

When, a few months later, Remak ('54) announced the discovery of radial striations traversing the whole thickness of the zona pellucida in the case of the rabbit's egg, and attempted to determine the cause of the appearance by a comparison with the conditions found in the egg membrane of a fish (presumably Gobio fluviatilis), Müller ('54^a, p. 256) was unable to agree with him in the conclusion that the appearance was probably due to "an alternation of canals and cylinders," but endeavored to show that it was "merely an optical expression of the summation and partial superposition of the images of the rods [Zapfen] as seen when viewing the vitelline membrane in profile." The images of the overlying rods which fall in one line would, in his opinion, cause the striations to appear much longer than the individual rods really were, and thus make the lines *appear* to traverse the whole thickness of the membrane.

Ransom ('56) maintained that there was present at an early stage in the growth of the ovum of Gasterosteus a very thin membrane, having a finely and regularly dotted structure; but he does not appear to have realized as yet that the dots were evidence of pore-canals. He also discovered that in older eggs the part of the membrane immediately surrounding the micropylar depression exhibited a number of cup-shaped pediculated bodies scattered over its surface. These have since been claimed by Kölliker ('58, p. 81) and subsequent authors to be the localized equivalents of the "Zapfen" layer discovered by Müller.

In the same category must also be placed the remarkable filamentous structures discovered by Haeckel ('55) on the eggs of several of the Scomberesocidæ. Although Haeckel described the filaments as lying inside the finely punctate vitelline membrane (zona radiata) and having no connection either with it or the yolk, there can be no doubt, as Kölliker ('58, p. 81) first showed, that they are really outside the zona. I have not yet had the opportunity of examining the eggs of any of the Scomberesocidæ, but conjecture that the sheath which envelops the bases of their filaments may be only a part of a membrane external to the true zona radiata, and comparable with that which Eigenmann ('90) has found in Fundulus.

Leuckart ('55, pp. 257–264), who in an appendix to his celebrated paper on the micropyle of insects' eggs deals, at least incidentally, with the structure of the egg membranes in fishes, was the first to perceive the true significance of Müller's discoveries in the perch. He retained the name chorion for the zona radiata, and from a study of the trout was fully convinced that the punctate appearance is due to "delicate tubules or canals which traverse the membrane perpendicularly, without opening, however, at its inner surface." The latter part of this statement has not been confirmed by subsequent observers. Leuckart, however, gave an excellent description of the structure of the zona radiata in the perch, for he not only recognized that it was composed of an outer thinner, firmer membrane, and an inner thick layer of viscid sarcode-like substance, but he also saw that the two layers were so intimately joined to each other that the canals were continued through both. While I prefer to regard these two layers as substantially a unit, basing my conclusions on a variability in the apparent independence of the outer layer and on the continuity of the pore-canals through both, I recognize that this is a minor point, and that already Leuckart was in possession of the important facts of structure. It was the presence of this "chorion" in addition to Müller's capsule with its coarser pore-canals which convinced Leuckart that the latter could not be considered the equivalent of the radially striate membrane in other fishes, such as the salmon and trout. I believe that Leuckart was less fortunate when he concluded that there was in Esox a layer immediately outside the "chorion" which was homologous with the Müllerian "capsule" of the perch; for in my opinion there can be no doubt that the layer in question is the same as that in which Eigenmann has found the pore-canals to be continuous with those of the deeper portion of the zona. Even Leuckart describes the canals as straight, not spiral as in Perca. In my judgment, therefore, this layer corresponds to the thin outer layer of the zona seen by Leuckart in the perch, rather than to the capsular layer described by Müller.

Although Volume V. (Supplementary Volume) of Todd's Cyclopædia of Anatomy and Physiology was not issued until 1859, the article "Ovum" by Allen Thomson was published much earlier, and in two parts, according to Gegenbaur ('61, p. 495). The first part (pp. 1–80) appeared in 1852, and the second part (pp. [81]–[142]), which contains the portion devoted to osseous fishes, in 1855.

Thomson ('59, pp. [99], [100], [103]), besides giving a very brief summary of previous work on the subject, treated at some length the structure of the zona radiata, basing his conclusions partly on the work of Ransom and partly on his own studies. On the strength of Ransom's work he claimed that "the structure [Eikapsel] described by Müller in the perch was peculiar to that fish, and belonged only to an outer covering superadded to the surface of the dotted membrane, which last resembles in all respects that of other fishes." "This outer covering," he adds, "appears to be of cellular origin; and Dr. Ransom thinks it may be due to the separation of the tunica granulosa along with the ovum." Thomson was able "to perceive the circle or lumen of the tubes" in the dotted membrane by using a high magnifying power, and also thought he could distinguish a hexagonal marking of the intervals between the pores (which he figures) in the salmon; he also pointed out that the size of these pores was only about one third of that of the tubes in the perch as described by Müller. The author was less successful, when, in explaining Fig. 67*, he said: "A granular or dotted appearance of these [granulosa] cells seems to indicate their conversion into the dotted membrane, which is probably formed in successive layers from the exterior. . . . The ovum (p. [103]) receives its firm porous membrane [zona] while within the ovarian capsule, but only in the latter part of the time of its formation." The origin of the membrane he is inclined to connect with the interior of the ovarian follicle; "but whether by exudation from it, or by amalgamation of the innermost layer of epithelial cells of the follicle," he has not been able to determine. The latter he believes the more probable, and that the membrane is the true vitelline membrane. I am entirely unable to comprehend how Thomson could have reconciled the two statements in the last sentence, for surely the vitelline membrane was not, even at that time, regarded as the product of anything but the ovum itself.

On ripe fish-eggs Reichert ('56, p. 89) was able to distinguish two membranes, both of which were formed within the follicle. The inner was the punctate membrane, but owing to the fineness of the markings it was impossible to determine whether they were the result of elevations or depressions of the surface. Reichert was unable to adopt without reserve the conclusion that the dotted appearance is due to radial canals, even though such an explanation was suggested by his finding the markings on the inner as well as the outer surface of the membrane in the case of Cyprinus carpio. He evidently reposes great confidence in Müller's explanation of the striation as an optical illusion, which in his opinion accounts for the appearances figured by Aubert. He is also uncertain in regard to the existence of a vitelline membrane, so that a positive conclusion as to the nature of the zona radiata was not reached. The smallest eggs possess a transparent homogeneous membrane without punctations, and too thin to be measured, which may be regarded as a vitelline membrane. With an increase in the size of the egg this membrane becomes thicker, and in Acerina punctate markings become visible on its outer surface when it is only about 2.8 μ thick. There is never any appearance which allows the supposition that the thickening is produced by secretions from the epithelium of the follicle. Since the punctate appearance becomes visible only after the thickening of the original membrane, it is to be concluded, says Reichert, that the punctate membrane of the ripe egg is not the original vitelline membrane, but a secondary egg-membrane, which, however, has been formed by the deposition of thickened layers ("Verdickungschichten") of the egg outside the vitelline membrane. But what has meantime become of the vitelline membrane is not stated.

Reichert distinguished on the majority of the eggs which he studied a second membrane outside the punctate one. In Esox it was clear, homogeneous, and viscid. In cyprinoids it had the velvety appearance already described by Müller, from whom the author differs in regarding this membrane as not a part of the punctate one. His reasons for this conclusion are, that it is as sharply marked from the punctate membrane as is the capsular membrane in the perch and may even be detached after treatment with chromic or nitric acids, that the rod-layer is not so firm as the punctate, and finally that the rods are much fewer than the punctations of the same egg. The rods are set in a clear, homogeneous layer, only their rounded ends protruding.

Reichert was evidently influenced by his belief in the probability that the capsular membrane in the perch owed its origin to the membrana granulosa, and consequently he left unsettled the question of the origin of this villous layer, although its intimate adhesion to the punctate membrane indicated a common origin with the latter.

Kölliker ('57, p. 197) found in 1856 that the porous membrane in the case of sturgeons' eggs presented conditions which favored the view that it was formed by the cells of the follicle, but he discreetly abstained from forming an ultimate judgment before eggs in all stages of development had been studied. Such studies he found the opportunity of beginning during a sojourn at Nice a few months later, and he continued them on fresh-water fishes during the beginning of the following year; the results were published in 1858.

Kölliker's ('58, pp. 80–93) observations embraced a large number of fishes, and — what is of more importance — he also studied eggs in several stages of development. With Reichert, he recognized two capsular egg membranes, which he called "Dotterhaut" (zona radiata) and "Gallerthülle" (the latter in Perca), but he dissented from Reichert's explanation of the striate appearance of the former, and demonstrated by means of *thin sections* that it was due to the presence of pore-canals. Kölliker also claimed that there was an outer thin, resistant layer of the porous vitelline membrane in the case of all fishes, — such as Leuckart alone had recognized in Perca, — and that this layer might retain the striate appearance even when the rest of the membrane had been made pale and apparently homogeneous by the use of reagents. It is in this outer layer that the viscid and fatty-looking "Zöttchen" are rooted with their slightly enlarged bases. Kölliker therefore regards the "Zöttchen" layer as belonging to the "Dotterhaut," rather than as a separate layer, and compares its elements to the peculiar appendages discovered by Haeckel in the Scomberesocidæ. To these he adds a description of peculiar mushroom-shaped appendages of the vitelline membrane in Gasterosteus and Cottus gobio. There is, however, one difference between the "Zöttchen" and the mushroom-shaped bodies; in caustic potash the former are greatly swollen and become pale, whereas the latter are made to shrink somewhat and to become darker.

In reference to the origin of this membrane and its appendages, Kölliker gives the first positive information which we have, for Reichert's conclusions were at best only theoretical and tentative. Both in the case of Gasterosteus and Cobitus barbatula he showed that the villous structures made their appearance before the zona radiata. In the case of the first-mentioned species, the mushroom-shaped bodies were distinguishable as minute wart-like points resting on the outer surface of a membrane so thin that it presented only a single contour. As these wart-like structures continue to occupy the outer surface of this vitelline membrane while the latter increases in thickness, it is not to be doubted, he says, that the increase is due to deposits upon the inner surface of the membrane. The warts continue to increase in size, while the membrane becomes still thicker and shows radial markings.

The first appearance of the villi in Cobitis is to be seen in eggs 0.08″ [0.08‴ ? = 175 μ] in diameter, where they appear as deposits or outgrowths on the external surface of a thin membrane (Reichert's primitive vitelline membrane); at first they are low and narrow, but they gradually increase in length, and also, though more slowly, in breadth. It is only when the villi have attained their full length that the porous layer begins to be formed by deposits on the *inner* surface of the thin membrane, but this proceeds with such energy that the porous layer soon exceeds in thickness the villous. At the same time the villi increase in breadth, though not in length, and the thin membrane persists as the outer layer of the porous membrane, which in the ripe egg bears the villi. Upon the first formation of the villi the appearance of a surface view of the membrane so closely resembles that of a porous vitelline membrane (zona radiata) with fine close-set pores, that one must follow the whole course of development, and convince himself of the late appearance of the porous layer before he can be certain that the fine points are due to the villi.

Kölliker believed that the formation of this peculiar "Dotterhaut" (zona radiata) of fishes could be easily understood as one of the so called secondary cell secretions, if there were on the inner side of it another membrane, which latter would then be regarded as the original cell membrane of the egg. Although he found some evidence of the existence of such a thin structureless membrane in Cobitis, he was unwilling to give much importance to that fact, but inclined to a belief in its existence, rather upon the theoretical ground that it would offer a satisfactory explanation of the radial pores as being the equivalents of pores in cuticular structures.

This assumption (p. 104) that the fine pore-canals are to be explained as resulting from the presence of fine openings in the cell membrane may be satisfactory enough for those cases in which a definite cell membrane is demonstrable previous to the appearance of the cuticular secretion; but it seems to me superfluous to assume the universal existence of a cell membrane in order to explain the conditions. Where no cell membrane exists, the same phenomenon may take place, and is no more difficult of explanation than in the case where there is a cell membrane with the supposed structure; for the latter must in its turn be explained as the result of localized activity on the part of the cell protoplasm in secreting its membrane. So, in the end, it comes to one and the same thing, whether we assume the presence of a cell membrane or not: the explanation must rest on the ability of protoplasm to localize its activities; but further than that we are at present unable to advance. Why or just how protoplasm is able to effect such a histological division of labor is still unexplained.

The important paper by GEGENBAUR ('61) on the structure and development of the vertebrate ovum adds nothing to our knowledge of the zona radiata in bony fishes, but is valuable for the way in which it illuminates the subject of the vitelline membrane.

The only articles of much importance during this decade were one by Buchholz and two by Ransom.

Rathke's ('61) posthumous work on the development of vertebrates evidently treats the subject from the standpoint of thirty years before, when little was known about the matter; and

Lereboullet ('61, pp. 120, 121, 123) does not greatly add to the knowledge of the zona radiata when he says that the chorion of the trout egg is thin and very soft at the moment the egg is laid, and does not present the resistance and elasticity which it acquires after it has remained for some time in the water. For the absorption of water and the passage of gases necessary for the respiration of the egg and embryo, the chorion is pierced, he says, by an infinite number of excessively narrow, short parallel tubes, which give a striate appearance to perpendicular sections of the shell.

Owen ('66, pp. 593–595), although following the accounts by Ransom and Thomson, fails to recognize one of the important points established by the latter author, for he does not distinguish between the "ectosac" in the perch, and that of salmon and other fishes. Moreover, this "ectosac" (evidently the zona radiata) "is composed of close-set series of hollow columns." (!) As Ransom ('67, p. 3) has since pointed out, Owen also erroneously states, possibly under the influence of Rathke's exposition, that the villi are formed after the ova escape into the cavity of the ovary.

Buchholz ('63, pp. 71–81, and 63^a, pp. 367–372) was the first to describe a very peculiar appearance of the egg membranes in Osmerus eperlanus. In addition to the porous membrane (zona radiata), which continues to invest the egg after it is laid, there is a second one *external* to the first, and like it traversed by similar pore-canals. Buchholz states that these canals are much more readily recognized to be pore-canals in the case of this fish than in that of other fishes (p. 73). When the egg has lain for some time in water, the outer envelope, or at least a portion of it, is found to be attached to the inner membrane around the circumference of the micropylar canal, whence it depends as a loose funnel-shaped frill with its originally inner surface now directed outward. The pore-canals which traverse these two membranes, instead of being cylindrical, are funnel-shaped, the wider end being directed outward. By treating the fresh ovarian egg with acetic acid the *outer* membrane, which at first lies closely in contact with the inner, is made to swell up with irregular foldings until it becomes entirely separated from the inner; but a striate appearance, which is visible for a moment, becomes quickly obliterated by the action of the acid. If, however, the eggs are first treated for twenty-four hours with very dilute chromic acid, and then with acetic acid, the radiate structure remains easily distinguishable in both membranes.

With regard to the formation of the two membranes, Buchholz argues that the outer is the older, since one often finds, in the earlier stages of their formation, that the inner is thinner than the outer, whereas subsequently they are both of equal thickness. The increase in the thickness of the inner membrane was observed between the middle of February and the middle of April, — the spawning time, — and meanwhile the outer membrane was found to be thinner over about one third of the surface. It is to be assumed, according to the author, that this attenuated portion finally disappears altogether, since the persistent portion which remains attached in the region of the micropyle is too small to have completely enveloped the egg. Even nearly up to the time of maturity there is no fusion between the two membranes, which must, therefore, take place rather late.

The homology of the outer membrane in Osmerus is not at once evident from this account by Buchholz. If one were to accept unquestioned his description, it would be most natural to regard it simply as a detached portion of the zona radiata, for he maintains that the two are identical in structure. There are, however, two other possibilities; it may be homologous either with the villous layer of Lepidosteus, or with the capsular envelope of the perch. I regret that I have not yet found the opportunity to acquire from personal study additional evidence in favor of one or the other of these explanations; but there are two or three things connected with the account given which incline me to believe that the outer membrane is the equivalent of the villous layer. The very fact of its becoming detached from the deeper layer and thrown into folds after the egg has lain in the water suggests a similar though less striking feature of the villous layer in Lepidosteus; and although there is no evidence that any such eversion of the membrane takes place in the latter case, or that it even becomes regularly attenuated on one side, as in the case of Osmerus, still I can imagine that a similar condition might be artificially produced in Lepidosteus, so far at least as regards the peeling off and eversion of the outer covering, and it is possible that a slightly different physical condition of the villi would cause them to adhere to each other so persistently as to allow the attenuation of the whole membrane on one side of the egg without separating the individual elements. Since Buchholz asserts that the pore-canals are more readily distinguished as such in this fish than in other instances, I infer that the markings which he observed must have been coarser than is usual. If his attention was mainly directed to the outer layer, and if, as I imagine, this is a villous layer, the reason of his statement would be obvious. Besides, he mentions that the canals are not cylindrical, but funnel-shaped, the wider end outermost; this, too, though suggestive of the capsular membrane, would be entirely compatible with the idea that he had under view club-shaped villi. And finally, the argument to show that the inner layer is produced after the formation of the outer is exactly applicable in the case of the villous layer, as the observations of Kölliker and my own conclusively show.

Ransom's ('67) account of the "yelk-sac" (zona radiata) in Gasterosteus is principally interesting to me from his asserting that the pore-canals as well as the villi increase in number during the growth of the egg, and from his consequent conclusions as to the method in which membranes are formed. The "yelk-sac," he says, is formed in very young ova ($\frac{1}{200}''$, or 125 μ, in diameter), in which it is easily recognized by the button-shaped villi attached to the outer surface surrounding the micropyle. The finely dotted structure is first discoverable in eggs $\frac{1}{140}''$ (180 μ) in diameter, and it is the same in character in these as in the ripest eggs. The membrane is composed of very fine concentrically arranged laminæ, each of which is marked by dots of equal size, so arranged as to mark (in surface view) the angles of equal-sized lozenge-shaped spaces, and corresponding in position in the successive laminæ so as to form vertically placed lines or striæ. In eggs .01 inch in diameter there were about 24,000 dots to the [linear] inch, and when the egg had attained .06 inch there were 11,000 to the inch; the distance between dots being scarcely more than *doubled*, while the diameter of the egg had been multiplied about *six* times. From this the author argues that there must have been an increase in the number of the dots during the growth of the sac, and therefore that the membrane does not increase by apposition of layers either from the inside or outside, either by the hardening of an exudation or by the conversion of the substance of the yolk into that of the yolk-sac. "It grows in some way by interstitial molecular deposit." A similar increase in the number of the button-shaped villi was also observed to occur during the development of the ova.

I do not recall that any one has corroborated or disproved these observations, or the deductions made from them; but I have shown that in the case of Lepidosteus there does not seem to be sufficient evidence to prove that there is any increase in the *number* of the *villi*. I believe that a careful investigation of the question in the case of the pore-canals would well repay one who should undertake it, for it could not be without influence upon theories on the method of the growth of membranes.

In his more extended paper RANSOM ('68) gives an account of the egg membranes of a number of fishes, but more particularly of Gasterosteus, Esox, and Perca. To what he had already stated about the "yelk-sac" of Gasterosteus he adds (pp. 440, 444, 448) that it is difficult, if not impossible, to determine the precise period at which it is formed. In eggs $\frac{1}{800}''$ (31.5 μ) in diameter it is not found, but is probably indicated by the smooth, hard outline which the yolk shows on its surface. "It is separable in eggs $\frac{1}{200}''$ in diameter, and may be seen in the fluids on the slide as a homogeneous-looking collapsed sac." With a power of 500 diameters the dots appear round, and with one of 3,000 they are but obscurely hexagonal; they are the same distance apart on the inner surface as on the outer. Besides these minute regular dots, there are larger and darker ones of a stellate form, which the author suggests may in some way be connected with the interstitial growth of the membrane. They occur irregularly at intervals of about $\frac{1}{3000}''$, and act like bodies of low refractive power; "at the cut edge they may be seen to pass radially about two thirds into the substance of the yelk-sac, gradually coming to a point and ceasing." I am not aware that any other observer has confirmed this appearance, which I imagine may be due to the presence of protoplasmic prolongations of the yolk into some of the pore-canals, just as in Lepidosteus the substance of the villi is traceable in many cases for some distance into the zona, although from the opposite direction.

"There are no facts known to me," says Ransom, "to point out whether the pabulum for the growth of this membrane is derived directly from the currents passing inwards, or from the material elaborated in the egg and passing out of it, or from both sources indifferently."

WALDEYER ('70, pp. 80, 81, 83), however, did not experience any such uncertainty concerning the source of the zona radiata, for while he continued to call it the "Dotterhaut" and could find no vitelline membrane inside it, he was very explicit in stating that it was *a cuticular formation produced by the follicular epithelium*, and that the pore-canals were occupied by delicate protoplasmic filaments which were in direct connection with the epithelial cells of the follicle on the one side, and with the finely granular yolk substance on the other. In his general conclusions concerning the eggs of vertebrates he says: "The complete homology of the zona pellucida [mammals] with the vitelline membrane of other vertebrates can . . . no longer be denied. The vitelline membrane is certainly a structure which does not belong to the primordial egg, but *is deposited upon it from without.*"

Like Waldeyer, Eimer ('72^a, pp. 417–428, Taf. XVIII. Figs. 9–13) regards the zona as a *cuticular* product, but, unlike him, he maintains that it is produced (precisely as in reptiles) by the egg, not by the granulosa. But the homology between the egg membranes in these two groups goes, in his opinion, still further. The delicate membrane described by Aubert and others as external to the zona in Esox and other fishes is to be regarded as a chorion, — which in reptiles is produced by the follicular epithelium. The trumpet-shaped structures of the outer membrane (Eikapsel) in the perch are formed from granulosa cells. They are the homologues of the trumpet-like structures which the author described for Coluber and placed in the category of "beaker cells." The villi which repose on the outer surface of the zona are, as held by Reichert, to be regarded as belonging to the second membrane, and not in accordance with Kölliker's views as the outer layer of the zona itself. In Eimer's opinion they are simply yolk substance which has emerged from the egg through the pores of the zona. Eimer even maintains that he has observed the protrusion and subsequent disappearance of such villi.

I have already (p. 54) given my reasons for dissenting from the use which His ('73, pp. 1–3) makes of the word "Eikapsel" to signify the zona radiata. According to His, the "capsule" in Salmo salar is from 33 to 35 μ in thickness, the pore-canals are straight, only 1.5 to 2 μ apart, and not funnel-shaped at either end. In Esox it is 16 to 17 μ thick, and has fine parallel (concentric) as well as radial markings (p. 13).

His ('73, pp. 17, 35, 36, *et passim*) also discovered that in numerous cases the young eggs possessed a peripheral layer of clear substance which exhibited fine radial markings after treatment with certain acids. This did not appear to be constantly present, nor necessarily of uniform thickness. He called it the *zonoid layer*, and thought there was probably a physiological connection between it and the porous egg capsule, but what the nature of that connection was, remained to be ascertained. Nothing in either figures or text allows one to draw conclusions as to the relative fineness of the striations in the zonoid layer and the "capsule."

In 1878 appeared three papers which dealt with the structure and development of the egg membranes of bony fishes.

A pupil of Waldeyer, Kolessnikow ('78, pp. 402, 403, 407–409), states that in both Perca and Gobio the "Dotterhaut" consists of two membranes, which are not easily separable either in the fresh state or after treatment with osmic acid; but on thin sections of eggs hardened in the latter reagent or potassic bichromate the two are sharply marked. This condition is held to indicate a gradual growth of the striate Dotterhaut, "*und zwar* ihrer äusseren Stäbchenschicht," which is the youngest formation, the *inner* layer being the older. Both layers consist of rods radially placed side by side, those of the inner layer being much finer, longer, and nearer together than the outer. It is evident, I believe, that the inner layer referred to must be the zona radiata, and the outer the "Eikapsel" of Müller. Fine granules, which are colored black by the osmic acid and are regarded as yolk granules, are found in the fine processes between the rods, as well as in the follicular epithelium itself. When the ovary hardened in alcohol is stained in hæmatoxylin, the inner surface of the follicular epithelium resembles ciliated epithelium, since the rods rest like cilia on the epithelium. On sections of a very young follicle (4.65 μ) the membrane, composed of rods, can be seen on the inner surface of the follicular epithelium. It is still thin and not easily detached, and the rods are fine and close together. When the follicle has reached a diameter of 465 μ, the membrane is much thicker, and composed of two layers. It is then to be seen that in some places the rods of the outer layer are continued into the inner layer, being consequently longer. In this case the rods gradually diminish in thickness as one passes from the follicular epithelium toward the yolk substance, but they are always sharply limited from the latter. Both layers, the author concludes, are cuticular formations of the follicular epithelium, and in no case is the inner layer to be regarded as a special membrane produced by the egg itself.

One of the best contributions recently made to this subject is that of Brock ('78, pp. 547–559), who gives, besides a condensed summary of previous observations, his own valuable results. Aside from the capsular membrane of the perch, which he calls "Gallertkapsel," and the related structures of other fishes, the author is able to recognize only one egg membrane, which, to avoid prejudging its genetic relation, he calls, from its most evident morphological peculiarity, the zona radiata, reserving the term *membrana vitellina* for egg envelopes, which are the equivalent of other *cell membranes* and therefore differentiations of the [egg] protoplasm. The outer lamella of the zona described by Reichert and by Kölliker he finds with varying distinctness in different cases. Being in some instances unable to discover it at all, he doubts its constancy. It may often be demonstrated by the use of acetic acid when not visible in the fresh condition. In the perch it has a striate appearance, and is much more coarsely marked than the true zona,[1] but in Serranus hepatus it is homogeneous.

The elongated club-shaped villi resting upon the outer surface of the zona were found in many cyprinoids, and also in Osmerus. They are not, as asserted by Eimer, expressed droplets of yolk substance; they are secondary appendages of the zona, which "have nothing whatever to do with either follicular epithelium or yolk." Brock finds the zonoid layer of His well developed in Alburnus lucidus, Salmo fario, and Perca fluviatilis. He is inclined to regard it as a general structural condition, often overlooked because distinctly shown only at certain periods in the development of the egg. It is often divisible into two layers, of which the inner remains homogeneous. When the zona radiata is removed from the yolk, the "zonoid" remains attached to the latter, to which it must therefore belong. Its striations are intermediate in fineness between those of the villous layer and those of the zona. Notwithstanding certain objections, Brock regards the follicular epithelium as the principal, if not the exclusive, source of nutrition and growth for the yolk, which are accomplished by means of cell processes sent by the granulosa through the zona into the yolk. The evidence, aside from that which Waldeyer produced for other groups, is to be found, says Brock, in the fact that, when the granulosa is separated from the zona by a secondary membrane (Perca, Serranus), it sends processes through the latter which are traceable up to the zona.

According to the opinion which I have formed, however, in regard to these so called processes, they are not outgrowths from the granulosa cells; on the contrary, the cells, retaining their original contact with the zona, are by the accumulation of the capsular secretion greatly attenuated.

In regard to the order in which the different membranes make their appearance, Brock comes to views diametrically opposite to those expressed by Kölliker. Leaving out of consideration the capsular membrane, which, as Brock rightly states, is late in being formed, and the outer lamella of the zona, concerning the origin of which he could dis-

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[1] It is not possible to say with certainty from his figure (Fig. 7, *f*) whether Brock regarded the striations of the outer lamella as less *numerous* than those of the rest of the zona. They are represented as *broader* than the latter, and as thickest at their peripheral ends, which agrees with Eigenmann's observations; but they certainly are not represented as *continuous* with the striations of the inner portion, and in this I believe that Brock is in error.

cover nothing, he asserts that he saw in all cases with the greatest distinctness that the zona radiata first appeared, and that when it had attained a certain thickness then for the first time the villi and the zonoid layer made their appearance, almost simultaneously. Unfortunately, Brock has given no details concerning the proof of this assertion, either in figures or description. I am tolerably certain, notwithstanding the very positive way in which he maintains his conclusion, that Kölliker was right, and that he is wrong, for I cannot believe that in so fundamental a matter there is such a difference between fishes as would be implied by admitting both views to be correct.

That the differences of opinion which the egg membranes have given rise to are not exclusively due to the study of different fishes, is clearly seen from the results reached by Kupffer, and soon after by Hoffmann, in the study of the herring's egg. KUPFFER ('78^a, pp. 177, 178) found the yolk to be closely invested by an egg capsule 6 to 8 μ in thickness, and the latter to be covered by a layer of viscid semifluid substance, which was found to be of nearly uniform thickness if the eggs were dropped into alcohol without contact with water. In water it soon becomes solid. The capsule consists of two firmly united layers, the inner one being finely striate radially, and alone equivalent to the porous capsule (zona radiata) of other eggs. The striation is due to pore-canals. These do not traverse the outer layer, which has concentric striations. A boiling ten per cent solution of potash dissolves the porous layer quickly, but does not affect the outer layer of the capsule, which is believed to be the same as that described for Esox by Aubert, and for other fishes by Kölliker. This difference in the two layers Kupffer regards as favoring Eimer's view, that one is produced from the egg and the other from the follicular cells.

On the other hand, HOFFMANN ('81, pp. 15–33), who has given the subject of egg membranes in fishes the most extensive treatment of any recent writer, differs materially from Kupffer in his account of the herring, although he offers a partial explanation of their differences in saying that Kupffer examined only fully mature eggs, and such as had been in contact with sea-water. Hoffmann makes the total thickness of the membranes in not quite mature eggs to be 32.5 μ. The outer layer, 10 μ thick, is not separable from the inner, although a dark line marks the boundary. Both layers are traversed by numerous pore-canals, which, to judge from his figures, appear to be finer (not necessarily nearer together) in the inner than in the outer layer; but whether they are continuous it is impossible to say on account of the sharp line separating the layers. The inner layer is composed of two parts, the outer part being often striated concentrically. The membrane of the fully ripe egg is different. Before contact with water the pore-canals of the outer layer are not so easily distinguishable as in immature eggs, and in this layer a great number of small lustrous spherules are now visible. In eggs which have been in contact with sea-water the outer layer is raised up from the inner, forming a viscid sheet 10–12 μ thick, which causes the adhesion of the eggs. The outer portion of the inner layer now exhibits a tendency to split into concentric layers, which obscures the radial pores, although they are still visible in the deeper part of this portion.

It appears to me probable that the two portions of the inner layer represent the whole of Kupffer's capsule, and that Hoffmann's outer layer is the equivalent of Kupffer's viscid semifluid substance. In view of the striate appearance of the outer layer described by Hoffmann, and the greater coarseness of the striations as compared with the inner layer, and also in view of its viscid nature, I am strongly inclined to believe the outer layer will ultimately be shown to be equivalent to the villous layer in other bony fishes.

Two layers resembling those of the herring were also found in Crenilabrus. Greater interest attaches, however, to the account of Leuciscus rutilus, in which, as the author says, one again finds the two layers of the zona radiata. But in the next sentence he shows that he does not distinguish sharply between zona and villous layer: "The outer layer here forms the well known *Zöttchenschicht.*" The villi are club-shaped, close set, and clothe the zona as a uniform layer, with the exception of the place where the micropyle is situated. In the extent of their distribution, therefore, the villi in Leuciscus differ from those of Lepidosteus, — which, though shorter, are not wanting in the periphery of the micropyle, — and also present a condition which is the complement of that exhibited by Heliasis, Gobius, etc., in which villous structures are restricted to the region of the micropyle.

Hoffmann arrives at these general conclusions: In adhesive eggs the zona radiata consists of two layers, of which the outer effects the adhesion. The latter may form a part of the whole zona, or may exist in the form of villi over the whole surface, or of long filaments limited to the micropylar region. "But whatever form this outer layer may assume, it always has a like origin with the rest of the zona; it is nothing else than a part of the zona itself, which sooner or later undergoes peculiar metamorphoses." Hoffmann recognizes the difficulty of determining how the zona radiata arises, whether as a product of the yolk or of the granulosa cells. An examination of maturing eggs shows him that its peripheral layers are always the most distinct. It is as though new layers were being deposited from within, and this leads to the conclusion that the zona radiata represents a true vitelline membrane.

Although Hoffmann seems to me to come very near the true solution of the problem, this presentation of the matter appears altogether unsatisfactory, because he insists that what he calls the zona is practically a unit in structure, and fails to recognize a fundamental difference between the outer villous layer and the "*true* zona radiata," as he terms the inner portion of his zona. From this last conclusion one would be led to infer that the zona (including both layers) was the result of a continuous process, and that there could not be any radical structural difference between villous and porous layer. But to my mind a common origin from the yolk by no means implies identity of structure, nor the continuous operation of the same formative process.

Ryder ('81^c, '82^c, '83) has contributed a good deal to our knowledge of the occurrence and functions of those modified forms of villi first described by Haeckel, but I think he cannot have given their structure very close attention or he would not have said ('83, p. 195) that "they are apparently composed of the same tough material as that which enters into the formation of the egg-membrane itself." He ('81^c, pp. 137, 138) regards it as probable that the egg membrane (zona radiata) is secreted from the cellular walls of the follicle.

Since writing the above summary of and comments on the observations of authors there have appeared a number of papers, some of them of considerable importance, which I was unable to utilize in forming my opinions of the nature of the membranes in Lepidosteus and other fishes.

Stockman's ('83) account of the egg capsule in Salmo describes the appearances of the pore-canals under a Reichert $\frac{1}{20}$ homogeneous immersion lens. In sections the limits of the pore-canals appear toothed, owing to the presence of minute folds which have for the most part a direction tangential to the capsule. These extend to the two ends of the canal, and consequently its mouth appears angular rather than circular. The substance of the capsule is beset with minute spaces which communicate with the pore-canals between the tooth-like projections, and are believed to have a function in the transmission of nutritive material to the egg.

Ryder ('84, pp. 3, 4, 11, Plate I. Fig. 5) states that the cod's egg is "covered by a vitelline membrane which is not porous or enveloped in adhesive material. It is thin, very transparent, and laminated, as has been stated by Sars, and at one point is perforated by a single minute opening, the micropyle." Ryder was unable to discover the lamination until after the action of osmic acid, and was uncertain whether it was a natural condition or the result of the action of the acid. "The cod's egg," he says, "is without the zona radiata found enclosing the egg proper of the shad, whitefish, and sculpin, and, inasmuch as it is unquestionably true that a micropyle perforates the zona in a number of these cases, it does not appear that sufficient grounds exist for the declaration that a micropyle perforates the zona radiata alone, in the face of the fact that the vitelline membrane only is perforated in this one instance." I have no doubt that the membrane in question possesses pore-canals, and that it is therefore a true zona radiata. I can confirm Eigenmann's observations in this particular, and believe that Ryder himself would have come to the same conclusion had he observed the membrane under the same favorable conditions.

In an extensive paper on the eggs of bony fishes OWSJANNIKOW ('85) describes the egg membranes of a number of the more common fresh-water forms. The most important part of his paper deals with the capsular membrane in Perca and the equivalent structure in other fishes; but the consideration of that part will best be deferred until I come to review the other papers which deal with that subject. I may here add, however, that he does not recognize the existence of a villous layer outside the zona, but regards the structure which immediately envelops the zona in Osmerus and other fishes as the equivalent of the capsular membrane of the perch. His description of a thin transparent membrane (membrana vitellina) inside the zona radiata in Salmo trutta is materially affected by the subsequent statement that it is not found in other cases (Lota, e. g.), and by the admission that it may have been an artificial product.

The pore-canals of the zona are often more deeply stained than the substance of the matrix, and by treatment with certain reagents minute points can be seen in the canals when highly magnified. In Lota vulgaris the zona is very thin, and the pore-canals in patches do not penetrate to its inner surface. It is generally stratified, the strata being laid down successively and all being perforated. The zona might, in his opinion, better be called *perforata* than radiata. Concerning its development in Gasterosteus the author says that the first trace of it is seen to be a very thin membrane without any pores. These appear when the zona has become thicker, but they are much finer than in the mature egg. After describing the condition in Acerina vulgaris (p. 18), the following statement is made as embodying the author's idea of what takes place in the formation of the zona. The granulosa cells secrete a substance (Zwischensubstanz), which surrounds the egg, one layer upon another. The porës in this substance arise by the growth into it of the points of the granulosa cells, or plasmatic processes from them. The way in which the author describes the mushroom-shaped villi which surround the micropyle in Gasterosteus shakes one's confidence in this part of his work. It is very probable, he says, that they are derivatives of the granulosa cells. After admitting that they have been described in a masterly way by Kölliker, he gives an interpretation of them that must appear to that author very remarkable. They are nothing less than individual cells (!), each with a nucleus that is stained red in carmine while the cell protoplasm resists for a time the action of the dye. From the base of the cell emerges a thread which can be traced into the zona. Very young eggs possess these appendages, as Kölliker maintained, but they are much smaller, and consist essentially of a nucleus which is attached by a thread and surrounded by a thin film of protoplasm.

If everything that is stainable is to be called either nucleus or cell protoplasm, then the current notions of what constitutes a cell will have to be abandoned; that will give room, it is true, for the admission into this category not only of the villi and the *yolk cells* of His, but also, I fear, of many other structures as well.

The author's views concerning the villous layer in other cases appear from his account of Coregonus. The granulosa cells send processes into the zona radiata. When they are removed from the immature egg, the zona appears to be covered with small more or less pointed "Zöttchen," which are therefore to be regarded as processes of the granulosa. Outside the zona radiata, Owsjannikow finds in many cases a thin viscid layer, which he suggests is derived from the oviduct in the case of S. trutta, but looks in Lota as though it resulted from the fusion of endothelial [granulosa ?] cells.

Solger ('85, pp. 330–332) is evidently inclined to bring the villous layer into connection with the presence of intracapsular corpuscles found in the perivitelline fluid. Without committing himself unreservedly to the views of Eimer, — that the villi are simply exuded drops of vitelline substance, — he confirms the statement that they are not of uniform length or shape in the case of Leuciscus rutilus, and says that Eimer must certainly have the credit of having especially emphasized the fact that after a certain epoch there exists a contrivance which prevents the further entrance of water into the intracapsular space.

I believe a comparison of the conditions in Lepidosteus with the early account by Kölliker will convince the author that the villi have no connection whatever with the interesting conditions of the perivitelline space which he has discussed.

Ryder ('86, pp. 18, 23, 30, 35, 36, and '87) has recently noted the existence of a zona radiata in several species of fishes, but without having given the structure special attention. In some cases the eggs when laid are covered with an adhesive material, the source of which is not alluded to. In the case of Ictalurus albidus (white catfish), there is an interesting condition of the egg membranes which he ('86, p. 47, and '87, p. 535) describes as follows: "The egg membrane is double, that is, there is a thin inner membrane representing the zona radiata, external to the latter and supported on columnar processes of itself which rest upon the inner membrane; there is a second one composed entirely of a highly elastic adhesive substance. The columns supporting the outer elastic layer rest on the zona and cause the outer layer to be separated very distinctly from the inner one. . . . This peculiar double egg membrane, with a well defined space between its inner and outer layers, is highly characteristic, and bears no resemblance to the thick, simple zona investing the egg of Ælurichthys, nor has anything resembling it ever been described, as far as I am aware, in the ova of any other Teleostean." Eigenmann ('90) has attempted to show by comparisons that the whole of this double membrane is probably a true zona radiata, and that the columns are protoplasmic substance which occupied the pore-canals before the separation of the two portions of the zona; but it seems to me more probable, from the "highly elastic adhesive" condition of the substance of the outer membrane, that it corresponds to the villous layer in other fishes. There can be no certainty, however, as to the real homology of the outer membrane until it has been subjected to a more careful study with especial reference to the time and manner of its production.

Cunningham ('86) has recently rediscovered what Buchholz found out upwards of twenty years ago about the peculiar egg membranes of Osmerus. It is unfortunate that Cunningham overlooked the valuable work of Buchholz, and the more surprising since he refers to Owsjannikow's paper, — which I should suppose he must have consulted, — in which Buchholz is cited. Cunningham gives figures and an account which practically confirms that of Buchholz as to the eversion of the outer membrane. According to Cunningham it serves as the so called suspensory filament by which the deposited eggs are attached. He calls the outer membrane the external zona, and agrees with Owsjannikow that it is traversed by pores which are larger and (according to his figure, about four times) farther apart than those of the internal zona. He makes no mention of the outer membrane differing in any other way from the inner, except that it is somewhat thinner.

SCHARFF ('87, '87ª) regards Beddard's zona radiata[1] in Lepidosiren as a "zonoid" layer, which he also finds present in Trigla gurnardus, where it has a thickness of 25 μ, while the true zona is only about 8 μ thick. "Both layers are striped, i. e. provided with minute radial pores," which are apparently continuous through both layers. The zona is firm, granular, and stains deeply; the zonoid is semifluid, usually devoid of granules, and stains only slightly. In ripe ova the latter disappears entirely. The zona is formed before the zonoid layer. In Blennius pholis there is no zonoid layer. Scharff "has no doubt that the egg membranes originate from the yolk," although, as far as I can understand, he advances no new arguments to prove the fact.

HENNEGUY ('88, p. 419) says that, notwithstanding the use of high powers, he has been unable to find in the trout the three layers of Owsjannikow or the denticulations described by Stockman.

A recent paper by CUNNINGHAM ('86ª) deals with the interesting question of the structure and origin of the egg membrane in *Myxine glutinosa*. Even when the eggs have become 11 mm. long there is no trace of any membrane between the yolk and the single layer of granulosa cells, which latter project irregularly into the surface of the former. Sections of an egg 16 mm. in length showed that the granulosa had become several cells deep, though not arranged in regular layers, and that there was beneath this, and in direct contact with the yolk, a thin homogeneous membrane.

It certainly is an interesting fact, to which Cunningham calls attention, that the epithelium is thicker at the poles than at the equator of the elongated ovum, and that the thickness of the membrane varies [directly] with that of the epithelium. W. Müller also observed the same fact.

In eggs 20 mm. long the follicular cells are elongated, and form practically a single-cell layer, with the nuclei at the ends nearest the yolk.

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[1] Compare Beddard ('86ª), and the summary of his paper given on pp. 66, 67.

The membrane at the poles presents thickenings which are shown by the later stages to be the beginnings of the peculiar polar filaments of the egg membrane. These thickenings project into corresponding depressions of the granulosa, the cells of which are so thinned at these places as to be barely discernible. When the egg has increased slightly in size (21 mm. long, 7 mm. in breadth), these thickenings affect the external appearance of the follicle. They have the form of finger-shaped processes covered with a single layer of granulosa cells, and project into the connective-tissue wall of the follicle. The statement that in the thinnest places (i. e. over the tips of the projections) the granulosa is only .02 mm. thick, while its thickness at the exact pole of the egg is .5 mm., is not borne out by the figure, and I therefore suppose that it is a typographical error for .2 mm. In either event, however, the granulosa does not regain its normal thickness over the ends of the rapidly growing filamentous projections of the membrane, and this fact may have significance in determining the source of the membrane. The author says that the membrane, if prepared in chromic acid, appears homogeneous even when highly magnified; but, if hardened in Perenyi's fluid, striæ perpendicular to the surface are to be seen with a high power. The striæ are not represented as rigidly straight and parallel; they may even branch, and are often moniliform. They have been traced to the outer surface of the membrane, where the author believes they are continuous with fibrils of the epithelium. He is also convinced that the membrane is homologous with the zona radiata of teleosts. I cannot agree with him, however, in the statement, " When there are two zonæ radiatæ, as in Perca fluviatilis and Osmerus eperlanus, according to Owsjannikow, these seem to be simply parts of one membrane differentiated in physical properties, but essentially similar in structure."

Cunningham believes it more probable that the zona is produced by the follicular epithelium than by the outermost layer of the yolk, and in the following manner: "The deeper part of the elongated epithelium cells is gradually changed into the zona radiata, the substance of the cells being partly transformed into the substance of the membrane, while threads of protoplasm, at more or less regular intervals, remain unchanged, and thus give rise to the pores of the membrane."

Against this view I would urge that a metamorphosis of the epithelial cells, especially if prolongations into the membrane occur at intervals, would be likely to result in the closest union between the membrane already formed and its generatrix, whereas it is exactly along this line that the artificial separation, which the author notes in all his eggs, takes place. Moreover, a diminished thickness in the epithelial cells corresponding to the most rapidly increasing part of the membrane (the filaments), *not at the close of the period of their formation, but at its beginning*, is the reverse of favorable to his "hypothesis."[1] And, finally, I would suggest that the zona is everywhere in contact with the substance of the ovum, and that the increased thickness of the membrane at the poles may be due to the accumulation there of a greater proportion of the active protoplasm than is found at the surface elsewhere. Perhaps it might be urged against this view that the explanation would be only partial, — that, while it might account for an increased thickness of the membrane at the formative pole, it would leave the condition at the opposite pole unaccounted for, and therefore could not fully satisfy the needs of the case.

I have recently sectioned the eggs of a rather poorly preserved specimen of *Myxine australis*, with a view to getting additional light on this question. Although the eggs had attained a considerable size, — 22 mm. long by 6 mm. in diameter, — still there was as yet no indication of the filamentous projections; in fact, I could not trace a membrane continuously around the egg. At the formative pole there was unquestionably a membrane about 3.5 μ thick; it was faintly marked like the zona radiata of teleosts, and it presented a deep micropylar infolding, with a cellular epithelial plug. Nevertheless this membrane gradually grew thinner in passing from the formative pole, until it could no longer be recognized. It had about the same extent as the protoplasmic cap. At this stage there was no more accumulation of protoplasm at the opposite pole than at the sides of the egg; *but there also was no more evidence of a zona radiata at this pole than at the sides of the egg.* That is all I can offer at present in reply to the possible objection which I have suggested. If it could be shown that the zona is developed at the nutritive pole of the egg without the presence of an accumulation of protoplasm there, and that the granulosa is more highly developed there than on the sides of the egg, I should admit that a strong case would be made against the view I defend.

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[1] It is true the author has offered an explanation of this; viz. that the filaments are formed from the cells *at the sides* of the process, where the epithelium is very thick, and that they are pushed up by the growth at the base. But I should imagine it would be difficult to explain how secretions from *lateral* cells could do anything more than increase the diameter of the process.

2. *Capsular Membrane.*

As I have already pointed out, the capsular membrane, since it was first described by Müller in the perch under the erroneous supposition that it was the same as the zona radiata of the salmon, has often been confounded with that membrane. In looking over the literature on the egg membranes of fishes, after I had worked out the structure of the villous layer in Lepidosteus, I was forcibly impressed by the resemblance of that layer to the descriptions that had been given of the outer membrane in the perch, and at first thought they might be homologous structures. It was particularly the account given by Ransom ('68, p. 455, Plate XVI. Figs. 30, 31) of the root-like prolongations of the tubules in the capsular membrane which suggested comparison. It therefore seemed necessary to examine carefully all that had been written on the egg capsule of the perch. The result has not confirmed my first supposition.

Müller ('54) himself gave an excellent account of the structure of the capsule, and accurately formulated the most interesting question concerning its morphological significance. He described the egg envelope (capsular membrane) as about 0.11 mm. thick; its outer surface as covered with six-sided facets, which average 19 μ in diameter. Each facet contained in its centre an open funnel, which was continued into a vertical tubule as long as the thickness of the capsule, and from 2.2 μ to 4.7 μ in diameter. In fineness these were comparable to dentinal tubules. They terminated on the inner surface of the capsule in funnel-shaped enlargements, just as they did on the outer surface. Upon eggs that had been boiled or hardened in chromic acid, it was possible to see that the tubules had a spiral course, but they also appeared narrower (1.1 μ) than in the fresh state. The tubules were filled with a thickish (albuminous?) mass, which in the fresh egg was clear, without deposits, and under pressure projected from the funnel like a rounded stopper or cylinder, but appeared to be coagulated by boiling and treatment with chromic acid. When one compressed the fresh egg to bursting, the oily substance of the yolk might be pressed into and through the tubules; thus was effected a delicate injection which might greatly distend them. Between the tubules, however, there was nothing pressed out, which proved that on its deeper surface between the tubules the capsule was closed. In the inter-tubular portions of the membrane, after the eggs are hardened, there were to be recognized, besides a gelatinous nearly invisible material, exceedingly delicate projections or filaments placed alternately and running across between adjacent tubules. These were thickest next to the tubule, and rapidly tapered to very fine threads. In his opinion, the method of the formation of the tubules might be made out during the winter. "*The question is, whether each of the tubes arises from a single cell, which becomes open, or whether the tubes are originally inter-cellular, and whether their walls result from the remnants of several cells in contact with each other.*"

A similar condition is maintained by Müller for Acerina vulgaris, but the membrane in this case was much thinner and the tubules consequently shorter.

LEREBOULLET ('54, pp. 242, 246) also discovered independently, perhaps even before Müller,[1] that there were in the perch what he called hollow closely interlaced piliform appendages (also called stiff curved filaments), which traversed the whole thickness of the shell, and to which he attributed the agglutination of the eggs into a network. He also saw besides these the much finer pore-canals.

In regard to the chemical nature of the capsular membrane, it was maintained by VON BAER ('35) and by LEUCKART ('55, p. 260) that it was an albuminoid substance. KÖLLIKER ('58) called it gelatinous, but HIS ('73, p. 15) proved that it at least closely resembled chondrin, and consequently claimed the right to call it a cartilage capsule.

REICHERT ('56, p. 93) was not able to add much to Müller's account of the structure of the capsular membrane. Concerning its origin he was at first inclined to believe that it resulted from [a metamorphosis of?] cells ("aus Zellen hervorgegangen"), and therefore to regard it as a product of the membrana granulosa. This conclusion was strengthened by finding the granulosa composed of *cylindrical* cells in the case of Esox, and that when this membrane appeared in the perch the granulosa cells had disappeared; but subsequently, finding that the follicular cells in the perch were round, and not finding any transitional stages from the epithelium to the membrane, he was compelled to leave the question unsettled. Reichert was certainly looking in the right direction, and evidently very near to a fair settlement of the question.

It remained for KÖLLIKER ('58, p. 90) to confirm this supposition of Reichert. He found that in February the capsular membrane had a thickness of 45 μ to 75 μ. The tubules, he says, are formed by the outgrowth of the epithelial cells of the follicle, so that the jelly which joins them can only be a substance secreted by these cells. These so called tubules were after all not hollow structures ("noch keine deutlichen

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[1] See Kölliker ('58), p. 81, foot-note.

Hohlgebilde"), but apparently solid pale processes of the epithelial cells, on which the anastomosing filaments found by Müller were visible. Kölliker did not doubt, however, that they were from the beginning hollow cell processes, but they still contained at the time of their formation cell contents, and only subsequently became clear. Their independent nature was shown by the fact that in chromic preparations they could be drawn out from the jelly without losing their union with the [rest of the] epithelial cells. As long as the eggs remained in the follicle the epithelial cells probably continued in union with the tubules; but at the liberation of the eggs the cell bodies probably fell off, with the exception of the walls, which were continuous with the tubules, and then constituted the hexagonal facets of Müller. Kölliker was able to produce a similar effect by artificially separating the cells from the capsule.

RANSOM ('68, p. 455, Plate XVI.), who does not seem to have been acquainted with the papers of either Reichert or Kölliker, compares the capsular membrane in consistence with fresh fibrine. "The striæ look like tubes, have a distinct double contour for each wall (Fig. 28), but are filled with a vacuolating material, and do not seem to convey anything into or out of the egg." The outer surface was thrown into folds which radiated from the ends of the "tubes," but the hexagonal markings seen by Müller could not be made out. The tubes, instead of being funnel-shaped, at their inner terminations divided into root-like processes, and were in some way intimately adherent to the outside of the dotted yolk-sac (zona). The clear matrix was elastic and concentrically laminated. "The appearance described by Müller, of oil granules passing through the tubes, may possibly have been due to vacuolation in them." Experiments with colored fluids to ascertain if there were any absorption of fluids along the "tubes" always gave negative results: the cleavage went on, the yolk-sac was dyed throughout, the clear matrix more than the tubes, the germinal mass not at all. Either, therefore, the tubes did not subserve imbibition at all, he contends, or in a much smaller degree than the clear matrix.

WALDEYER'S ('70, p. 81) conclusion about the origin of the zona from the granulosa appears to me to have resulted, in part at least, from the fact that he was unable to discover any essential difference between it and the capsular membrane of the perch. He says the latter does not differ from the former in the principle of its structure. He rightly adds, that "here [capsule] it is to be seen with the greatest distinctness that the filaments are connected with the subsequently somewhat degenerated remnants of the follicular cells." He further adds, that occasionally it appeared as though there were between these two membranes a thin flat expanse of granular protoplasm in which the filaments terminated.

His ('73, pp. 14, 15), who examined perch eggs in April, also confirms the opinion of Kölliker, and gives a figure to show the relation of the radial processes to follicle cells. The radial streaks consist, he says, of a turbid substance, which stains in osmic acid, and is continuous with conical nucleated bodies which form a continuous layer between the follicle wall and the outer surface of the capsule. Kölliker is therefore right in considering the layer as "granulosa," and the capsule as its product.

Brock ('78, p. 556) gives the following clear, and I believe correct, account of the capsular membrane of the perch. The follicular cells, which at first are in close contact with the young egg, are raised up from the zona radiata by the developing gelatinous layer, and with the advancing growth of that layer are drawn out on the side toward the egg into long processes which can be followed up to the zona. In older eggs these follicular cells, separated by considerable intervals, (an indication that their multiplication soon stops,) lie in shallow depressions of the gelatinous capsule, and with their lower pointed ends continuous with the processes. These appear to end at the zona with conical enlargements, but the author will not affirm that this is a constant feature. Brock also maintains that Serranus hepatus has a very similar gelatinous capsule. The follicular cells, however, are very peculiar. They form a network of thin flat cells, which are in contact with each other only by means of lateral processes, while perpendicular processes, which are sometimes branched and exceedingly fine, can be traced through the jelly to the zona radiata. Concerning the development of the capsule, nothing is known.

So far as regards the capsular membrane of the perch, Hoffmann ('81, pp. 19, 20, 27–29) comes to totally different conclusions from Brock, and expresses views which seem to me untenable. In October, ovarian eggs from 600 to 700 μ in diameter possess a membrane 5 μ thick, which is composed of two layers of nearly equal thickness. "The inner is the true zona radiata; the outer is composed of very numerous, small knob-like projections, which stand very close together and *correspond exactly to the villi of the cyprinoids.* On the free surface of the conical villi lie the granulosa cells."

It cannot be denied that the figure cited (*l. c.*, Taf. I. Fig. 9) corroborates the description given. But there is one fact which I should imagine would have caused the author more concern than it seems to have done. If his drawing accurately reproduces the conditions, there must have been about *four times as many villi as there were granulosa cells.* That in itself alone might not be of any significance to the author, especially as he disclaims any genetic connection between the granulosa and the underlying capsular membrane, but it does seem as though it should have received some explanation in view of the ultimate relation (Taf. I. Fig. 10) which Hoffmann admits to exist between the radial fibres of the capsular membrane and the cells of the granulosa. This is what he says about the later (February) stage of the egg: The zona itself is seen to consist of two layers, the inner much thicker than the outer. From the latter there arise with small triangular bases long peculiar fibres only 1 μ in thickness, which are stained in osmic acid precisely like the outer layer of the zona from which they arise. The outer ends of these fibres are thickened, even more than their inner ends, and form a continuous layer, between which and the zona radiata the fibres themselves are stretched like so many columns. "Over the proximal[1] ends of these fibres the granulosa cells are arranged *in such a way that a granulosa cell fits into* EACH *thickened end* (Taf. I. Fig. 10)."

If after the [ovarian] eggs have lain in water a short time they are transferred to osmic acid, it becomes very easy, says Hoffmann, to isolate both this layer (formed by the expanded ends of the fibres) and the granulosa in the form of large shreds ("Lappen"). From such preparations one can readily convince himself, he says, that the expanded ends of the fibres form a continuous sheet, and *are not processes of the granulosa cells,* as one is at first inclined to assume, and that *the fibres are, as the examination of the early stages proves, only the greatly grown-out conical villi.*

That which seems to me to need explanation is, Why is the numerical relation between the villi and the granulosa cells so different at different stages in the growth of the egg, and why does this relation become such an invariable one in the later stages of development?

It might be answered, in reply to the first question, that the granulosa cells undergo rapid multiplication, and that cell division occurring twice for all granulosa cells between October and February would explain the altered relations; but is it not more reasonable to suppose that, through some unexplainable accident, Hoffmann has been led to attribute eggs to a perch which were taken in October from some other

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[1] It is not clear to me in what sense the author can use the word "proximal" of the ends of the fibres which are directed *away* from the centre of the egg.

fish possessing a villous layer, than to ignore the evident constancy between villi and granulosa cells, and to assume an extensive multiplication of the latter in eggs during the period of their growth from a diameter of .7 mm. to .75 mm.?

The latest paper dealing with the capsular membrane of Perca is that of OWSJANNIKOW ('85, pp. 3–8), who reaffirms Müller's claim that the "tubules" are hollow structures, and corroborates Ransom's discovery that their inner ends are divided into branches which penetrate the pore-canals of the zona radiata.

On gold or silver preparations of ovarian eggs, one finds the granulosa cells[1] bounded by broad lines of a precipitate, so that there must be present a large amount of intercellular substance. The cells themselves lie, as previous authors have shown, over the beginnings of the corkscrew-shaped canals. These "beginnings," in the fully developed egg, are not at all cells, and have no nuclei; they are little funnels, with the narrow end sunk as it were into the jelly. The finely granular substance lies more compactly in the bottoms of the funnels; it is scantier on the margin, and in many places extends beyond the rim, of the funnel. This tissue (granulosa cells) often presents the appearance of stellate cells joined together by numerous processes, and separated from one another by abundant intercellular substance. The more closely packed molecules at the bottom of the funnel had given occasion, he says, to the assumption that there was a nucleus there

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[1] In his account of the Graafian follicle, not always readily harmonized with his figures, and sometimes obscure, he claims the presence of a greater number of epithelial (or endothelial, for he recognizes no difference between the two) cell layers than have usually been admitted. Thus, if I rightly understand the explanation of Fig. 4, Taf. I., there are in Perca, e. g., two epithelial cell layers between the vascular layer and the capsular membrane, — an outer layer of flat endothelium and a deeper layer of cylindrical granulosa cells; but in the text (pp. 4–8) he speaks of only two cell layers, an inner and an outer granulosa (!), which are separated by the vascular layer. From his description of the latter as the source of the new eggs, there can be no doubt that it is the "germinal epithelium" of authors. I can reconcile this apparent contradiction between text and figures only on the assumption that Figure 4 *and its explanation* belong to a period in Owsjannikow's studies when he was not as yet convinced of the error of taking "the granular accumulation in the bottom of the funnels of the 'tortuous canals' for a cell nucleus." That he ultimately supposed that to be an error appears from his description, and the statement (p. 5), "Die am Grunde des Kelches dichter an einander gelagerten Moleceln gaben die Veranlassung dort einen Kern anzunehmen."

In the case of Osmerus, moreover, he recognizes the existence of *two* layers of large flat endothelial cells in addition to the granulosa. (Compare his explanation of Fig. 8, Taf. I.)

From the bottom of the funnel there proceeds to the zona radiata a strongly and evenly twisted canal, which breaks off easily at the bottom of the funnel. Owsjannikow cannot agree with Kölliker, who maintained that in February these were solid fibres, because already in November and December he finds them hollow. The superficial layer of the gelatinous mass, as well as that part which immediately surrounds the canals, appears to be more compact than the rest of it, and the vicinage of the funnel is more deeply stained by aniline red or gold chloride than the other parts. The granules which occupy the canals or the funnels never enter into the gelatinous substance when the canals are ruptured, but escape into spaces which surround the canals. The inner end of the canal does not terminate in a pointed manner, as figured by His, but is often enlarged into a funnel, and sometimes divided into two or three fibres, — in one case into so many that it looked like a brush. On one occasion these branches were traced through the zona. These processes are to be seen only in stained specimens (gold chloride followed by aniline blue), because, having the same refractive power as the substance of the zona, they are otherwise undistinguishable. The fine molecules which lie on the inner surface of the zona were found to be deeply stained, and the author concludes that the dye must have penetrated through the spiral canals. The function of these canals must consist in the transportation of nutritive material to the yolk. They arise out of the granulosa cells, are similar to those seen by Eimer in the adder, and are not processes of the zona radiata, as affirmed by Hoffmann. The lateral processes from the canals were also seen by Owsjannikow, but he has for them another interpretation. The matrix (Zwischensubstanz) appears to lie in layers parallel to the surface. Upon its being swollen by any fluid, narrow *fissures* are formed between these layers, which join the canals and appear as processes from them.

Besides the difficulty of trying to comprehend how fissures could arise as a result of the swelling of a gelatinous mass, — it would seem that the reverse process ought to be more favorable to their appearance, — the sufficient answer to this last claim is, that the transverse processes are more deeply stained than the remaining portions of the matrix, which could hardly be the case if they were simply fissures.

It was in the hope of ascertaining something more about the interesting capsular membrane in the perch, that I advised Mr. Eigenmann ('90) to include that fish in his studies on the development of the micropyle. I believe it will be seen from his results that there is still very good reason for maintaining that the tubular or columnar structures of the capsular membrane, which have been the objects of so much study, are derived from the granulosa cells, one from each cell, and that the process by which the capsular membrane is formed is neither simply a cell secretion nor exclusively a cell metamorphosis. Although Eigenmann has not succeeded in getting stages which show clearly all the steps in the formation of the capsule in Perca, he has shown that there exist conditions in the later stages of the development of the egg in Esox (Eigenmann, '90, Plate III. Fig. 37) which seem to me of considerable importance in interpreting the conditions in Perca. In Esox the cells become elongated, and the central (axial) portion retains the granular and stainable properties of unmodified cell protoplasm. This axial portion is not cylindrical, but conical; its base is directed outward and contains the nucleus. The peripheral portion — which is more and more abundant as one approaches the zona — is more homogeneous than the axial part, and reacts with dyes in a different way. The cell boundaries have been previously lost. The boundary between these two constituents of the cell is not at first sharp, so that this phase of the process may perhaps be regarded as one of metamorphosis rather than of secretion.

I believe that Perca must pass through some such stage as this during the earlier part of the process which produces the capsular membrane. I imagine that the distinction between the axial and the peripheral portions of the cell becomes more and more sharply defined as the thickening of the capsule goes on. Meanwhile the axial portion does not long retain the indifferent condition, but is metamorphosed, especially at its periphery, into a highly refracting substance, so that there is reason for regarding the structure as tubular. This metamorphosis advances till it has practically obliterated the cell, even though a nucleus with a small amount of enveloping protoplasm may still be made out at its distal end in very late stages of ovarian growth. At any time before this, and after the distinction between a funnel part and a tubular part has arisen, the less modified distal portion of the cell may doubtless be easily separated from the secreted gelatinous substance and also from the metamorphosed cell process. Such at least is the view which I have formed, after a comparison of the granulosa in Perca and in Esox.

It would certainly be remarkable if the perch were the only representative of bony fishes in which such a process took place. I believe that there are a few cases already known which may prove upon renewed inquiry to be essentially the same as the perch. The similarity to the perch shown by Acerina vulgaris was recognized by Müller ('54, p. 189). "In Acerina," he states, "the egg membrane has the same structure [as in Perca], only it is much thinner, and consequently the tubules are only short, not longer than the breadth of the areas." Ransom ('68, pp. 453, 454) also says of this species that its "yelk-sac has an outer layer or 'Eikapsel.'" But he adds, that "the outer layer appears to be continuous with, and similar in structure to, the yelk-sac proper." However, Owsjannikow ('85, pp. 17, 18, Taf. I. Fig. 13) has given an account of it which points still more strongly to the resemblance claimed by Müller. That part of his account is especially significant in which he states that in some preparations the follicle cells have the form of very narrow cylindrical epithelium; the broad end of the cell is directed outward, the pointed end inward toward the zona. The cells, he adds, lie in a transparent non-staining layer, similar to that in which the spiral canals (in the perch) are located. Finally, a third condition is described in which the cell form is lost. The structure begins with a broad short funnel, and passes at once into a narrow, straight hollow fibre which imbeds itself in the zona radiata.

The peculiar follicular layer described by Scharff ('88, p. 69, Plate V. Fig. 15) in the interesting egg of the shanny (Blennius pholis) also appears to have begun to undergo a modification in the same direction that leads in the perch to the formation of a capsular membrane. The substance which Scharff calls "interstitial" is, I believe, *morphologically* the same as the gelatinous secretion of the follicular cells in Perca.

3. *Micropyle and Micropylar Plug.*

Since DOYÈRE ('50) discovered, in 1850, an aperture leading through the membrane of the egg of Syngnathus, and gave to it the name of micropyle, there has been a good deal of attention given to that structure.

Independently of each other, and probably without knowledge of Doyère's discovery, RANSOM ('56) in England and BRUCH ('55^a) in Germany rediscovered, in 1854, this structure in fish eggs, and both applied the name which had meantime become current through Müller's ('51 and '54^b) discovery of a similar canal in the egg membrane of Holothuria, to which he also gave the name of micropyle, borrowed from the usage of botanists.

Ransom in fact succeeded in observing in the egg of the stickleback the passage of spermatozoa through this opening; Bruch was less fortunate with Salmo fario and S. salar, although he made special effort to discover if such a passage took place, and particularly emphasized the fact that the orifice of the micropyle is of exactly the same size as the spermatozoön.

These discoveries were soon (1855) confirmed by LEUCKART ('55, pp. 257–264) and REICHERT ('56, pp. 83, 84, 98–104, Taf. IV. Figs. 1–4) on the Continent, and by THOMSON ('59, p. [100]–[104]) in England. Their observations established the fact of the existence of the micropyle in numerous fishes, and under several modifications of form. Ransom had given a fairly accurate account of the structure of the micropylar region, but Reichert especially insisted upon the differences between an invaginated portion of the membrane and a passage through the latter in the case of cyprinoids. He distinguished three regions, — an approach (Eingang), a fundus, and a neck or cylindrical canal, the length of which was diminished from what it would otherwise have been by a reduction in the thickness of the membrane to one third its normal dimension.

It was, however, the interest in fertilization stimulated by Newport's researches on the impregnation of the ovum in Amphibia, and by Keber's paper, "De introitu spermatozoorum in ovula," etc., Königsberg, 1853, that gave paramount importance to these discoveries, and attracted general attention to them.

Perhaps it is not surprising, in view of this fact, that the *ovarian* egg was less studied, and that the relation of the micropyle to the granulosa cells in its vicinity was not especially examined; and yet those observers who concerned themselves with the questions relating to the origin of the different egg envelopes must have been very near to inquiring what share the granulosa had in producing so remarkable a modification in the egg membranes. KÖLLIKER ('58, p. 92), although only incidentally making observations on the micropyle, established the fact of its existence in a large number of cases. He regarded it simply as an enlarged radial pore of the secondary vitelline membrane (zona), which might be produced, he thought, by a process of resorption.

Several subsequent writers have concerned themselves only with questions relating to the form and position of the micropyle, and its probable function. Thus BUCHHOLZ ('63, p. 72) compared the micropyle in Osmerus eperlanus to a crater the floor of which is closed except for a minute canal in the middle of it, which traverses the thickness of the wall; RANSOM ('68), besides adding some unimportant details to his earlier description of Gasterosteus, described briefly the micropyle in a large number of other (fresh-water) fishes, claiming that it always terminated in a small elevation lying directly over the germ, and concluded, as the result of experiments (pp. 459–462) made on Gasterosteus pungitius, that "the function of the micropyle is to admit the spermatozoids to the surface of the yolk"; and His ('73, pp. 3, 4) described with some detail the structure of the micropyle in Salmo salar and S. fario, in both of which he recognized a shallow depression ("Mulde") surrounding the crater, which in S. fario terminated in a deep funnel, and this in the canal. He also showed that only one spermatozoön at a time can pass the micropylar canal, which terminates somewhat eccentrically over the germinal disk.

Hoffmann ('81, pp. 33–36) has confirmed for a large number of fishes the observations that the inner end of the micropyle terminates in a papillary elevation of the zona radiata, and that in the ovarian egg it lies directly over the germ. From a comparison of the dimensions of the spermatozoa and the calibre of the canal, he also draws the conclusion that not more than one spermatozoön can traverse the micropyle at a time. In nearly all the micropyles figured by Hoffmann the canal is a tubular passage without any special enlargement; but in the case of the herring's egg — which is the one most carefully described — the outer half of the passage is enlarged into a conspicuous bulbous cavity (Taf. I. Fig. 19), which, so far as I recall, has been seen in only one other instance, that of Petromyzon as figured by Calberla. But the greatest interest attaches to the conditions figured for Leuciscus rutilus (Taf. I. Fig. 20). In this case there exists a distinct plug of granulosa cells occupying the depression in the egg membrane at the micropylar region. Since the author does not mention the fact in the text, it is probable that he attached to it no importance. I think, however, it is the first clear proof published of the existence of a specialization in the granulosa of the micropylar region in any teleost. On account of the rather diagrammatic rendering of the granulosa cells, it is not possible to be very confident about the existence of a specialized micropylar cell, but the fact that a single cell forms the apex of the plug favors that view, and I shall be surprised if such a structure is not hereafter demonstrated in this European fish.

Of the more recent writers on the micropyle, Owsjannikow ('85, pp. 11–13, Taf. I. Figs. 5–7) describes for Osmerus eperlanus a micropylar apparatus composed of two portions, an external and an internal, corresponding respectively to the two membranes which envelop the egg, — the "external zona radiata" (which corresponds, in his opinion, to the outer [capsular] layer in Perca) and the "internal zona radiata." The apparatus has the form of a crater-like depression, about as described by Buchholz and Ransom. Where the membranes take the direction of the crater, they form folds with the pointed ends directed inward. But of more interest are his statements, that the zona radiata externa takes the greater share in the formation of the crater, and that "*other tissues, especially the endothelium and granulosa cells, participate in the same.*" Owsjannikow was thus, I believe, the first person after W. Müller ('75) to call attention to an intimate relation between granulosa and micropyle.[1] But there are some elements of uncertainty about his descriptions and figures that seem to baffle every attempt to reduce them to harmony. The most perplexing thing about his description is the use of the term "endothelium," which is at first used for Osmerus in the following connection (p. 10): "Die Graafschen Follikel der Osmeruseier bestehen aus Endothel, Gefässen, Bindegewebe und Follikelzellen." In the description of other eggs (Perca) the word "Endothel" is also used as though applied to cells which lie *outside* the vascular layer, and even as though including the germinal epithelium of other authors (p. 4). Unless an endothelium having a very different position from that previously described by him is meant, when he says that it participates (as well as the granulosa) in the micropylar structure, I believe that the author has fallen into some error; for I am of opinion that neither the connective-tissue layer with its blood-vessels nor the germinal epithelium shares directly in the formation of the micropylar apparatus. Neither do I believe that there exists *inside* the connective tissue any layer of cells except the granulosa. Moreover, I do not think that any layer of endothelium, either inside or outside the vascular layer, has been figured as sharing in the formation of the micropyle. It does not help matters in the least to add that the author discountenances (p. 4) any attempt to draw a distinction between *epithelium* and *endothelium;* for after saying that "Endothel *und* Granulosazellen" share in this formation, he proceeds with a description which certainly allows the assumption that there is only *a single layer of cells involved,* to which, however, he gives successively the names of granulosa, endothelium, and epithelium.

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[1] Although published several years ago, Owsjannikow's studies were not made until some time after I had demonstrated the conditions in Lepidosteus which have been described above. His paper, as well as the more recent one of Cunningham, has therefore had no influence in determining the course or the results of my studies on Lepidosteus, nor did it influence me to suggest a comparative study on the eggs of bony fishes, such as Mr. Eigenmann has undertaken; for I had already proposed that question to one of my students before either paper was published.

On "teased" preparations Owsjannikow often finds the zona deprived of its cellular covering, but ordinarily the detached cells are to be found in the form of a continuous membrane, on which a conical projection is to be seen. The form of the projection corresponds exactly to that of the external micropyle; it is hollow; the cells at the entrance to the micropyle have the form of a crown, and become smaller and smaller toward the bottom of the crater. I am not sure that I fully understand the figure (Taf. I. Fig. 6) which the author refers to in this connection, but it appears to me to be a view of an egg from the animal pole; the granulosa cells of the crater, having been detached, are seen partly in side view, but somewhat obliquely, as a conical structure, and the pore-canals of the *external* zona of the crater are visible where the granulosa cells have been lifted. In the middle of the figure is an optical section of that portion of the zona which forms the internal projection, and in its centre the micropylar canal. There is apparently a single layer of cells, and this I take to be the granulosa. It is to be regretted that the author has not furnished us with a strictly radial section through the micropyle and the accompanying structures on a sufficiently large scale to enable one to determine what becomes of the membrana propria of the theca folliculi in the region of the micropyle. One would infer, from the statement that this granulosa cone was hollow, that the theca must follow the course of the crater; but if it does, it must be different from all other known cases. Not even in Perca does the membrana propria suffer any deflection or infolding due to the participation of the granulosa cells in the formation of a micropylar structure. I have also been considerably perplexed by Owsjannikow's Figure 5 (Taf. I.). At first I took it to represent a strictly diametric section of the egg and its membranes. With that understanding of it, I imagined that the large oval body just above the micropyle might possibly represent a single micropylar cell in some way loosened from its natural position; but more careful study leads me to believe that this is a figure representing in part an optical section, in part a surface view, and that the oval structure presents an oblique view of the external entrance to the funnel-shaped cavity of the crater, still lined with granulosa cells, while all the rest of the figure represents a view of the egg as it would be seen in optical section. Owsjannikow states that the inner micropyle can be regarded as a somewhat enlarged canal of the zona, and claims that it subserves the nutrition and growth of the egg; for he has traced from the inner end of the canal a row of granular bodies which were continued in the yolk as a fine thread, which at last disappeared. This row of granules and its thread-like continuation the author regards as a product of the granulosa cells. But at present there are no data, he says, either teleological or phylogenetic, which can explain the remarkable structure of the external micropyle.

RYDER ('86, p. 30, Plate VII. Fig. 35), who has demonstrated the existence of the micropyle in the eggs of several fishes, speaks of the passage through the capsular membrane in Perca Americana as "a wider pore-canal which leads to the micropyle." It is evident from Eigenmann's ('90) account and from his figures that this statement is inaccurate.

CUNNINGHAM ('86^a, pp. 59, 61–63, etc., Figs. 2–4, 12) has shown much more satisfactorily than W. Müller ('75) did, that in Myxine glutinosa the follicular epithelium plays an important part in the formation of the micropyle. Of an egg 16 mm. in length he says: "At the exact pole of the egg there is a differentiated portion of epithelium, where a proliferation of the latter has taken place. This portion is composed of polygonal cells, which are little or not at all elongated, and towards the egg it runs out into a thin cylindrical process which penetrates the next layer [zona radiata] as shown at *e. p.* [Fig. 2]. . . . This process penetrates the vitelline membrane [zona radiata], occupying a tubular cavity in the latter, and passing through it to form a hemispherical projection on its inner surface. . . . This cellular projection is covered by a thin membrane continuous with the vitelline membrane, and is not in immediate contact with the germinal disk. . . . There is thus at one pole of the nearly ripe ovum a tubular canal extending through the chorion [zona radiata], but not open internally, filled up by a cylinder of cells projecting from the follicular epithelium. . . . It is evident," the author adds, "that this aperture is to form the *micropyle* in the ripe ovum. . . . It is probable that careful investigation would show that in all Teleosteans whose ova possess a micropyle that structure is produced by a projection of cells from the follicular epithelium." Cunningham also believes it "at least possible that in all vertebrates the micropyle will be found on investigation to be produced in the same way as in Myxine, namely, by the growth of a cellular process from the follicular epithelium towards the vitellus while the vitelline membrane is being formed."

In an egg 20 mm. in length "the proliferation and differentiation of cells at the pole in the follicular epithelium have disappeared, but the cylinder of cells, though reduced in size, still remains in the micropyle, and is evidently destined to keep the latter open until the maturation of the ovum is complete." This egg was from material obtained in

December. Older eggs, obtained at the end of January, although only slightly larger than the December egg (21 mm. long), presented a very different appearance at the micropylar region. Cunningham says of the latter: "The micropyle is somewhat narrower, and the cells present in it at previous stages have disappeared almost completely, only a little débris remaining. The micropyle seemed also in these ova to be open internally, though of this point I am not absolutely certain. If there is a membrane closing the inner end, it is an extremely thin one."

Cunningham has shown conclusively, I believe, that the granulosa has much to do with the modifications of the egg membrane in the micropylar region, but there are several particulars concerning which his description and figures leave me in a rather unsettled state of mind. In the first place, the author does not seem to distinguish with sufficient sharpness between a funnel-like region, which may be partly the result of an infolding of the membrane, and a *passage through* the membrane, which I have called the micropylar canal. It seems to me possible that his uncertainty as to whether the micropyle is closed at its inner end at a *late* stage may be due to this fact. The disproportion between the calibre of the "micropyle" and the size of the spermatozoa[1] is not alluded to, but at once suggests to me that the structure in question may be the equivalent of the micropylar funnel only. I should be quite certain that it was so, were it not that the drawing of the latest stage (Fig. 12) — which is not sufficiently explained — admitted two interpretations. In this figure the whole passage is divided into two portions of about equal length, but of very unlike calibre. The inner half is a narrow canal with parallel walls, about one third of a millimeter in diameter (actual size about 10 μ); the outer half is *abruptly* widened to 6 or 8 mm. in diameter, and gradually increases toward the granulosa to 10 mm. (nearly 300 μ actual size). There are no granulosa cells, however, in either portion of the passage. The almost flat-bottomed outer half of the passage would appear to be the equivalent of the micropylar funnel in bony fishes, and I should certainly have so regarded it if Cunningham had not evidently considered the inner narrower portion as a part of the "micropyle" of previous stages from which the cells had disappeared, leaving "only a little débris." If the author is right in this assumption, that the *narrow* part of the ap-

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[1] In its narrowest place the "micropyle" of the author's Figure 3 is represented as 5 mm. in diameter in the drawing, which, being magnified 280 diameters, makes its actual diameter about 18 μ, whereas the actual diameter of the head of a spermatozoön (Fig. 14) is not over 7 μ.

paratus was once occupied by granulosa cells, then either the true micropylar *canal* has not been seen in Myxine, or it is formed in a different manner from that of bony fishes, for in no case is it occupied in the latter by a plug of cells from the follicular epithelium. The former alternative is, in my opinion, the more probable. This conclusion I base partly on the appearance of Cunningham's Figure 3, and partly on the conditions presented by sections of an ovarian ovum of Myxine australis, which I have studied since reading Cunningham's paper. In his Figure 3, the plug of granulosa cells which is sunk into the yolk is *completely* enveloped in a uniform layer not more than 2 μ thick, which not only separates its deep end from the yolk, but also its sides from the membrane called by him vitelline membrane, or zona radiata. What the significance of the part of this thin membrane lining the chimney-like elevation of the zona radiata may be, I cannot say, unless it is reflected at the upper edge of the chimney to form the outermost layer of the zona; but the portion which separates the yolk from the granulosa plug I regard as the equivalent of the first formed portion of the zona radiata, and believe that the true micropylar canal will be found in the form of a minute passage through that membrane. My principal reason for this opinion rests on the condition of this region in the egg of M. australis. This egg was about 22 mm. long and 6 mm. in diameter. It was enveloped by a follicular epithelium composed at the animal pole of a single layer of cells, averaging about 10 μ thick. Over the region of the flattened germinative vesicle the granulosa was gradually thickened to about 25 μ, and from the middle of the thickening a solid plug of cells about 35 μ long and 25 μ in diameter projected into the yolk. The membrana propria of the follicular theca passed over the micropylar region without being at all infolded, so that the total thickness of the granulosa, measured from the apex of the plug, was about 60 μ. The cells of the plug were not well preserved, but appeared to be of about the same relative size as in Cunningham's Figure 3, — i. e. of one half or one third the diameter of the plug. There was no enlargement at the apex of the plug as seen in his figure. Between the granulosa and the yolk, and in contact with both, was a highly refractive thin (3.5 μ) membrane, which at first appeared homogeneous, but in which I believe I have detected at intervals radial markings. This membrane became thinner at some distance from the pole.

The whole apparatus had such a striking resemblance to the micropylar apparatus in Lepidosteus that I cannot doubt that the granulosa plug represents the same structure in Lepidosteus, and that consequently the micropylar canal is to be found at the bottom of the funnel-shaped infolding produced by the plug.

4. *Micropylar Cell.*

Although no other cases are yet known in which a single cell of the follicular micropyle-plug is so evidently differentiated from its neighbors, as in Lepidosteus, still it is clear from the results of Eigenmann's ('90) studies that the existence of such a specialized cell is not uncommon.

The most interesting question relative to the micropylar cell is that of its physiological signification.

That it sustains some intimate and constant relation to the micropyle itself can scarcely be doubted. Perhaps its primary function is fulfilled by serving as a source of passive resistance to the forming membranes in the region of the micropyle — a kind of mould for them — during the process of their formation, therefore a mechanical device for producing a micropylar funnel. In that case it would doubtless often be more than the single micropylar cell on which devolved the function; it would be rather that plug-like accumulation of granulosa cells, with the micropylar cell at its apex, which attains such an extensive development in Lepidosteus and some other cases.

But the very fact that one cell is generally, if not always, differentiated more than the rest, suggests that the function referred to may not be the only one, — perhaps, indeed, not the primary one. There are two other possible functions which are naturally suggested in this connection.

Concerning the first of these it may be said that it still remains to be shown to what extent the micropylar canal and its funnel are the result of an exclusively progressive process of development; whether, in other words, any part of this structure is produced by a process of *resorption*. Such a process would not be without a parallel. At least Leuckart ('55, pp. 108, 216, 247) has asserted in most positive terms that the micropyle of certain insects' eggs is not to be found in the chorion from the very beginning of its formation, but that it arises after the production of the chorion by means of resorption[1]

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[1] More recently, it is true, Korschelt ('84, pp. 421, 422) has shown that the micropyle in Meconema varians is formed by a single cell, and he apparently leaves no room for a process of resorption, since he says: "Die Entstehung des Canals ist wohl so zu denken, dass die Zellen schon frühzeitig einen Fortsatz ausstrecken, der

The fact that Eigenmann ('90) has been unable to discover a micropyle in the earlier stages of the formation of the egg membranes in fishes may also point in the same direction. I would not wish, however, to place too much weight upon such negative evidence; it requires extensive and indeed the most exhaustive examinations to make such testimony satisfactory. Especially am I compelled to this reserve, in view of the fact that Cunningham ('86[a]) has found the micropylar apparatus well developed at a relatively early stage in the formation of the zona in the case of Myxine glutinosa; but it will be observed that he says (p. 61): "This cellular projection *is covered by a thin membrane continuous with the vitelline membrane, and is not in immediate contact with the germinal disk.*" An actual opening does not exist, therefore, at the time of which he speaks. Although I have found in M. australis at an apparently early stage in the development of the zona a deep infolding of that membrane, as described above, still I have not satisfied myself in the single specimen sectioned that there is an *orifice* through the membrane at this stage of development. But on this observation I would not care to speculate were it not confirmed by Cunningham's studies on more extensive and I presume histologically more favorable material, for I know how easily one may be deceived as to the existence of so minute a structure as that with which we have to deal.

The facts which I have given above do have a certain weight with me as rendering it possible that in Myxine at least the micropylar canal, in the strict sense of the word, is not present until near the maturity of the ovum, and that consequently it may be the function of the cell nearest to the bottom of the funnel — the micropylar cell — to absorb so much of the already formed membrane as is necessary to allow the

anfangs nur kurz ist, später mit dem Dickerwerden des Chorions und dem entsprechenden Zurückweichen der Epithelschicht aber länger und länger wird."

In his final paper, Korschelt ('87, p. [43] 223) suggests a method of reconciling Leuckart's views with his own observations: "Die Beobachtung Leuckart's, nach welcher die Mikropylkanäle sich nicht von Anfang an am Chorion finden sollen, liesse sich vielleicht mit unseren Befunden in Uebersinstimmung bringen. Wir erkannten in mehreren Fällen, dass die Masse des in der Bildung begriffenen Chorions eine durchaus weiche und plastische sein muss. Deshalb wäre es leicht denkbar, dass die Zellfortsätze, welche die Kanäle entstehen lassen, nicht von Anfang der Bildung an vorhanden wären, sondern erst später in die weiche Masse des jungen Chorions hinein entsendet würden. Dann würde es ein Stadium geben, in welchem das Chorion eine ununterbrochene Oberfläche besässe. Seine Ansicht, das die Mikropylkänale an dem bereits gebildeten Chorion durch Resorption entstehen sollen, dürfte Leuckart wohl aufgegeben haben, sobald er die Entstehungsweise der Porenkanäle der Eischale kennen lernte."

passage of the spermatozoön.[1] I realize that this is a speculation on very narrow foundations; for even if it could be shown to result from absorption, it might be the protoplasm of the ovum, not the granulosa cell, which accomplished the work. There are, however, still more serious objections to this view, which, though not disproving it, render it very doubtful. The fact that in general pore-canals and orifices in cuticular secretions are the results, not of resorption, but of the previous existence of protoplasmic projections, makes it probable, without positive proof to the contrary, that the same would be true in this case.

The serious, and indeed, as it seems to me, insurmountable objection to considering the whole micropylar funnel as the result of absorption, is the condition which it exhibits in Lepidosteus, where there is a very gradual diminution in the thickness, not only of the zona, but also of the villous layer. It is not probable that any process of absorption could result in diminishing the thickness of *both* layers so evenly without affecting their mutual relations, unless perchance it should be imagined that the zona was absorbed through the agency of the yolk, and the villous layer by means of the granulosa cells. But even that assumption would not help the case very much, for it would still have to be explained why the shorter villi retained all the parts of the longer ones, *and in nearly the same proportions.*

While, then, the conditions clearly preclude the possibility of looking upon the micropylar apparatus in general as resulting from a process of absorption, it by no means follows that the micropylar *canal* may not be produced by such activity.

The other purpose which it has occurred to me the micropylar cell may subserve, is to facilitate the penetration of spermatozoa. Not precisely that a minute drop of slimy substance, resulting from the degeneration of this cell and covering the orifice of the narrow canal, would offer less resistance than water, but that its presence might prevent the occlusion of the orifice by the accidental introduction of impenetrable substances without itself offering any serious obstacle to the free entrance of the fertilizing element.

If one were to attempt a phylogenetic explanation of the *micropylar funnel and canal* in bony fishes, he might reason somewhat as follows.

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[1] In Cunningham's Figure 3, the cells of the granulosa plug which form the layer nearest the yolk — four of them being cut in the section figured — are all larger than the remaining cells of the plug, but I am unable to say that any one of them is the largest of all.

The development of a persistent egg membrane impervious to spermatozoa would evidently be possible only with the concomitant production of one or more orifices; for without such provision no egg could be fertilized, and the transmission of such tendencies would clearly be impossible. That would necessitate the development of the micropyle by what I should call an exclusively *progressive* method. It would not imply any *regressive* or resorbent process. How, then, could one find reason for claiming any such process? I believe it would only be necessary to assume that the zona radiata in its original development subserved some useful end *during the development of the ovum*, in order to form an idea of the possible course of events which has led to the present condition. Imagine eggs at oviposition provided with a zona radiata which remained or had become penetrable by spermatozoa; such eggs would be in the most favorable condition for fertilization, but on account of the condition of the zona they would be poorly *protected* against external agencies. If, under these conditions, a *portion* of the zona radiata in some eggs should become more resistant, even to the point of preventing the entrance of spermatozoa, the eggs thus modified would be better protected from injurious external influences than those which remained in the original condition, and yet they would be almost as readily fertilized as the latter, *provided* some portion of the zona remained, as at first, penetrable. In short, the advantages of such a changed condition would be greater than the disadvantages, and consequently in the long run the more favorable condition would predominate. Evidently the *optimum protection* would have been reached when a region no larger than that absolutely necessary to admit a single spermatozoön had been left for that purpose. But this process of restriction in the area accessible to the spermatozoa may easily have been accompanied by another process, which may have begun as early as the former. The zona was assumed to remain, or *become* at the time of oviposition, penetrable to spermatozoa. It seems to me entirely reasonable that a process tending to modify a portion of the zona and make it more readily penetrable should be set up in the ovary, and that such eggs in the matter of fertilization alone would have some slight advantage over eggs less easily penetrable. If now these two tendencies were operative at the same time, — the one serving to soften a part of the zona, in order to make it the more readily penetrable, the other to harden *another portion*, to make the egg less subject to adverse environment, — the former would become localized by the encroachment of the latter until at length there would be only a limited area in which the process of softening went on; but this might be — as we must believe often happens in the animal economy — correspondingly intensified, until an activity which in the beginning resulted in only a feeble modification in the condition of the zona ultimately terminated in its complete liquefaction and absorption.

A single argument, which it seems to me may have some value, in support of this hypothesis, is to be drawn from the condition of the zona in Elasmobranch fishes. So far as I now see, the complete disappearance of the zona at the maturity of the egg would be entirely in harmony with the hypothesis. The condition there at any rate seems to me to favor the assumption previously made, that the zona originally had a function distinct from that which it now appears to possess in protecting the embryo after fertilization. For if not, it must be an inheritance from ancestors which, like bony fishes, had their embryos thus protected. There are probably few who would defend the idea that the Elasmobranchs are descended from bony fishes, and the evidence of common ancestors with eggs thus protected still remains to be found.

If, as the Russian naturalists assert, there are several micropylar openings in the egg-shell of sturgeons, it may be that those fishes present a condition which is intermediate between an extensive region of penetrability and the extreme restriction which now prevails in bony fishes.

The funnel portion of the micropylar region is certainly the less essential and least constant part of the structure. It may reasonably be considered, I believe, a secondary condition, and the explanation of its development might lie either in the fact that it served, in a passive way, to direct the motion of a greater number of spermatozoa toward the actual orifice, or, possibly, that it served to preserve from accidental removal the protective products of the degenerated micropylar cell.

Thus the micropylar *canal* might be regarded as the result of two to a certain extent antagonistic tendencies, — the fittest solution of a problem requiring the fulfilment of two conditions. The micropylar *funnel* could obviously be regarded as a partial compensation for the diminished opportunities for fertilization caused by a restriction of the area available for that purpose, and might have arisen simultaneously with the restriction, or only after the latter had attained its present maximum.

Summary.

1. The first food of young gar-pikes after the absorption of the yolk is mosquito larvæ; later, they feed on small fishes.

2. Very cold water is injurious to young gars.

3. The arched form which the body sometimes exhibits is probably always the result of an abnormal condition.

4. The acts of catching and swallowing the prey are complicated. Fishes are usually swallowed head first.

5. The young gar-pike lives at the surface of the water after the absorption of the yolk-sac.

6. The emission of bubbles of gas through the gill slits is accompanied by a rolling of the body to one side and a forward movement.

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7. Analyses of the bubbles of gas emitted by young fishes, and also of a limited amount of atmospheric air which had been used by the fishes in respiration, showed a reduction of the oxygen to 10–15 per cent, and the presence of only a small amount of carbon dioxide, 0–1.7 per cent.

8. Air which had been previously deprived of its carbon dioxide gave no evidence of containing even a trace of that gas, after having been respired. Consult the text for the conditions of experimentation.

9. It is probable from these experiments that the air-bladder respiration serves to oxygenate the blood, but that the elimination of carbon dioxide is effected in some other manner. It is possible that the two elements of the respiratory function of higher vertebrates were *successively* transferred to the air-bladder, that of oxygenating the blood being the first to be transferred.

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10. There are two egg membranes in Lepidosteus, — a zona radiata and a villous layer, — and they are intimately joined together. Both are radially striate, the zona finely, the villous layer coarsely.

11. Balfour and Parker were mistaken in stating that there was a homogeneous non-striate membrane inside the striate zona, and also in supposing that the pyriform bodies (villi) of the outer covering were metamorphosed follicular cells.

12. The outer layer is not as thick as the zona, and is made up of radially compressed and folded columnar structures, the villi.

13. Each villus is composed of three parts, — head, stalk, and roots. The roots project into the pore-canals of the zona.

14. The zona presents the usual structure of that membrane in bony fishes. The pore-canals are slightly enlarged and spiral at their peripheral ends.

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15. The egg of Lepidosteus has a single micropyle, but it has been overlooked by previous observers.

16. The micropylar apparatus embraces a funnel and a canal. The funnel results from an infolding and a reduction in thickness of both the villous layer and the zona radiata. The micropylar canal is a narrow passage of uniform calibre (2 μ) and circular cross section, through the thinnest part of the two membranes, namely, at the bottom of the funnel.

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17. The granulosa of the mature ovarian egg consists of a single layer of polygonal cells, except in the region of the micropylar funnel, where it forms a plug of cells completely filling the funnel.

18. The membrana propria of the theca folliculi is not infolded in the region of the micropylar funnel.

19. A single large granulosa cell, the "micropylar cell," forms the apex of the plug, and occupies the bottom of the funnel.

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20. The villous layer is formed before the zona radiata. It first appears at the surface of the yolk in eggs about 0.4 mm. in diameter, and reaches one third its maximum thickness a year before the egg matures. It is produced by the ovum, not by the granulosa cells.

21. The number of the villi is not increased during the growth of the villous layer.

22. The zona radiata is likewise the product of the ovum, and its formation requires less than twelve months.

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23. The name "capsule" should not be used for the zona radiata; it ought to be restricted to membranes outside the zona, which, like that of the perch, are the product of the granulosa.

24. An egg membrane comparable structurally and genetically with the *zona radiata* of bony fishes is to be found in representatives of all the groups of fishes except Amphioxus. It is fugitive in selachians and Lepidosiren, and probably in viviparous teleosts. *The zona is produced by the ovum, not by the follicular cells*, and is traversed in all cases by

pore-canals, which rarely (Myxine?) branch. J. Müller is wrongly credited with their discovery.

25. An egg membrane genetically, but not always structurally, comparable with the *villous layer* of Lepidosteus, is found in several other cases: *possibly* in Petromyzon, probably in selachians and Lepidosiren, and certainly in several teleosts. *This membrane is also produced by the ovum, and earlier than the zona.*

There is some reason to believe that it exists in the herring and the smelt (Osmerus). Hoffmann is probably in error in attributing the presence of villi to the eggs of Perca in October, and Owsjannikow certainly is in asserting that the mushroom-shaped villi in Gasterosteus are individual cells.

26. The *capsular membrane* is produced, as originally defined by J. Müller, by the follicle or follicular cells. It has often been confounded with the zona, and also with the villous layer.

27. Although the "tubules" in Perca have been described as possessing root-like prolongations which penetrate the pore-canals of the zona, they are genetically unlike the villi found on other eggs, being produced by the granulosa cells alone. Hoffmann's statement to the contrary rests on insufficient evidence.

28. A comparison of the condition of the granulosa in Blennius pholis and Esox with that in Perca, is believed to shed some light on the probable steps by which the capsular membrane is produced.

29. The most important paper on the *origin* of the zona and the villous layer I believe to be that of Kölliker, whose conclusions I have confirmed in the case of Lepidosteus. All authors who have maintained that either zona radiata or villous layer is the product of the granulosa I believe to be in error; and in particular I maintain that the reasons assigned by Cunningham to prove that the zona in Myxine is produced by follicular epithelium are not adequate to establish his proposition.

30. It is doubtful whether the micropylar *canal* has been seen in Myxine. What W. Müller called the micropyle was the micropylar funnel, and possibly that which Cunningham describes as the micropyle does not embrace the canal.

31. What I have called the *micropylar plug* of granulosa cells was first seen by W. Müller in Myxine; later it was figured, but apparently not appreciated, by Hoffmann in Leuciscus; it was described as hollow by Owsjannikow in the case of Osmerus, who recognized its relation to

the micropylar funnel; and finally, it was described more fully for Myxine by Cunningham. Eigenmann has found the same structure in a number of bony fishes.

32. The *micropylar cell* has never before been recognized. Eigenmann has now found it in a number of osseous fishes, — Perca, Pygosteus, Esox, etc. I believe it may fairly be assumed to exist in the greater number of those fish eggs which possess a micropyle, and that it has an important function in connection with the formation of the micropyle or the fertilization of the egg.

33. I have made the following suggestions as to the possible functions and history of the micropylar apparatus: The *micropyle*, being evidently a provision for the fertilization of the ovum, may have its present structure as the result of two to some extent conflicting tendencies; one induced by the advantages of protection to the egg, the other by the necessity of some provision for the penetration of the fertilizing element. But the best protection is not compatible with penetrability of the membrane at all points. Any reduction in the extent of the penetrable surface would be favorable to protection. An optimum condition would be reached when the penetrable area is reduced to a minimum, and that is the diameter of the head of a spermatozoön.

The *funnel* may be a partial compensation for such reduction.

The *micropylar plug* may *mechanically* determine the presence and form of a funnel.

The *micropylar cell* may serve to form the *canal* by resorption, *or* to prevent the occlusion of the canal by less penetrable matter at the time of oviposition.

Cambridge, April 7, 1889.

Postscript.

Since the completion of the present paper, I have received from the author, Dr. J. Beard, a copy of his "Preliminary Notice, — On the Early Development of *Lepidosteus osseus.*" This paper was presented to the Royal Society of London, April 20, 1889, and was printed in the Proceedings of the Society, Vol. XLVI. pp. 108–118, May 16, 1889.

Dr. Beard's material was procured by him in the spring of 1888 from the same place as that which supplied Mr. Agassiz and myself, — Black Lake, New York.

In this preliminary notice the author does not devote much attention to the egg membranes. What he says about *the inner egg membrane* coincides with the views which I have expressed. He says that it "is not composed of two layers either in Lepidosteus or in the sturgeon. It is a simple zona radiata, the striæ reaching to the innermost portions of the membrane. The division into two layers, sometimes seen, is the optical effect of thick sections."

I cannot agree with his conclusions regarding the external layer, and am confident that his final paper will not contain proof of the accuracy of his statements. He says: "The *pyriform bodies* are certainly modified cells, each with the remains of a nucleus at its outer end. These modified cells have degenerated into a sort of glue, which causes the excessive stickiness of the newly laid eggs. . . . In the ovarian egg these 'pyriform bodies' are probably nutritive cells to the ovum, for their outer ends near the nuclei contain a number of minute yolk particles."

As far as regards the external layer, it is difficult to conceive how our views, whether morphological or physiological, could have been more divergent.

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EXPLANATION OF FIGURES.

All the figures were drawn with the aid of the camera lucida, and were made from preparations of *Lepidosteus osseus*.

ABBREVIATIONS.

cap.	Head of villus.	*pd.*	Stalk of villus.
c.-t. cp.	Connective-tissue corpuscle.	*rx.*	Root of villus.
fus. mat.	Maturation spindle.	*st. vil.*	Villous layer of egg membrane.
gran.	Granulosa.	*th. fol.*	Membrana propria of theca folliculi.
m py. can.	Micropylar canal.	*vac.*	Vacuole.
m py. cl.	Micropylar cell.	*vit.*	Vitellus.
nl.	Nucleus.	*vs. g.*	Germinative vesicle.
nl. gran.	Nucleus of granulosa cell.	*z. r.*	Zona radiata.

PLATE I.

All the figures of this plate were made from material that had not been hardened, and all the figures except Figs. 7 and 11 are magnified 472 diameters.

Fig. 1. A surface view of a small portion of the villous layer of egg membrane.

" 2. The appearance presented by the same layer when the region near the boundary between it and the zona radiata is in focus. Some of the roots of the villi are seen between the stalks.

" 3. The zona radiata when the focusing is a little below its outer surface. A few pore-canals are occupied by roots of villi and appear darker.

" 4. A portion of a radial section after being treated with weak hydrochloric acid. Two of the villi much more elongated than the others.

" 5. A radial section of a fresh egg-shell, showing the relative thickness of the zona radiata and the villous layer.

" 6. A portion of the same with the villous layer removed, but leaving its roots in the spiral pore-canals. Examined in glycerine.

" 7. Portions of the villous layer removed from the zona radiata and much swollen in water. The roots appear like a fine fringe. × 145.

" 8. The appearance of the pore-canals after treatment with hydrochloric acid. The most of them, especially toward the margin of the figure, should have been drawn larger but faint. A few are conspicuous from the presence of roots of villi.

" 9. *a* to *h* and *j* to *n*, isolated villi in various stages of elongation after imbibition of very dilute hydrochloric acid; *i*, after soaking in water only.

" 10. A fragment of the zona radiata deprived of the villous layer and treated with weak hydrochloric acid until all the pore-canals except those containing villous roots had disappeared. The zona, having become soft, was partly crushed, so that the roots were seen obliquely, the ends toward the top of the plate being the ones torn from the stalk.

" 11. Optical radial section of the micropylar region of a fresh egg, the wall of the membrane beyond the micropyle being projected on the same plane. × 145.

" 11^a. Optical cross-section of the same, at plane *a* of Fig. 11.

" 11^b. Optical cross-section of the same at plane *b* of Fig. 11.

E. L. Mark del.

B. Meisel lith.

ABBREVIATIONS.

cap.	Head of villus.	*pd.*	Stalk of villus.
c.-t. cp.	Connective-tissue corpuscle.	*rx.*	Root of villus.
fus. mat.	Maturation spindle.	*st. vil.*	Villous layer of egg membrane.
gran.	Granulosa.	*th. fol.*	Membrana propria of theca folliculi.
m py. can.	Micropylar canal.	*vac.*	Vacuole.
m py. cl.	Micropylar cell.	*vit.*	Vitellus.
nl.	Nucleus.	*vs. g.*	Germinative vesicle.
nl. gran.	Nucleus of granulosa cell.	*z. r.*	Zona radiata.

PLATE II.

All the figures of this plate are magnified 515 diameters.

Fig 1. Portion of a radial section through the shell of a deposited egg preserved in 5 per cent potassic bichromate. Stained in carminic acid dissolved in 80 per cent alcohol. Mounted in benzole-damar.

" 2, 3. Isolation preparations of villi from a mature egg pressed from female. The egg was let fall into 90 per cent alcohol, afterwards soaked in water, and then stained in acetic-acid carmine. Examined in glycerine. The heads were of a much brighter rose-color than is shown in the lithographic print. In Fig. 3, *c*, the villi are seen edgewise; in *a*, *b*, and *d*, flatwise.

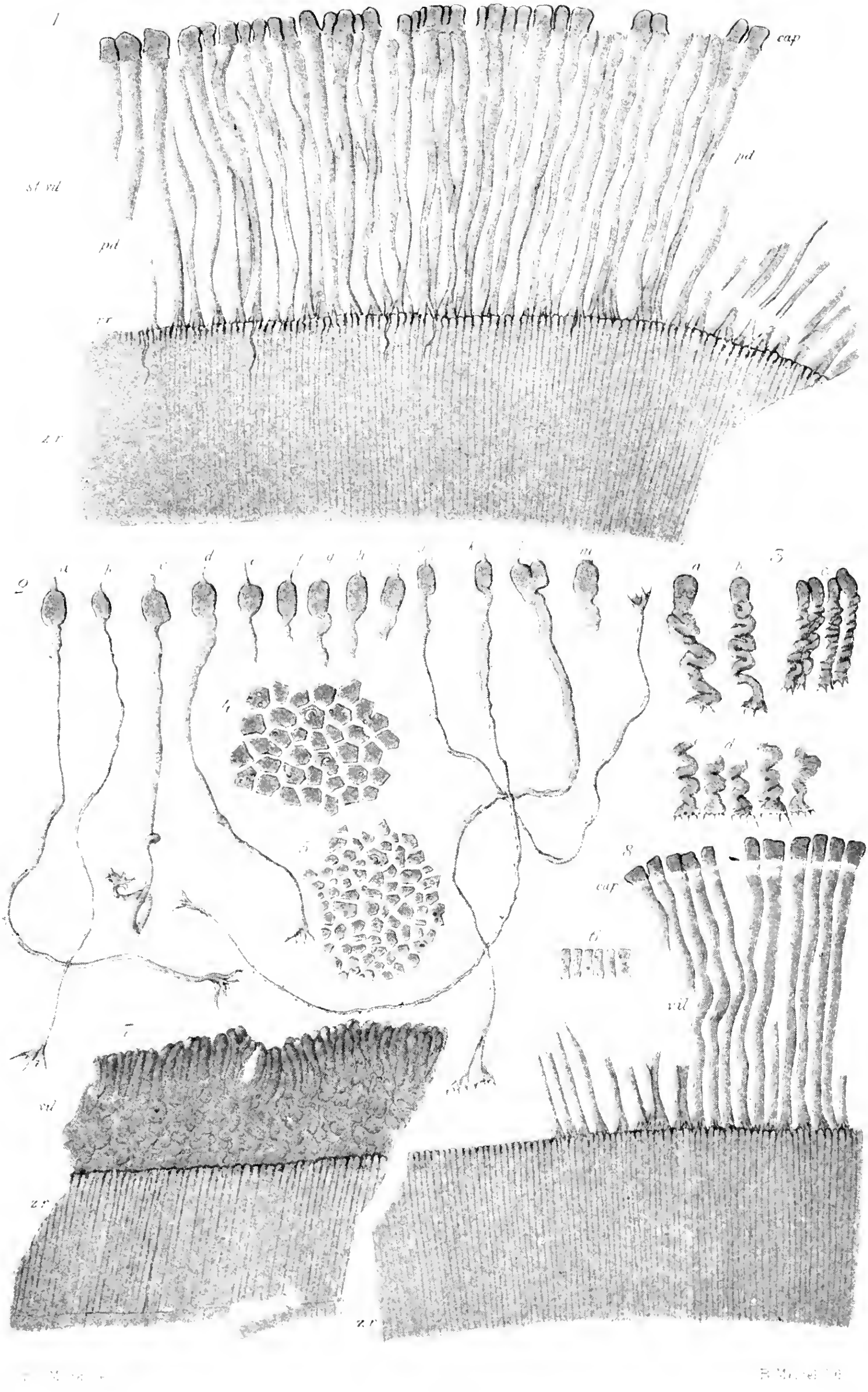
" 4–6. Sections through villi of a mature ovarian egg which was preserved in 0.25 per cent chromic acid forty-eight hours, washed in water six hours, and further hardened in grades of alcohol. Stained in alcoholic borax-carmine (Grenacher). In Figs. 4, 5, the ends of the villi are seen; in Fig. 6, the sides.

" 4 Sections of stalks from the pole opposite the micropyle.

" 5, 6. Sections of stalks from near the micropyle.

" 7. Radial section of an egg preserved in Perenyi's fluid (4½ hours) followed by alcohol and stained in alcoholic borax-carmine, showing an inner portion of the zona radiata partly detached from the outer portion. It is not a separate membrane.

" 8. Radial section through zona and villous layer of an egg preserved in 5 per cent potassic bichromate, stained in carminic acid in 80 per cent alcohol, and mounted in benzole-damar.


1
cap
st vil
pd
pd
zr
z r
2
a
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m
3
a
b
c
d
4
5
6
8
cap
vil
7
vil
z r
z r

ABBREVIATIONS.

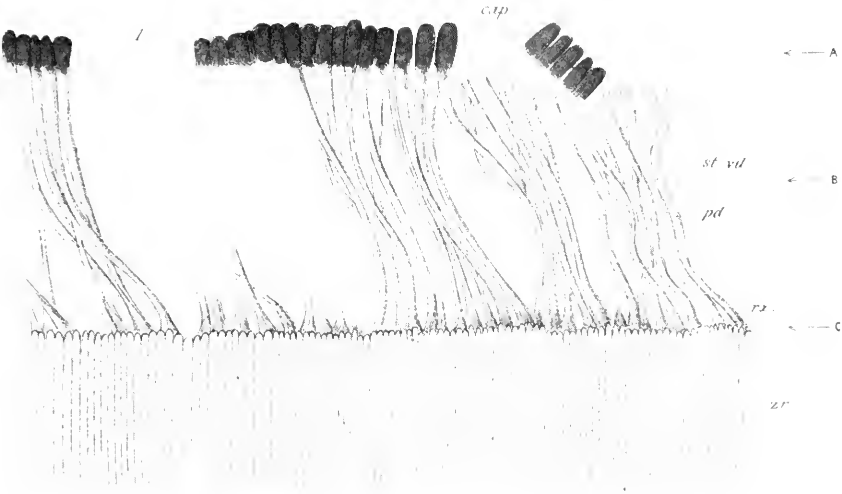
cap.	Head of villus.	*pd.*	Stalk of villus.
c.-t. cp.	Connective-tissue corpuscle.	*r.x.*	Root of villus.
fus. mat.	Maturation spindle.	*st. vil.*	Villous layer of egg membrane.
gran.	Granulosa.	*th. fol.*	Membrana propria of theca folliculi.
m py. can.	Micropylar canal.	*vac.*	Vacuole.
m py. cl.	Micropylar cell.	*vit.*	Vitellus.
nl.	Nucleus.	*vs. g.*	Germinative vesicle.
nl. gran.	Nucleus of granulosa cell.	*z. r.*	Zona radiata.

PLATE III.

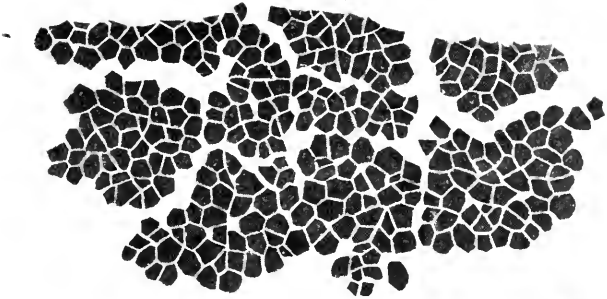
All the figures of this plate were drawn from the shell of an egg preserved in cold corrosive sublimate (4 hours) followed by alcohol, stained in Kleinenberg's hæmatoxylin, sectioned in paraffine, and mounted in benzole-damar. All except Fig. 3 magnified 515 diameters.

Fig. 1. Radial section, the heads of some of the villi broken off.
" 2. Tangential section through the heads, at A of Fig. 1.
" 3. Similar section of four heads more highly magnified to show the deeply stained peripheral portion. × 750.
" 4. Tangential section through the middle region (B of Fig. 1) of the stalk.
" 5. Section parallel to preceding through the region of the roots of the villi (C of Fig. 1). The lower portion of the figure cuts through a deeper part of the membrane (zona) than the upper portion does. The middle portion shows the branching roots of the villi as they enter the pore-canals.

1



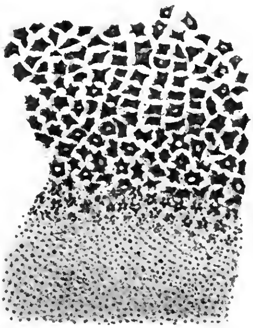
2.(A.)



3.

4 (B)

5.(C)



E.L. Mark del

B. Meisel lith

ABBREVIATIONS.

cap.	Head of villus.	*pd.*	Stalk of villus.
c.-t. cp.	Connective-tissue corpuscle.	*rx.*	Root of villus.
fus. mat.	Maturation spindle.	*st. vil.*	Villous layer of egg membrane.
gran.	Granulosa.	*th. fol.*	Membrana propria of theca folliculi.
m py. can.	Micropylar canal.	*vac.*	Vacuole.
m py. cl.	Micropylar cell.	*vit.*	Vitellus.
nl.	Nucleus.	*vs. g.*	Germinative vesicle.
nl. gran.	Nucleus of granulosa cell.	*z. r.*	Zona radiata.

PLATE IV.

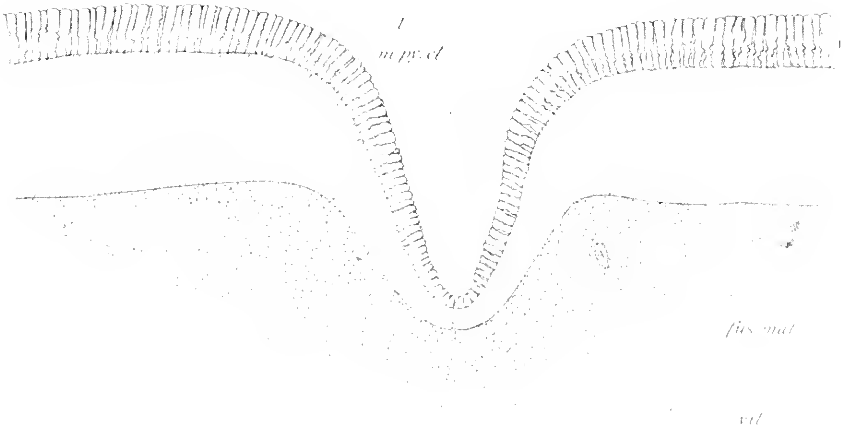
Fig. 1. Radial section through the micropyle and micropylar funnel, showing the micropylar cell and a portion of the maturation spindle of an egg "stripped" from the fish, preserved in 0.5 per cent chromic acid (5 hours) followed by washing in water, and hardened in alcohol. Stained in picrocarmine. × 515.

" 2. The second section preceding that shown in Figure 1, and passing nearly through the middle of the maturation spindle. × 515.

" 3. View of the animal pole of an egg preserved in Merkel's fluid. The germinal disk was rather more than half as broad as the diameter of the egg, and its outline should have been represented more distinctly by the lithographer; it was of a yellowish color, but much lighter than the rest of the egg. The micropylar funnel is seen exactly over the centre of the disk. × about 10.

" 4. View of the micropylar funnel and contained micropylar cell of the egg, a section of which is shown in Figure 1. × 158.

" 5. Radial section through the micropylar region and germinative vesicle of an ovarian egg preserved in alcohol. A portion of the granulosa still adheres to the outer surface of the villous layer. Stained in alcoholic borax-carmine. × 158.


1
m py cl
vit.
z.r
fus mat
vit
m py can.


2
z.r
fus mat


.3.


4


5
gran.
vit.
z.r.

ABBREVIATIONS.

cap.	Head of villus.	*pd.*	Stalk of villus.
c.-t. cp.	Connective-tissue corpuscle.	*rx.*	Root of villus.
fus. mat.	Maturation spindle.	*st. vil.*	Villous layer of egg membrane.
gran.	Granulosa.	*th. fol.*	Membrana propria of theca folliculi.
m py. can.	Micropylar canal.	*vac.*	Vacuole.
m py. cl.	Micropylar cell.	*vit.*	Vitellus.
nl.	Nucleus.	*vs. g.*	Germinative vesicle.
nl. gran.	Nucleus of granulosa cell.	*z. r.*	Zona radiata.

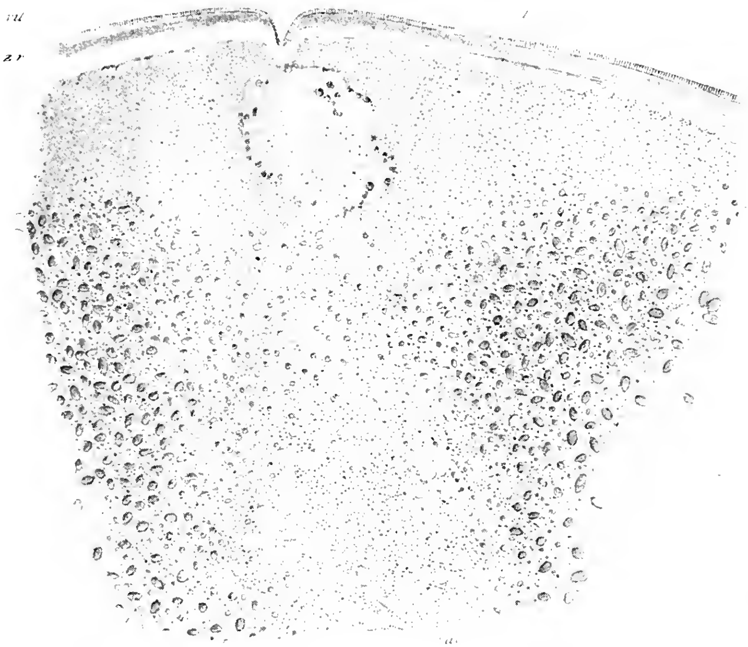
PLATE V.

Fig. 1. Radial section through germinative vesicle and micropylar funnel. Owing to a distortion of the section, the curvature of the part of the membrane shown is less than it should be. The finely granular and vacuolated portion of the yolk (*vac.*) beneath the germinative vesicle is in the centre of the egg. The egg was from an ovary which was hardened in 0.25 per cent chromic acid and stained in alcoholic borax-carmine. × 72.

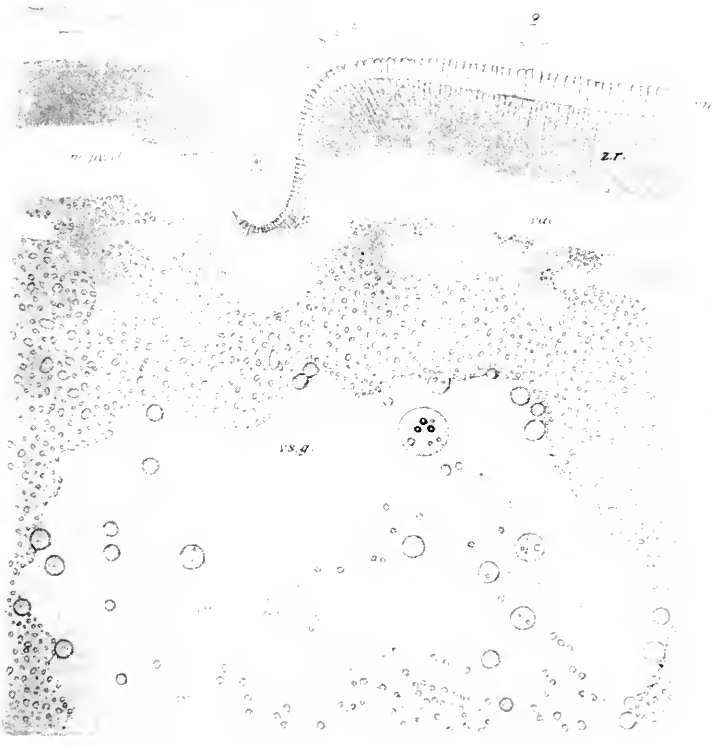
" 2. The section following that shown in Figure 1, more highly magnified. × 335.

" 3. Radial section through villi and granulosa of an ovarian egg preserved in alcohol and stained in alcoholic borax-carmine. × 515.

" 4. Surface view of a portion of the granulosa from the same egg as that of Figure 3. × 515.


vil
z.r
1


vil
gr.m
th.gl


2
vil
z.r.
vit
vs.g.

ABBREVIATIONS.

cap.	Head of villus.
c.-t. cp.	Connective-tissue corpuscle.
fus. mat.	Maturation spindle.
gran.	Granulosa.
m py. can.	Micropylar canal.
m py. cl.	Micropylar cell.
nl.	Nucleus.
nl. gran.	Nucleus of granulosa cell.
pd.	Stalk of villus.
rx.	Root of villus.
st. vil.	Villous layer of egg membrane.
th. fol.	Membrana propria of theca folliculi
vac.	Vacuole.
vit.	Vitellus.
vs. g.	Germinative vesicle.
z. r.	Zona radiata.

PLATE VI.

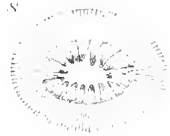
All figures on this plate are magnified 515 diameters.

Fig. 1. Radial section through the micropylar canal, somewhat oblique to its axis. The egg was preserved in 5 per cent potassic bichromate and stained in carminic acid dissolved in 80 per cent alcohol.

" 2–4. Three successive tangential sections through the bottom of the micropylar funnel and the micropylar canal of an egg stripped from the fish preserved in 90 per cent alcohol, and stained in alcoholic borax-carmine. The zona radiata is closely enveloped by the yolk.

" 5–8. Tangential sections through the deeper portions of the micropylar funnel of an ovarian egg hardened in 0.25 per cent chromic acid and stained in alcoholic borax-carmine. In Figures 5 and 6 the sections pass through the deep portion of the zona radiata which is not infolded to form the funnel, but in Figures 7 and 8 only that portion of the zona is cut which projects as a conical elevation into the substance of the yolk. Only alternate sections were drawn.


1.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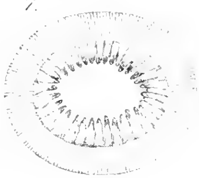
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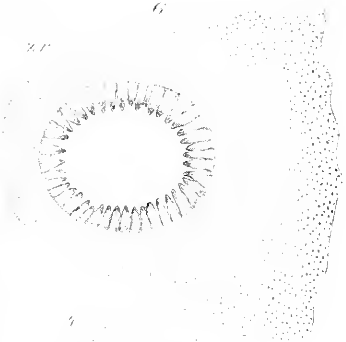
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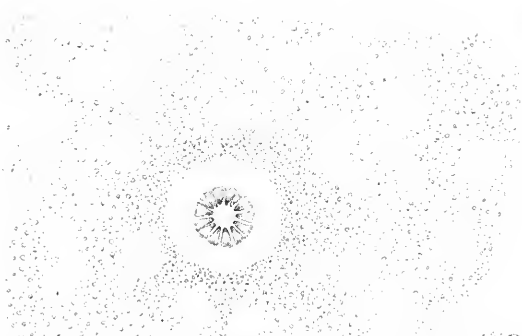
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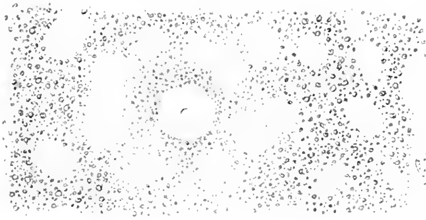
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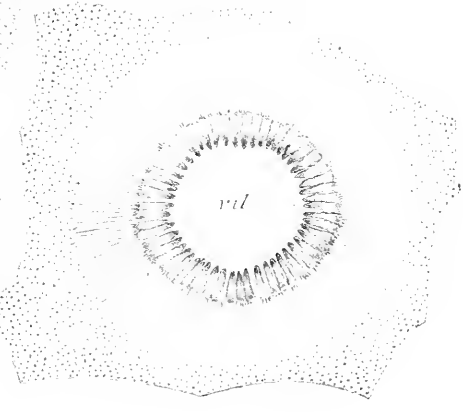

5.

ABBREVIATIONS.

cap.	Head of villus.	*pd.*	Stalk of villus.
c.-t.cp.	Connective-tissue corpuscle.	*rx.*	Root of villus.
fus. mat.	Maturation spindle.	*st. vil.*	Villous layer of egg membrane.
gran.	Granulosa.	*th.fol.*	Membrana propria of theca folliculi.
m py. can.	Micropylar canal.	*vac.*	Vacuole.
m py. cl.	Micropylar cell.	*vit.*	Vitellus.
nl.	Nucleus.	*vs. g.*	Germinative vesicle.
nl. gran.	Nucleus of granulosa cell.	*z. r.*	Zona radiata.

PLATE VII.

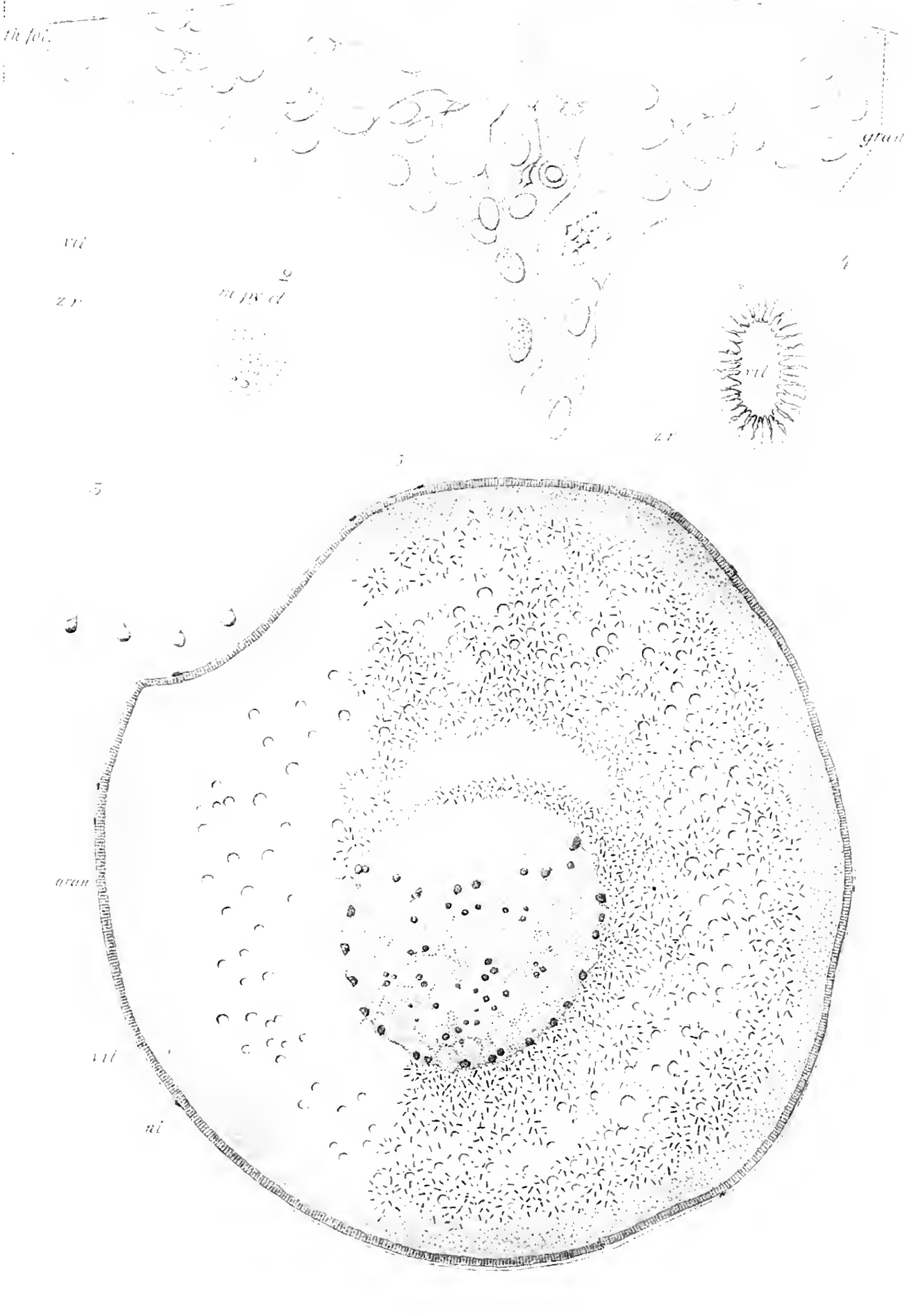
Fig. 1. Radial section through the granulosa plug which fills the micropylar funnel. From an ovarian egg preserved in 0.25 per cent chromic acid, and stained in picrocarminate of ammonia. × 515.

" 2. Micropylar cell and outlines of the egg membranes in the region of the micropylar funnel, from an ovarian egg preserved in alcohol. Radial section. × 515.

" 3. Four spermatozoa, dried on the slide. × 472.

" 4. Micropylar funnel; *optical* cross-section as seen from the yolk side of the egg membranes; showing the oval form of the funnel which is sometimes met with. × 515.

" 5. Section of an ovarian egg through the germinative vesicle. Only one membrane besides the granulosa present; it is the villous layer. Preserved in 0.25 per cent chromic acid (48 hours). Stained in alcoholic borax-carmine. × 158.


rh fol.
gran
vit
z r
2
m pr el
4
vit
z r
5
3
gran
vit
ni

ABBREVIATIONS.

cap.	Head of villus.	*pd.*	Stalk of villus.
c.-t. cp.	Connective-tissue corpuscle.	*rx.*	Root of villus.
fus. mat.	Maturation spindle.	*st. vil.*	Villous layer of egg membrane.
gran.	Granulosa.	*th. fol.*	Membrana propria of theca folliculi.
m py. can.	Micropylar canal.	*vac.*	Vacuity.
m py. cl.	Micropylar cell.	*vit.*	Vitellus.
nl.	Nucleus.	*vs. g.*	Germinative vesicle.
nl. gran.	Nucleus of granulosa cell.	*z. r.*	Zona radiata.

PLATE VIII.

Figures 1 and 2 are from sections of an ovarian egg about 0.4 mm. in diameter which was hardened in chromic acid. × 510.

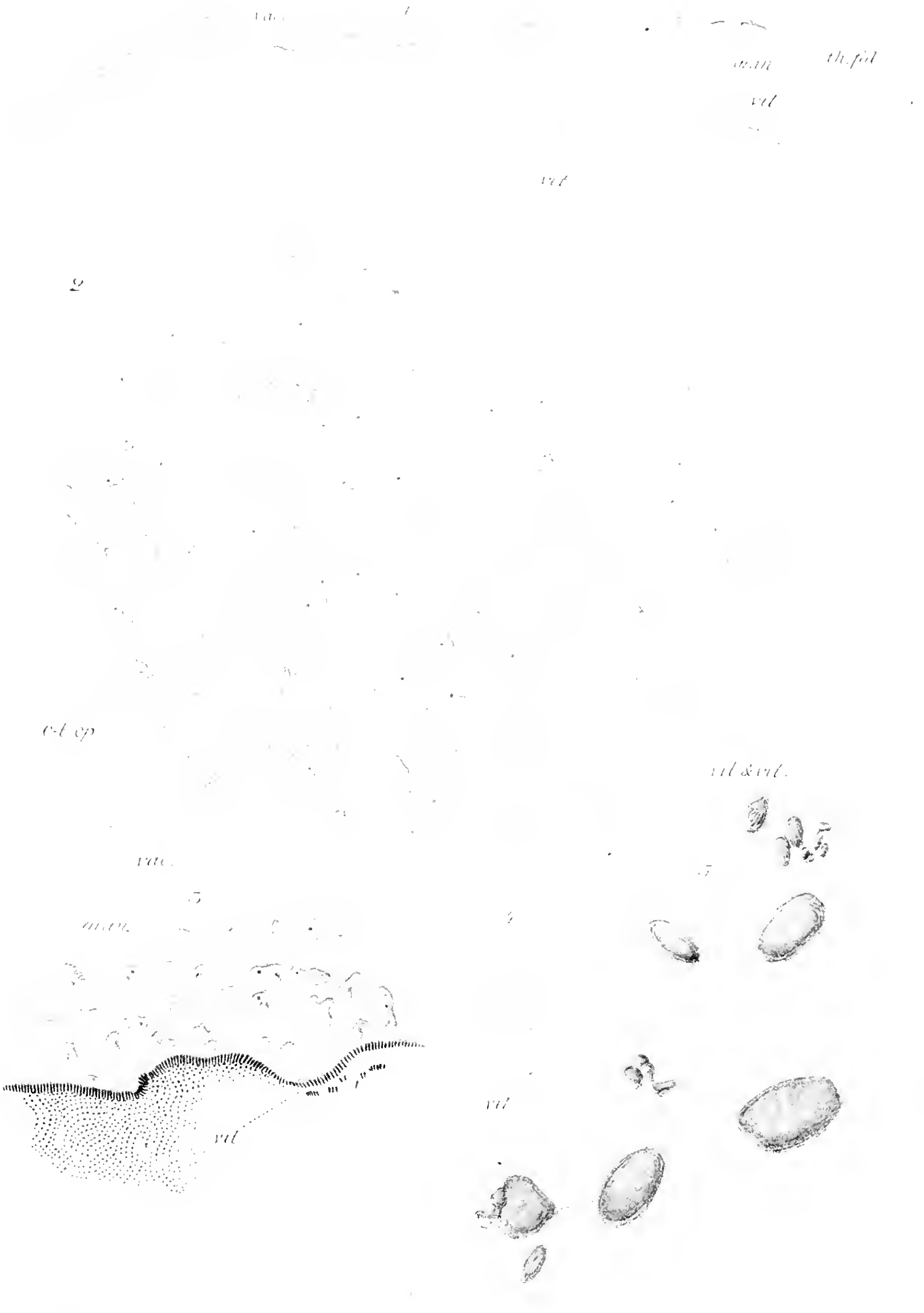
Fig. 1. Part of the peripheral portion of a radial section in which the earliest observed trace of the villous layer has made its appearance. The membrana propria of the theca and the follicular epithelium are artificially separated from the yolk and villous projections.

" 2. Tangential section from the same egg. The section embraces connective-tissue cells of the stroma, as well as follicular epithelium, and has also cut off a portion of the periphery of the yolk, with its villous projections, which last give it a dotted appearance. The nuclei of the epithelium are often lobed. *vac.* indicates vacuities evidently due to depressions in the surface of the yolk, not to vacuoles in its substance.

" 3. Portion of a section which, owing to the wrinkled condition of the surface of the egg, affords a surface view of the granulosa, as well as a radial section and surface view of the villous layer. Some of the detached villi are seen at one side. The nuclei of the granulosa cells still have irregular lobed forms. Chromic acid preparation of an egg about 0.6 mm. in diameter. × 510.

" 4. View of the villi as seen from the surface of an egg after it has lain for some time in water. × 472.

" 5. Amber-colored bodies found at the outer surface of the villous layer of the egg membrane. × 472.



E. Meusel, lith.

ABBREVIATIONS.

cap.	Head of villus.	*pd.*	Stalk of villus.
c.-t. cp.	Connective-tissue corpuscle.	*r.x.*	Root of villus.
fus. mat.	Maturation spindle.	*st. vil.*	Villous layer of egg membrane.
gran.	Granulosa.	*th. fol.*	Membrana propria of theca folliculi.
m py. can.	Micropylar canal.	*vac.*	Vacuole.
m py. cl.	Micropylar cell.	*vit.*	Vitellus.
nl.	Nucleus.	*vs. g.*	Germinative vesicle.
nl. gran.	Nucleus of granulosa cell.	*z. r.*	Zona radiata.

PLATE IX.

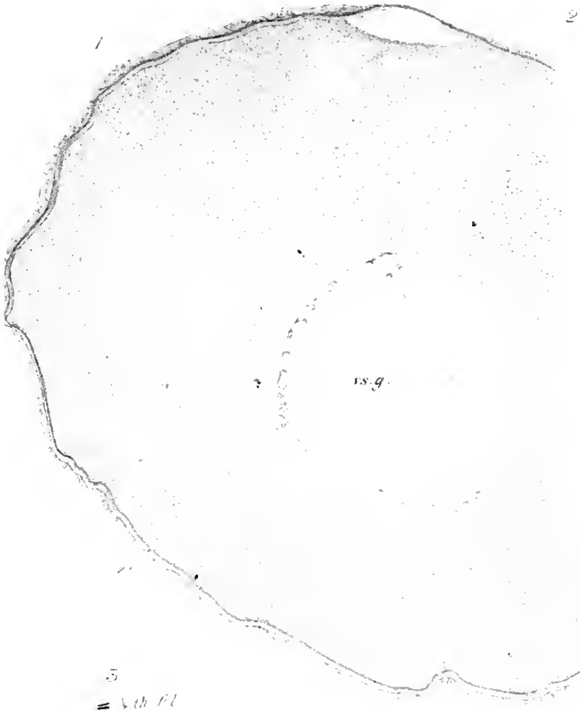
Fig. 1. Section of an ovarian egg about 0.6 mm. in diameter through the germinative vesicle. The villous layer is at all points in contact with the yolk; but it is separated from the granulosa at intervals. The egg was hardened in 0.25 per cent chromic acid and stained in alcoholic borax-carmine. × 158.

" 2. Portion of a radial section through a mature ovarian ovum, hardened in 0.25 per cent chromic acid, showing the penetration of the roots of the villi into the pore-canals of the zona radiata. × 515.

" 3, 4. Radial sections of ovarian eggs preserved in alcohol, showing stages in the formation of the villous layer. The eggs were somewhat more than 0.5 mm. in diameter, and were stained in alcoholic borax-carmine. × 510.

" 5. A portion of Figure 1 enlarged. The outlines of the granulosa cells, especially on the side toward the villi, are much too sharp. × 515.

1


vs.g.
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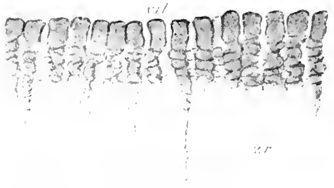
2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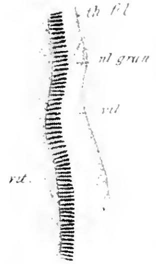
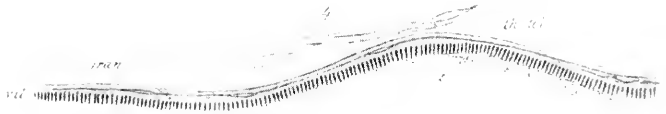

th fol
nl gran
vit
vit.

4


th fol
gran
vit

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th fol.
gran
vit
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vac.

No. 2. — *On the Egg Membranes and Micropyle of some Osseous Fishes.* By Carl H. Eigenmann.[1]

At the suggestion of Dr. E. L. Mark, I undertook the study of the development of the micropyle and egg membranes in some of the bony fishes.

The eggs of the following species were examined: Amiurus catus, Tachisurus sp. (?), Catostomus teres, Notemigonus chrysoleucus, Carassius auratus, Clupea vernalis, Alosa sapidissima, Fundulus heteroclitus, F. diaphanus, Apeltes quadracus, Pygosteus pungitius, Lepomis megalotis, Morone americana, Esox reticulatus, Anguilla anguilla rostrata, Cyclogaster[2] lineatus, Gadus morrhua, and Hippoglossoides platessoides. In many of these species the eggs were not in a condition favorable for tracing the development of the micropyle or even the membranes. My account will be confined to the eggs of Amiurus catus, Notemigonus chrysoleucus, Clupea vernalis, Fundulus heteroclitus, Pygosteus pungitius, Perca americana, Morone americana, Esox reticulatus, and Cyclogaster lineatus.

I am indebted to Dr. Mark for the use of his manuscript abstracts of the papers on egg membranes published before 1881.

It has long been known that fish ova are provided with a membrane, the zona radiata. The eggs of certain fishes have, in addition to and outside of the zona radiata, a second membrane which bears in some cases long filaments, in others short processes which serve to attach the eggs to foreign bodies.

Fundulus heteroclitus and F. diaphanus.

The fact that the eggs of some fishes are provided with long filaments was first noted by Haeckel ('55). He found them on the eggs of many species of Scomberesocidæ, but mistook their position, describing them as thin fibres lying inside the egg membrane (zona radiata). A connection of the fibres with cells could not be traced.

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[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the Direction of E. L. Mark. — No. XVI.

[2] Liparis of authors.

Kölliker ('58) corrected the mistake made by Haeckel as to the position of the filaments.

Hoffmann ('81) found filaments on the eggs of Heliasis, Gobius, and Blennius.

Ryder ('82[c]) described the filaments of the eggs of Belone longirostris, and, in passing, mentioned the probability of their existence in the eggs of Mugil albula. He afterwards ('86[a]) found them on the eggs of Fundulus heteroclitus, and has also ('83) shown that the eggs of Menidia (Chirostoma) notata are provided with four of them.

I have examined eggs of Fundulus heteroclitus at intervals of about two weeks from October, 1887, till June, 1888. The eggs undergo scarcely any change between October and April. A series taken between April 1st and June 1st shows all the stages covered by the longer period. The filaments can best be studied in fresh material. They appear in the form of hyaline threads, which are more highly refractive than any other part of the egg membrane. In an ovary of October 27 there were filament-bearing eggs in three stages of development.

In the smallest eggs — about 0.16 mm. in diameter — in which filaments can be seen (Plate I. Fig. 1) they appear as hyaline dots, or as conical bodies with rounded bases, uniformly distributed over the entire surface. They either lie wholly below the granulosa, or the tips of the longer ones may lie in between the granulosa cells (Fig. 8). In this stage the diameter of the threads is much greater than the thickness of the membrane, which can scarcely be distinguished in sections. I was not able to discover sheaths enveloping the filaments such as Haeckel describes for the Scomberesocidæ. In other slightly larger eggs belonging to this same stage of development (Figs. 2–4, 6) the filaments are no longer conical, but appear in the fresh condition as short, curved threads equally blunt at both ends.

In the second stage, the eggs being intermediate in size between those just mentioned and the largest, the threads (Fig. 5) are of about the same thickness as those of the earlier stage, but they are much longer, and taper near the free end. They do not seem to be closer together than in the smaller eggs. The filaments are bent in a more or less regular manner, first to one side and then to another. On stained sections it was to be seen that the threads usually follow the margins of the granulosa cells, and that they are correspondingly curved (Fig. 6).

On the largest eggs — about 0.4 mm. in diameter — the filaments are much longer, and cover about as much of the surface of the egg as they leave exposed. They are so long and so tortuous that it is almost impossible to follow a single filament throughout its whole length. It often happens (Fig. 10) that several filaments are parallel to each other for a considerable distance. In sections the filaments are found to lie in between the bases of the granulosa cells, and also to rise between these cells nearly to their outer surfaces.

In the ripe ovarian eggs the basal ends of the filaments pass directly through the granulosa layer, and the greater part of the filament thus comes to lie between the outer portions of the granulosa cells, or even quite outside of them (Fig. 9). The regularity of their windings cannot be seen as well as in eggs of the third size. The filaments are of varying lengths, but most of them are several times as long as the diameter of the egg. The distances between filaments are not materially altered during the growth of the egg; but since the surface of the egg increases during its development to many times the size which it had when the filaments first appeared, the total number of the latter must also be greatly increased. The earliest stages in the formation of new filaments would be difficult to find after the egg has reached its second stage, because they would be hidden by the larger filaments.

In ripe eggs forced from the ovary, the filaments extend out from the egg for some distance, and then form a network, several filaments deep, over the whole surface (Fig. 11).

Concerning the origin of the filaments it may be said that they do not have any connection with the granulosa cells at any stage of their growth (Figs. 3, 4, 6, 8). In tangential sections it is seen that they arise at places corresponding to the boundaries between two or three cells. In a ripe egg examined in the fresh state under pressure (Fig. 12) indistinct processes are seen to radiate from the base of each filament, forming a stellate figure. In no case, either in fresh specimens or sections, could the filaments be traced into the substance of the zona radiata. They are outgrowths from a thin membrane which lies outside of the zona and is formed before the latter, not processes of the zona itself.

When the filaments first make their appearance, the egg membrane, as stated above, is much thinner than the diameter of a filament, and the granulosa cells are lens-shaped, barely touching by their margins (Fig. 8). In the largest eggs found in the ovary of April 2d, the granulosa was about 8 μ thick, but the egg membrane had only reached the thickness of 2 μ. That it is radially striate is rather to be inferred than directly seen. In places the outer surface shows slight elevations at regular distances, which I believe to be prolongations of granulosa cells sunk into the radial canals (Fig. 13). While the largest eggs of April 2d were only about 0.4 mm. in diameter, and therefore scarcely exceeded in size those of October and November, the largest ovarian eggs of May 2d measured over 0.8 mm. Between May 1 and June 1, — by which time the eggs have reached their full size, — the growth is still more rapid. The egg membrane of early May eggs measures about 6.5 μ in thickness, and has distinct pore-canals.

There exists an exceedingly thin outer membrane overlying the zona radiata. It was discovered in the examination of fresh ripe eggs, in which the striation of the zona itself could be seen much better than in sections of hardened eggs. In one instance, in which the zona of a fresh ripe egg was ruptured, this overlying membrane was left intact. It is with this membrane that the bases of the filaments are continuous.

In view of this condition in Fundulus, and of the fact that other process-bearing eggs (Cyprinidæ and Gasterosteidæ) possess a thin outer membrane, it would be interesting to re-examine the eggs of the Scomberesocidæ, of Menidia, and of Mugil to find whether they do not also possess this structure.

The outer surface of the fresh ripe egg of Fundulus heteroclitus shows a network of lines (Fig. 7). This appearance is doubtless due to the presence of superficial ridges, which in radial sections have the appearance of minute projections fitting in between the bases of the granulosa cells (Fig. 9). Where two or more lines meet, there is a thickening. The whole arrangement bears a superficial resemblance to the appearance presented near the surface of the zona in the perch (Fig. 31). In the case of the latter, however, the thickenings correspond in position to filaments, each of which corresponds to the middle of a granulosa cell, whereas in Fundulus heteroclitus the thickenings correspond in position to the boundaries between granulosa cells. From the position of the filaments in Fundulus it is probable that, like the ridges, they are outgrowths of the outer structureless egg membrane. It is evident from what has been said that there is a fundamental difference between the filaments found in Perca and those in Fundulus. In Perca they owe their origin to the granulosa, and are formed after the zona has nearly reached its full growth; in Fundulus, on the contrary, they owe their origin to the activity of the egg itself, and they begin to be formed before the zona.

Pygosteus pungitius.

After Haeckel had described the long filaments peculiar to the eggs of the Gobiesocidæ, Kölliker ('58) described external appendages in the eggs of Abramis brama, Chondrosteus nasus, Squalinus argenteus, Cobitis barbatula, Gobio fluviatilis, Cyprinus rufus, and Gasterosteus pungitius. In all these species he found the appendages inserted in a very thin membrane, which ultimately lies just outside the zona radiata and which makes its appearance before the latter.

The most important paper on Pygosteus is that of Ransom ('67). He studied Gasterosteus pungitius and G. leiurus, and found that the eggs of the two species do not differ greatly. He says that in the oviduct the eggs are surrounded by a viscid layer, and that the zona radiata lies below this layer. The zona is in contact with the yolk except in ripe eggs, in which a thin homogeneous membrane covers the yolk and follows the constrictions at the time of cleavage. The micropyle and the dotted appearance of the egg membrane were first made out in eggs $\frac{1}{140}''$ thick, and in eggs $\frac{1}{200}''$ in diameter the membrane could be separated from the yolk. The button-shaped processes can be made out in eggs somewhat less than $\frac{1}{140}''$ (0.17 mm.) in diameter. They are attached to the outer surface of the yolk-sac by a bright, highly refractive point. In the case of the smallest ova there are on an average seventy buttons, in that of the largest two hundred and seven. They serve to attach the egg to foreign substances. Ransom describes and figures the micropyle.

Owsjannikow ('85) found that in ovarian eggs the granulosa cells cover the micropyle. In fully grown eggs only a single membrane is present, while in the younger ones the zona seems differentiated into two layers, owing to the fact that the zona is laid down by successive additions. The pores do not appear till the membrane has attained considerable thickness, and they are then much finer than in the ripe egg. The mushroom-shaped processes are maintained by him to be cells that possess nuclei which are colored red with carmine. From the base of the process a thread can be traced into the zona radiata. In young eggs the processes consist principally of a nucleus attached to a filament. He does not believe that they are derived from the zona, but thinks they come from the granulosa; why he thinks so is not stated. Inside the zona he has found the zonoid layer of His.

I have examined ovarian eggs of fishes taken in November, December, and April. A few days after the spawning, in early April, the ovaries contain a considerable number of eggs (about 0.55 mm. in diameter) in which the formation of the yolk is well advanced. These are evidently destined to be laid before the recurrence of the next annual spawning season, for they are much larger than any of the ovarian eggs found in December. These eggs show no signs of degeneration, and their presence can therefore hardly be explained as due to their failure to pass off with the first lot of eggs laid; nor can they be eggs which properly belong to the first set of spawn, as their size in comparison with that of the mature eggs (1.1 mm.) sufficiently proves. Therefore I believe that, as Ransom has inferred, these fishes deposit eggs more than once during the season.

The ovaries are most available for study after the first set of eggs are deposited. As in the case of Fundulus heteroclitus, all stages of growth are shown in ovaries taken during a period extending from one or two months before till a short time after spawning, — the months of March and April.

In eggs 0.15 mm. (Plate I. Fig. 14) or more in diameter there are two membranes, — an outer more highly refractive, and an inner striated one. In many sections the two are artificially separated (Fig. 15^a). In ripe eggs the outer membrane had either entirely disappeared, or its structure had become so much like that of the true zona that the two could not be distinguished from each other. Their total thickness is from 15 to 18 μ. In many sections of ripe eggs an outer layer, much thicker than the outer layer seen in the earlier stages of development, was in places separated from the rest of the zona. If it represents the outer membrane of the earlier stage, then the latter must undergo a great change in its later development, for it is now much thicker, and is traversed by the same pore-canals as the deeper portion.

The rivet-shaped processes which are found in the region of the micropyle are inserted, as Kölliker says, in the thin membrane which lies outside the zona, and which is formed before the latter makes its appearance. They take a much deeper stain than the thin membrane, but I have seen nothing which would warrant one in claiming that they contain each a nucleus. The smallest egg in which these processes could be seen had a diameter of about 0.14 mm.; only a single thin, structureless membrane was to be made out in this stage. The largest eggs examined had a diameter of about 1 mm.

When the processes make their appearance, the granulosa is so thin that it is difficult to determine from surface views whether they lie above or below it; but radial sections show that they lie below. There is no such constant relation between the processes and individual cells of the granulosa as to suggest the origin of the former from the latter; but at a later stage the heads of the rivets occupy nearly the same plane as the nuclei of the granulosa cells (Fig. 16), and therefore appear to have an intimate connection with the granulosa cells. When the granulosa is torn from the egg membranes, as, owing to the shrinkage of the egg, it frequently is, the processes no longer show the same sharp outer margins. Their edges are often frayed, and are not stained as deeply as when the granulosa and the membranes are in their normal relations to each other. With the separation of the granulosa the thin outer membrane is sometimes torn (Fig. 15^a); and whether torn or not, it is often separated from the inner membrane. This may be due to the fact that the processes are from the beginning adhesive, and have thus acquired an intimate secondary relation to the cells of the granulosa. In such sections it can be clearly seen that the rivet-shaped processes are joined to the outer membrane and not to the zona, though their bases have projected into the zona for a greater or less distance. When the granulosa is torn from the egg membranes, the processes always, even in the smallest eggs, remain attached to the membranes rather than to the granulosa. I have been able to find neither the nuclear structure within nor the prolongations from these processes which Owsjannikow has described.

I have not succeeded in finding the micropyle in eggs that were much less than 0.4 mm. in diameter; in such the zona has an average thickness of about 5 μ. The portion immediately surrounding the micropyle shows a considerable local thickening. Owing to the variation in the thickness of the zona in different regions of the same egg, and to the inconstancy of the position of the micropyle in relation to this variation, it sometimes happens that the zona at the micropylar region has already reached a thickness of 10 or 11 μ.

It is a noticeable fact, that at this earlier stage the micropyles of nearly all the eggs were cut radially when the sections were made in planes perpendicular to the axis of the ovary. Furthermore, the micropyles uniformly lie in the half of the egg opposite the side of attachment.

In the vicinity of the micropyle the zona becomes thickened by the elevation of its *outer* surface, the deeper surface undergoing no change of direction. At a distance of about 10 μ on either side of the micropylar canal it attains its greatest thickness, and then its outer contour curves inward until it becomes continuous with the wall of the micropylar canal. The inner end of the canal is sometimes slightly enlarged (Figs. 19–22).

At this stage the pore canals of the zona radiata do not seem to be modified in direction in the region of the micropyle; they are all radially arranged. The outer membrane could not be distinguished in this region; it probably is entirely wanting in the area immediately surrounding the micropyle. The granulosa cells are two or three layers deep in the vicinity of the micropyle, and a single cell larger than the others is always to be found directly above the canal. It usually sends a prolongation into the canal itself (Figs. 18, 21).

In eggs about to be laid, the greatest thickness of the zona in the vicinity of the micropyle is approximately 24 μ, and the thickening in this region is not so conspicuous as at the earlier stage. The zona bends inward slightly, so that its inner surface no longer forms a simple curve. The micropylar passage through the zona presents three regions: a shallow funnel-shaped depression, which occupies the outer third of the layer; a narrower tubular portion, which is a prolongation of the bottom of the funnel, and is rounded at its lower end; and finally a very narrow canal, which traverses the inner sixth or eighth of the zona, and opens at the apex of the low elevation of the inner surface (Fig. 18).

The outer or funnel-shaped portion is wholly filled even at this advanced stage by the single large micropylar cell which was seen at the earlier stage (Figs. 18, 21).

Perca.

.ne egg of the perch has been a favorite subject for study. Almost every writer on teleostean ova has examined it. Von Baer ('35, pp. 6, 7) first described it as having a double membrane, the outer portion being traversed by long narrow dark spots ("dunklern Flecken").

Müller ('54) gives a fuller account. He separates the membrane into an inner, the zona radiata, and an outer, the capsule. The outer surface of the zona is described as being covered with exceedingly small cylindrical projections. These are doubtless nothing but the elevations between the pore-canals, which are rather wide on the outer half of the zona. The capsule is radially traversed by small spiral tubes, which are enlarged and funnel-shaped at both ends. Transverse filaments are sometimes seen between these radial tubes. On applying pressure, yolk granules were forced into the spiral tubes, but in no case was any yolk matter forced between the tubules; from which he concludes that the capsule must be closed between them.

Kölliker ('58) discusses the origin of the "tubules." He considers them to be outgrowths from the follicular cells, and the substance between them as a secretion from those cells. He denies the statement made by Müller, that they are hollow, but has seen the anastomosing filaments described by him. The tubules are independent of their jelly matrix, and in chromic acid preparations they can be separated from the latter. When the eggs are deposited, the granulosa cells probably fall off, leaving shallow depressions having polygonal outlines, from the centres of which "tubules" arose.

Ransom ('68) described the canals passing through the outer portion as having a double contour for each wall, and as filled with material containing vacuoles; but they do not seem to him to convey anything either fluid or solid into or out of the egg. This outer layer is separable only by tearing it from the yolk-sac (zona), and does not leave a distinct outline. The tubes divide at their inner terminations into branch-like roots, and adhere closely to the zona radiata. The internal ends are not expanded as Müller described, and it is rarely that filaments pass from one to the other. He supposes that the granules seen by Müller were only vacuoles. The eggs when deposited are arranged in the form of hollow tubes with the micropyles all turned to the inside.

His ('73) mentions having seen the micropyle, but neither figures nor describes it.

Brock ('78) describes the zonoid layer, and finds its striations intermediate in fineness between those of the villous layer and those of the zona. Judging by his drawing of Alburnus lucidus there are about three striations in the zonoid layer to four in the zona. The latter, he says, makes its appearance before the villous layer.

Hoffmann ('81) finds that in October the zona and the villous layer are of equal thickness. The latter is said to be composed of numerous small projections which correspond exactly to the villi of the Cyprinoids. At the free ends of the villi lie the granulosa cells. In February the zona is differentiated into two layers, of which the inner is four times as thick as the outer. There arise from the outer layer long fibres with triangular bases and with their distal ends expanded to form a continuous layer on which the granulosa cells rest. Each filament corresponds to, but is not a process of, a granulosa cell.

Owsjannikow ('85) recognizes the usual divisions of the egg membrane. The contents of the distal ends of the filaments are granular, which has given rise to the belief that they are nuclei. The filaments end externally in funnel-shaped enlargements described by Müller. He succeeded in forcing granular matter from the yolk into their deep ends. The latter divide and enter the pores of the zona, through the whole thickness of which they can be traced. He states (p. 7) that, contrary to Hoffmann's belief, the filaments are derived from the granulosa. In a subsequent part of his paper (pp. 29–31), where he gives an account of the development of the ovarian egg, his statements seem to be conflicting as to the relation of the spiral canals to the granulosa cells, but at the end he repeats that the canals are outgrowths of cells as stated by Kölliker. The interstitial matter (Zwischensubstanz) is arranged in lamellæ which are parallel to the surface of the egg. By the swelling of the lamellæ fissures arise which have the appearance of processes from the canals.

I have studied the ovarian eggs of Perca killed in October, February, and May. It is probable that the formation of the egg membranes is less advanced in the American species of this latitude than in the European species at a corresponding season.

Contrary to Hoffmann's statement that in October the capsular layer and zona are of equal thickness, not a trace of the capsular layer, distinct from the granulosa, could be found at this time of the year. The zona is well developed, and is differentiated into two layers of about equal thickness. The outer layer is radially striate, while the inner appears to be structureless. The granulosa cells lie immediately in contact with the zona radiata (Fig. 23, Plate III.). I have not been able to find the micropyle in October eggs.

In February the zona remains practically as it was in October, but vacuoles — which may be caused by the method of treatment — are to be seen in the inner portion (Fig. 25, Plate III.). They are much flattened radially, and thus suggest an approach to a stratified condition of this portion of the zona. The radial striations of the outer half of the zona are more strongly marked than at the earlier stage, and much fainter striations may also be seen traversing the inner half. The latter, though less distinct, are just as numerous as, and continuous with, those of the outer half. At this date the capsular layer is already well developed, but it has attained only half the thickness which it has in May.

Up to the month of May the thickness of the zona radiata has not changed, but the pore-canals can now be more readily traced passing entirely through it. They still remain much more evident in the outer than in the inner half of the zona. This is due to the greater calibre of the canals, not to their being farther apart in the outer half.

The different descriptions of the capsular layer are in part due to the fact that it presents different conditions according to varying circumstances. The radially arranged spiral structures traversing this layer arise as funnel-shaped tubules, one beneath each cell of the granulosa.

In the early stages of their development the tubules have a more or less spiral course, while in the later stages they become more nearly straight. In February eggs (Fig. 25, Plate III.) their inner ends are slightly expanded, and terminate in a thin structureless film overlying the zona. In radial sections of eggs taken in May, they often appear triangular at the base, and their contents divide into branches which enter the pores of the zona. The "filaments" connecting the canals are sometimes much more abundant than at others. In the vicinity of the micropyle one finds on tangential sections (Fig. 31, Plate II.) that the tubules at or near their bases are joined to each other by what appear like slender filaments, but these may be the cut edges of nearly perpendicular membranes. This results in the production of an irregular network with meshes of variable size and shape, at the angles of which the spiral tubules are located.

The micropyle was seen in eggs taken in February and in May. Immediately surrounding it, the zona radiata is thickened by a slight elevation of its internal surface (Fig. 24, Plate III.). The micropyle consists of a funnel-shaped opening in the zona with the wide end directed outward. In some cases the inner end of the canal also flares slightly. In a February egg in which the micropylar region was somewhat distorted (Fig. 26, Plate II.) the micropyle seems to have been composed of two regions, separated from each other by a distinct shoulder, the inner end of the outer portion being much wider than the outer end of the inner portion. The granulosa cells and their tubules are greatly crowded above this region (Fig. 24, Plate III.). At some distance on either side of the micropyle it is to be seen that the outer funnel-shaped ends of the canals begin to be more elongated than in other parts of the egg, and continue to increase in length up to the micropyle. The nuclei of the granulosa cells, which are situated near the bottom of the funnel-shaped expansions, also become more and more elongated as one approaches the centre of the micropylar region, and at the same time they come closer to the zona radiata. The effect of this is to produce in radial sections through the micropyle the appearance of an immense funnel-shaped depression in the whole capsular layer (Fig. 24). But the appearance is misleading; there is no such broad depression; the granulosa cells of this region extend outward beyond their nuclei until they reach the theca folliculi at the same level that the neighboring cells do. The thickness of the capsular layer is therefore not changed in the vicinity of the micropyle, and the theca folliculi does not bend inward, but stretches over this region with a uniform curvature. The granulosa cells stain more deeply than the inter-tubular substance of the capsular layer. This peculiarity is very serviceable when one is searching for the micropyle. Notwithstanding the absence of a broad depression, there is a narrow irregular canal left in the centre between the modified granulosa cells, which can best be seen upon sections tangential to this part of the egg. (Figs. 27–32, Plate II. Compare Explanation of Figures.) The appearance is similar to what one might imagine would result if the central cell of this region had dropped out of its original place. That such a cell has not wholly disappeared, but has simply lost its peripheral connection with the wall of the theca, is rendered probable by the presence of a peculiar cell at the bottom of this canal. Directly over the micropyle, in contact with the zona and filling more or less completely its micropylar depression, lies a single cell of large size. Its nucleus is more nearly spherical than the nuclei of the other cells, and it is not stained as deeply as they are. (Fig. 24, Plate III.; and Figs. 26, 31, Plate II.) There can be no doubt that it is a peculiarly modified granulosa cell.

Morone americana.

The egg membrane of the white perch has never been described, but Ryder ('82) has described the micropyle.

There is only a single membrane, the zona radiata, but it is composed of two distinct layers, both of which are traversed by pore canals. The eggs examined were taken from fishes caught in February, April, and May. In February the ovary contained eggs in four stages of development; in the older stages there are well developed membranes. Eggs of 0.16 mm. in diameter have a single homogeneous membrane 1.2 μ thick. When they have reached a diameter of 0.28 mm. the zona is composed of two layers (Fig. 33, Plate II.), a very thin inner and a thicker outer one; together they measure 39 μ in thickness. By the time the eggs have reached a diameter of 0.40 mm. (Fig. 34, Plate II.) the total thickness of the membrane is more than doubled; that of the outer layer is 49 μ and that of the inner 39 μ. The outer layer is formed first and takes a deeper stain. It does not increase much in thickness after the appearance of the inner layer, and in the older eggs it contains vacuoles. The inner is at first apparently homogeneous, but with its great increase in thickness there appear in it the radial striations characteristic of the zona. The granulosa cells are small and low, and have flattened nuclei situated in the middle of the cell.

Esox reticulatus.

The egg membrane of Esox was first described by Aubert ('53). He says the shell of the egg is a thin, transparent punctate membrane, which closely envelops the yolk and in sections exhibits radially placed streaks. After lying in water some time, an outer very thin granular membrane makes its appearance.

Lereboullet ('54) describes two membranes, the outer of which is pierced by microscopic tubes. The inner is a simple extremely thin and amorphous envelope, which has no homologue in the perch.

Reichert ('56, p. 94) states that the membrane discovered by Aubert surrounding the zona radiata is to be found on all eggs of this species, but that it is in the fresh condition entirely homogeneous.

Kölliker ('58, pp. 84 and 85) maintains the existence of a thin outer, resistant layer in all fish eggs, and was able to isolate it in fresh eggs of Esox.

Ransom ('68) says that in Esox the egg membrane is similar to that of Gasterosteus; he also, as I think erroneously, supposes the thin outer membrane to be homologous with the "Eikapsel" of the perch. He figures the micropyle.

Finally, His ('73) described for the zona radiata concentric as well as radial striæ.

The eggs examined by me were taken from the ovary in February. Leaving out of consideration the smallest eggs, 0.063 mm. and less in diameter, which have no membrane except the granulosa, the ovary contained eggs in three stages of development, respectively about 0.50, 1.00, and 1.50 mm. in diameter. In eggs of the first stage the zona radiata is about 3 μ thick and very faintly striate. There is no evidence of its being differentiated into concentric layers. At the micropyle (Fig. 35, Plate III.) it reaches a thickness of 7 μ. Very generally the yolk is more or less retracted from the zona by the action of the hardening reagents, so that a narrow space, which varies a good deal in thickness over different parts of the egg, is left between the two structures. Spanning this interval are numerous fine threads, which have the appearance of being prolongations of the substance of the yolk continued into pore-canals of the zona. This is a condition which remains at subsequent stages, and will therefore be discussed further on. The granulosa cells are still thin, and their nuclei much flattened.

In the second stage (Fig. 36, Plate III.) the zona has a total thickness of 11 or 12 μ, and is distinctly differentiated into two layers, the outer of which is only about one fifth as thick as the inner. The latter is faintly stained, and distinctly striate radially ; the outer is deeply stained, and striations are usually not to be seen in it, but on favorable sections, especially such as are very thin, the striations may frequently be made out to pass continuously through the whole thickness of both layers. Upon this point there is not the least doubt, so that it is certain the outer layer in question is truly a part of the zona, and I have been unable to find in ovarian eggs any membrane intervening between this and the granulosa cells. In sections of the micropylar region, the inner portion of the zona radiata exhibits vacuoles elongated in the direction of the pore-canals. In this region the latter are not strictly radial, but converge towards the outer end of the micropylar canal. Inside the zona there is a region to be seen which bears some resemblance to a membrane with coarser (more distant) striations than those of the zona. It varies in thickness on different parts of the egg, and corresponds, I believe, to the sub-zonal space seen in the eggs of the first stage ; but it may represent the zonoid layer of His.

The membranes of eggs of the third or oldest stage (Fig. 37) differ somewhat from the conditions just described. The vacuoles of the zona radiata, found in the second stage near the micropyle only, are here found over all portions of the egg ; they are always most numerous near the inner surface, and are not found at all in the outer fifth of the membrane. They are more or less regularly arranged in series parallel with the surface of the zona. Kölliker ('58, p. 84) attributed the presence of such vacuoles in the pore-canals to the effect of fresh water on the zona.

The granulosa cells in the second and third stages have nearly spherical nuclei, which lie at their distal ends (Fig. 37, Plate III.). Below the nuclei, tapering columns of granular protoplasm extend to the zona radiata. These columns are separated by less deeply stained tracts of substance, but the boundaries of the columns are not sharply marked. The appearance is as though the columnar cells were being gradually metamorphosed into an intercellular substance. This condition is evidently an approach to that found in Perca.

The micropyle was found in eggs of both the first and second stages. In the first stage (Fig. 35, Plate III.) the zona is twice as thick around the micropyle as in other regions. This thickening results in a considerable elevation of the inner surface of the zona, the outer surface being only very slightly changed. The micropyle is a wide canal, the outer third of which tapers rapidly and is continuous with the inner two thirds, which taper only slightly from without inward. The micropylar canal is partially filled with a plug of substance which appears to be continuous with the yolk. The granulosa cells overlying the micropyle do not appear different in size from those which envelop the rest of the egg, but a single cell is sometimes seen to overlie the micropyle in addition to the regular layer of granulosa cells. In the second stage (Fig. 36, Plate III.) the micropylar canal is narrower than in the first; it no longer tapers gradually from the outside inward, but is slightly narrowed at two points, one near the outside and one at its deep end. By the retraction of the yolk from immediate contact with the zona near the micropylar canal in the case of one of the eggs, a space was formed through which could be traced a cord of substance continuous with that which occupied the canal itself. The portion of the substance which traversed this space was funnel-shaped, with the wide end next the yolk. The thickness of the zona does not now differ so greatly in different regions as at the first stage. At some distance from the micropyle in the egg last mentioned (Fig. 36), the inner surface of the zona was raised rather abruptly; nearer the micropyle it was slightly depressed, but the margin of the canal was raised in the form of a low cone, which thus occupied the centre of a very shallow inverted crater, the rim of which was formed by the outer circular elevation. Above the micropyle in the granulosa was a large spheroidal space nearly filled with a granular mass somewhat denser than the yolk. The mass was slightly contracted, leaving a narrow space at its periphery. I am in doubt whether to regard it as a cell or not, since no nucleus could be detected. On both sides of this granular mass there were several highly refractive homogeneous bodies (Fig. 36, *x x*). It is however doubtful if they have any significance in relation to the micropyle. The granulosa cells at this stage are tall and have elongated nuclei, which are broad at the exterior end, and taper towards the egg membranes.

Notemigonus chrysoleucus.

The ovary of this species contained ova in four stages of development on May 9th. In all but the smallest eggs the zona radiata was present. The largest had a diameter of 0.6 mm., and the zona varied gradually from a thickness of 2 μ on one side of the egg to that of 4 μ on the opposite side. The pore-canals are very fine, being almost invisible in balsam preparations.

The micropyle was observed in only a single case; it was found in the middle of the thickest portion of the membrane, which is exactly in the middle of the attached side of the egg. The direction of the inner surface of the zona was not altered in the vicinity of the micropyle (Fig. 38, Plate II.), but its outer surface exhibited a broad circular depression, by which the thickness of the zona was diminished about one half. The micropyle proper at the centre of the depression appeared as a narrow canal of uniform calibre. Between the zona and the yolk there was a narrow space, probably formed by the contraction of the yolk; beneath the micropylar region, this space was abruptly enlarged into a hemispherical depression. Across this space the radial strands of protoplasm characteristic of almost all the spaces between the zona and the yolk were plainly visible.

The granulosa, which over all other parts of the egg is composed, as usual, of a single layer of cells, is thickened in the region of the micropyle. As the direction of the long axes of their oval nuclei show, the cells near the margin of the micropylar depression in the zona have their peripheral ends inclined toward the axis of the micropyle. The cells which fill up the depression have larger and more elongated nuclei, and the obliquity of the latter has become so great that the depression appears to be filled with a granulosa layer two or three cells deep. It would seem that in this case the single micropylar cell found in other eggs was represented by a number of enlarged granulosa cells.

Clupea vernalis.

The chief interest in the egg membranes of this species centres in the presence of a thin, highly refractive, structureless membrane overlying the zona radiata of eggs in an advanced stage of development (Fig. 39, Plate III.). This outer membrane is intimately connected with the granulosa cells, so that it usually retains its connection with the granulosa when the latter is artificially separated from the zona. In all such cases slender striations extend from it to the zona radiata. The appearance of these markings is such as to show clearly that they are prolongations of the substance of the outer membrane, and there can be little doubt that the projections penetrate the pore canals of the zona radiata, from which they are partially withdrawn by the artificial separation of the two membranes. This structural condition suggests an explanation for similar appearances *below* the zona radiata in Esox and other fishes, and between the zona radiata and an *inner* layer in Amiurus (Fig. 45) and Ictalurus. It will be more fully discussed later.

Cyclogaster lineatus (*Liparis lineatus* Auct.).

The ovaries of this species contain ripe eggs in May, the time at which I examined them. The largest eggs were about 0.63 mm. in diameter, the membrane averaged about 0.043 mm. in thickness. The zona radiata seems to be filled with small spaces connected by the much finer radial canals (Fig. 40, Plate III.), the spaces causing the latter to appear moniliform. Near the inner and outer margins of the zona the canals are simple tubes, as in most other fishes.

The eggs next in size are 0.25 to 0.30 mm. in diameter; their zona is always only half as thick on one side as it is on the opposite, the change in thickness being nowhere abrupt. In eggs of this stage the zona is traversed by simple pore-canals, which are indistinct near its outer surface. In some cases (Fig. 41, Plate III.) the transition from the inner to the outer portion is so abrupt that the zona appears to be composed of two layers of unequal thickness, — an outer, thinner, more nearly homogeneous and unstained, and an inner which is thicker, more distinctly striate, and usually faintly stained.

The micropyle (Figs. 42–44, Plate III.) was observed only in eggs about 0.16 mm. in diameter. As in the case of Pygosteus it seems to lie in a plane perpendicular to the long axis of the ovary. The micropyle is a long narrow tube, with parallel sides, in a local thickening of the zona. This increase in the thickness of the zona affects the outline of the inner surface more than that of the outer, and is entirely independent of the above mentioned gradual change in thickness between opposite poles of the egg. It is produced principally by additions to the inner surface of the zona. The outer surface is slightly elevated at a little distance from the micropyle, but is abruptly depressed immediately over it. The regularity in the arrangement of the nuclei of the granulosa cells is disturbed in the immediate vicinity of the micropyle, where the whole layer is slightly thickened. Usually an enlarged single nucleus lies immediately above the micropylar canal (Fig. 44).

On the Number of Egg Membranes.

The views held concerning the number of egg membranes in teleosts have been many and various. Authors have generally been agreed about the presence of a membrane perforate with radial canals, the zona radiata; but doubts have been raised by Ryder whether this membrane is always present. He ('82^c) found no striations in the egg membrane of

Belone longirostris or ('84, p. 457) the cod, and states ('85, p. 145) that the eggs of Gambusia patruelis do not possess any membrane. I have found striations in the membrane of the fresh cod egg. It may be stated here that the striations of the zona sometimes show plainest in fresh eggs, sometimes not until reagents have been applied. Haeckel says, for forms related to Belone longirostris, that the membrane is structureless, but that it is covered with minute black dots. These dots were doubtless pore-canals seen from the surface. The zona radiata of Osmerus eperlanus was found by Buchholz ('63) to consist of an inner and outer portion, joined together in the micropylar region only. On deposition of the eggs the outer membrane is turned wrong side out, and serves to attach the eggs to foreign substances. These conditions have been redescribed by Cunningham ('86). Hoffmann ('81) found that the zona is differentiated into two layers in all adhesive eggs, the outer portion being ultimately transformed into a viscid mass.

Ryder ('86) describes a peculiar arrangement of the egg membranes of Ictalurus albidus. He says: "The egg-membrane is double, that is, there is a thin inner membrane representing the zona radiata, external to the latter and supported on columnar processes of itself, which rest upon the inner membrane; there is a second one composed entirely of a highly elastic adhesive substance. The columns supporting the outer elastic layer rest on the zona, and cause the outer layer to separate very distinctly from the inner one." I have found similar conditions in Amiurus catus (Plate II. Fig. 45), but am inclined to think that the two membranes represent the outer and inner portions of the zona radiata; for the outer shows the striations peculiar to the zona, and the columnar layer is of varying thickness. The inner membrane, being closely associated with the yolk, would cling to it when the yolk contracts; the protoplasm in the pore-canals being partially withdrawn would give rise to these columnar processes. Where the two membranes were separated for a considerable distance, the columnar structure was destroyed. Similar conditions obtain in the eggs of Clupea vernalis, but in this case the columnar structures lie between the zona radiata and a thin *outer* homogeneous layer which is in contact with the granulosa. There cannot be the least doubt concerning the meaning of the columnar layer in Clupea vernalis, for the two membranes lie directly in contact in some parts of the egg. The peculiar structures in Ictalurus and Amiurus doubtless have an origin and meaning similar to that of Clupea.

The eggs of all the species of fishes examined by me possess a perforate zona radiata. The radial striæ could never be made out on the first appearance of the egg membrane. The absence of striæ in these younger eggs may be accounted for by assuming, as Reichert has suggested, that the zona radiata is a later growth, and that the imperforate membrane of younger eggs is a different structure, or that during the earlier stages the material composing the membrane is less dense, allowing the food material to have ready access to the yolk. Granting for the moment that the zona grows by apposition of layers from within, the latter view is the more probable, because in the perch the inner portion of the zona is not perforate even after the outer is distinctly so, and in most cases the pore-canals are much more distinct and wider in the outer than in the inner portion of the zona. The meaning of the pore-canals, in the intra-ovarian egg at least, needs little discussion. In most of the sections prepared, where the granulosa cells are slightly raised from the zona radiata, processes of the granulosa cells can be seen to enter the pore-canals.

Various membranes have been described for different fishes as overlying the zona radiata. The peculiar capsular layer of the perch has been seen by all authors who have examined the eggs of this fish. It was first described by Von Baer ('35).

Rusconi ('36) describes a thin membrane overlying the ovarian eggs of Cyprinus. Aubert ('53) saw an outer membrane on eggs of Esox which had lain in water some time. Kölliker ('58) succeeded in isolating this membrane in the case of Esox.

Reichert ('56) discovered that whenever processes are present, as in many cyprinoids, they are set in a thin outer membrane. Kölliker ('58) confirmed this statement, and added that this outer membrane is developed before the zona radiata. Reichert also found that the membrane of the smallest membrane-bearing ovarian eggs is not striate, and concluded that the zona radiata must be a secondary formation.

Vogt ('42) was the first to describe a membrane within the zona radiata. He found that in the eggs of Coregonus palea and Salmo umbla this membrane cannot be readily seen until after the eggs have been in water for some time, and that it passes (p. 29) gradually into the germ. Ransom ('56) found a similar structure in eggs of Gasterosteus pungitius, in which this inner membrane takes part in cleavage. Eimer ('72^{a}) claims to have isolated this vitelline membrane, which he saw in trout, pike, white-fish, and perch. Oellacher ('72) also succeeded in separating it in the brook trout. I believe that the structures described by Vogt, Ransom, Eimer, and Oellacher are, as others have maintained, not to be considered as vitelline membranes, but as the superficial part of the protoplasm of the egg.

His ('73) found that the cortical layer of the yolk in many ovarian eggs is more finely granular than the rest of the yolk, and that it is radially striate. This outer portion of the yolk he called the *zonoid layer.* Many others have seen similar structures. According to the accounts of some authors the zonoid layer is found only in eggs which are not mature, and even then it is not always present.

The condition of the egg membranes in Amiurus and in certain stages of Esox has suggested the idea that similar appearances may in some cases have given rise to a belief in the existence of a zonoid layer when there really was none. A partial withdrawal of the egg protoplasm occupying the pore-canals produces an appearance which at first sight suggests the presence of a striate membrane internal to the zona; in fact, I at first supposed it to be a distinct membrane, and was the more easily misled because in some cases it seems to be of nearly uniform thickness. However, more careful study showed that it was not a membrane, and that the appearance was due to fine threads of highly refractive substance stretching across a space between the inner surface of the zona and the yolk. There are two things especially which make it impossible for me to believe that this is a normal condition: the great variability in the thickness of the supposed membrane in different parts of the same egg, and the fact that the radial striations are due to a substance which is more highly refractive than the substance, if any, filling the intervening spaces. If, on applying reagents, there is great contraction of the yolk, either it is torn from the protoplasm in the pore-canals, or the protoplasm contained in the pore-canals is suddenly withdrawn from them and distorted; in either case, there would be no appearance of a zonoid layer. If, however, the protoplasm should not be withdrawn from *all* the pores, but should in the case of many remain stretched across the space between the zona and the yolk, as might no doubt frequently happen, we should find the supposed zonoid layer more coarsely striate than the zona, a condition described by recent authors. Such an origin of the zonoid layer might also explain its absence in ripe eggs. After the egg has attained its full size, the connection of the yolk substance with the canals would naturally be less intimate than at an earlier stage, and then a contraction of the yolk would not be accompanied by the stretching of any filaments across the space thus produced.

Scharff ('87 and '87^{a}) has recently described, within the zona radiata in young eggs of Trigla, a zonoid layer, which subsequently disappears.

The eggs examined by me may be divided as follows : —

I. Eggs with a single membrane, the zona radiata.
  a. Zona radiata a single layer of uniform structure. Notemigonus chrysoleucus, Carassius auratus.
  aa. Zona radiata differentiated into an inner and outer layer. Morone americana, Esox reticulatus, Cyclogaster lineatus, Amiurus catus.

II. Eggs with a zona radiata and a thin homogeneous outer layer.
  b. Outer membrane without appendages. Clupea vernalis.
  bb. Outer membrane bearing filiform appendages. Fundulus heteroclitus, F. diaphanus.
  bbb. Outer membrane with short appendages. Pygosteus pungitius.

III. Eggs with a zona and a thick outer layer produced by a secretion from and metamorphosis of the granulosa cells. Perca americana.

Origin of the Egg Membranes.

Concerning the origin of the different egg membranes of fishes several views have been held.

Vogt ('42) and Vogt and Pappenheim ('59) maintained that the zona radiata is formed by the compression of a layer of cells surrounding the egg; Reichert ('56), Kölliker ('58), Gegenbaur ('61), and Eimer ('72^a), that it is derived from the yolk; Thomson ('59) and Waldeyer ('70), that it is derived from the follicular epithelium; Ransom ('67) argued that it cannot grow by apposition of layers from within or without, and that it must grow by interstitial deposition of material. Whether this material comes from ingoing or outgoing currents, he was unable to determine.

I think that the zona is undoubtedly derived from the yolk. Kölliker found that in all the filament-bearing eggs studied by him the zona radiata was formed after the filament-bearing membrane. I have found the same to be true in Fundulus. In the case of Morone the outer layer of the zona does not become much thicker after the inner layer has begun to be formed, whereas the latter continues to grow rapidly. In the case of Cyclogaster lineatus, where the outer layer of the zona shows columnar structures, these do not bear any definite numerical relation to the overlying cells of the granulosa. The outer portion of the zona is almost always more uniform in its structure, and stains deeper, than the inner portion.

Reichert ('56) and Kölliker ('58) are inclined to believe that the capsular layer of the perch is derived from the granulosa cells, an opinion with which Hoffmann ('81) does not agree. It certainly does not make its appearance till after the zona is well developed; if it were derived from the yolk, its substance would first have to traverse the zona radiata. How the nourishment for the egg could pass into the latter through the pore-canals, and the formative substance of the villous layer at the same time pass out through them, is scarcely conceivable. Moreover, at the distal end of each of the villi lies the nucleus of a granulosa cell, there being as many villi as there are cells, a fact which proves beyond a doubt the intimate relation of the two structures.

The membrane just external to the zona in Clupea vernalis may be considered homologous to that in Gasterosteus, Fundulus, and many Cyprinoids, even though it does not in Clupea bear appendages as it does in Gasterosteus. From the development of the appendages in Fundulus and Gasterosteus it is evident that this membrane has no connection with the granulosa cells. In these cases each of the appendages does not correspond to a single cell as in the perch, nor to any definite number of cells. If Reichert is correct in saying that the homogeneous membrane found in young eggs is a different structure from the zona radiata, the membrane under consideration may perhaps be looked upon as the primitive membrane described by him. It is certain that it appears before the zona, and I am inclined to think that it is derived from the yolk.

CAMBRIDGE, December, 1888.

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EXPLANATION OF FIGURES.

ABBREVIATIONS.

cp.	Blood corpuscles.	*pr j. i cl.*	Intercellular ridges.
fil.	Filaments of Fundulus.	*spa.*	Space below micropyle.
fil. vt.	Filaments of vitellus.	*tbl.*	Tubules of the capsular membrane in Perca.
gran.	Granulosa.		
m py.	Micropyle.	*thc. fol.*	Theca folliculi.
m py. cl.	Micropylar cell.	*vac.*	Vacuole.
nl. gran.	Nucleus of granulosa cell.	*yk.*	Yolk.
nl. m py.	Nucleus of micropylar cell.	*z. r.*	Zona radiata.
po. can.	Pore-canals of the zona radiata.	*z. r.′*	Zona radiata externa.
pr c.	Rivet-shaped processes of zona.	*z. r.″*	Zona radiata interna.

All the figures were made with the aid of the camera lucida, and all except Figs. 1, 2, 5, 7, 11, and 12 from preparations mounted in benzole-balsam. Figs. 39–41 were drawn by Dr. Mark, and the others by the author.

PLATE I.

Figures 1–13 *are of Fundulus heteroclitus.*

Fig. 1. Surface view of one of the smallest filament-bearing eggs of October 27. Diameter of egg, 0.16 mm. Examined fresh. × 750.

" 2. Surface view of another egg of the same size and date, with somewhat larger filaments. Examined fresh. × 425.

" 3. Tangential section of an ovarian egg 0.15 mm. in diameter. The ovary was hardened, December 23, in Flemming's chromic-osmic-acetic mixture, and subsequently stained with hæmatoxylin. × 425. The section is seen from its inner surface.

" 4. Tangential section of an egg from the same ovary with longer filaments. × 425. This section is also seen from its inner surface.

" 5. Surface view of an egg of October 27, about 0.23 mm. in diameter. Examined fresh. × 425.

" 6. Tangential section of an egg 0.25 mm. in diameter, from the same ovary as Fig. 3. × 750.

" 7. Surface view of a ripe (June) egg from which the granulosa cells had been removed, showing the network of ridges between their bases. Examined fresh. × 750.

" 8. Radial section of the egg represented in Fig. 3. Transsections of filaments are seen at *fil.* × 425.

" 9. Radial section of an egg of May 2, about 0.8 mm. in diameter. Preserved in Perenyi's fluid, and stained with picrocarminate of lithium. × 750.

" 10. Tangential section of an egg of December 23, about 0.4 mm. in diameter. From the ovary mentioned under Fig. 3. × 425.

" 11. Radial optical section of a ripe egg shortly after being forced from the ovary (June 1). Examined fresh. × 50.

" 12. Base of one of the filaments of the ripe egg. Examined fresh under pressure. × 750.

" 13. Radial section of an ovarian egg of November 23. Preserved in Perenyi's fluid, and stained with Grenacher's alcoholic borax-carmine. × 750.

Figures 14–22 *are of Pygosteus pungitius,* all except Figure 20 being of eggs from a single ovary, which was cut transversely.

" 14. Radial section through an ovarian egg 0.15 mm. in diameter. The ovary was preserved in Perenyi's fluid, April 18, shortly after spawning, and subsequently stained in picrocarminate of lithium. × 750.

" 15. Tangential section near the micropyle. × 112.

" 15a. Radial section of an egg 0.37 mm. in diameter. × 750.

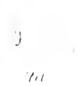
" 16. Radial section of an egg 0.33 mm. in diameter. × 750.

" 17. Radial section of an egg 0.37 mm. in diameter. × 750.

" 18–22. Radial sections through the micropyles of eggs, about 0.4 mm. in diameter. In Figs. 20 and 21 the micropyle is cut obliquely. × 750.

" 20 is from an ovary hardened April 4, i. e. some time before spawning.

1.



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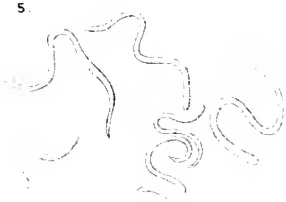

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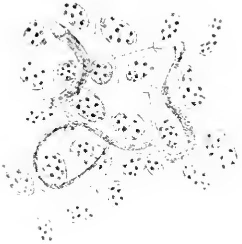

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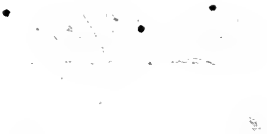

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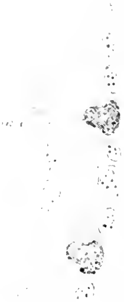

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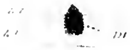

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ABBREVIATIONS.

cp.	Blood corpuscles.	*pr j. i cl.*	Intercellular ridges.
fil.	Filaments of Fundulus.	*spa.*	Space below micropyle.
fil. vt.	Filaments of vitellus.	*tbl.*	Tubules of the capsular membrane in Perca.
gran.	Granulosa.	*thc. fol.*	Theca folliculi.
m py.	Micropyle.	*vac.*	Vacuole.
m py. cl.	Micropylar cell.	*yk.*	Yolk.
nl. gran.	Nucleus of granulosa cell.	*z. r.*	Zona radiata.
nl. m py.	Nucleus of micropylar cell.	*z. r.′*	Zona radiata externa.
po. can.	Pore-canals of the zona radiata.	*z. r.″*	Zona radiata interna.
pr. c.	Rivet-shaped processes of zona.		

PLATE II.

Figures 23, 24, *and* 25 *are on Plate III.; Figures* 26–32 *are from Perca.*

Fig. 26. Radial section through the micropyle of an ovarian egg from *Perca* killed in February. × 750.

" 26^{a}. Section through the micropyle (?) of an egg of *Perca*. × 750.

" 27–32. A series of sections tangential to the surface of an egg of *Perca* 1 mm. in diameter at a point somewhat to the right of the micropyle. The portion of the sections to the right of the micropylar region lies deeper than the portion to the left. In

" 27, the cells lying to the right of the region of the micropyle are crowded, and have a curved band-like arrangement. The cells which contain dark points and lie farther to the right are from the central portion of the section, and are therefore cut across deeper than the others. In the second section,

" 28, only the deeper, filamentous prolongations of these cells are seen. In

" 28, 29, a median pit can be traced through the centre of the column of cells which occupies the micropylar region. In

" 30 the nucleus of the enlarged micropylar cell (*nl. m py.*) is seen. In

" 31 is seen the enlarged mouth of the mycropyle (*m py.*) and a few cells which lie somewhat higher and to the left of it.

" 32 is a section through the zona radiata and micropylar canal.

This egg was preserved February 15 in Perenyi's fluid, and was stained with Czoker's alum-cochineal. × 425.

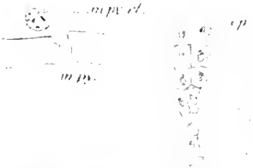
" 33. Radial section of an ovarian egg of *Morone americana* 0.28 mm. in diameter. Hardened in chromic acid February 25, and stained with picrocarminate of lithium. × 750.

" 34. Radial section of a larger egg (0.4 mm. in diameter) of *Morone americana* from the same series of sections as that of Fig. 33. × 750.

" 38. Radial section through the micropyle of an egg of *Notemigonus chrysoleucus* 0.63 mm. in diameter. Ovary of May 5, killed in Perenyi's fluid, and stained with picrocarminate of lithium. × 750.

" 45. Radial section through the egg of *Amiurus catus*. Ovary of May 9, killed in Perenyi's fluid, and stained with picrocarminate of lithium. × 750. The radial markings have been accidentally omitted and *fil. vl.* placed for *fil. vt.*

26.



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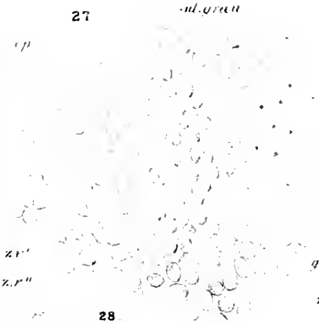

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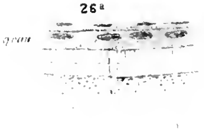

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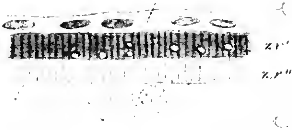

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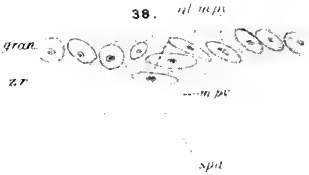

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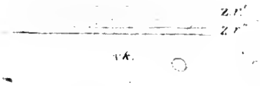

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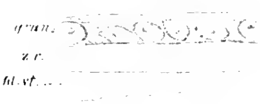

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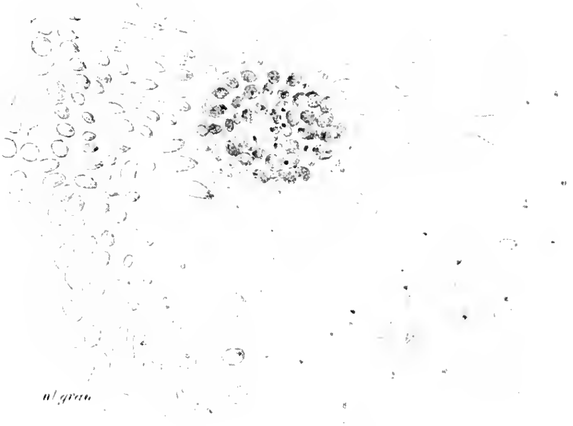

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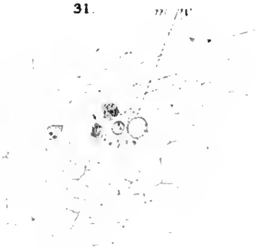

ABBREVIATIONS.

cp.	Blood corpuscles.	*pr j. i cl.*	Intercellular ridges.
fil.	Filaments of Fundulus.	*spa.*	Space below micropyle.
fil. vt.	Filaments of vitellus.	*tbl.*	Tubules of the capsular membrane in Perca.
gran.	Granulosa.	*thc. fol.*	Theca folliculi.
m py.	Micropyle.	*vac.*	Vacuole.
m py. cl.	Micropylar cell.	*yk.*	Yolk.
nl. gran.	Nucleus of granulosa cell.	*z. r.*	Zona radiata.
nl. m py.	Nucleus of micropylar cell.	*z. r.′*	Zona radiata externa.
po. can.	Pore-canals of the zona radiata.	*z. r.″*	Zona radiata interna.
pr c.	Rivet-shaped processes of zona.		

PLATE III.

Fig. 23. Radial section of an egg of *Perca* in October, 0 5 mm. in diameter. The ovary was hardened in 0.25 per cent chromic acid, and subsequently stained with Czoker's alum-cochineal. × 750.

" 24. Radial section through the micropyle of an egg of *Perca*. The ovary was preserved in Perenyi's fluid, May 9, and stained in carminate of lithium. × 425. The definite line at the outer margin of the zona radiata should have been omitted.

" 25. Radial section of an egg of *Perca*, 0.9 mm. in diameter. From an ovary hardened in February. × 425.

Figures 26–34 *are on Plate II.*

" 35. Radial section through the micropyle of an egg of *Esox*, 0.47 mm. in diameter. Ovary of February 23 killed in chromic-osmic-acetic mixture, and stained with picrocarminate of lithium. × 750.

" 36. Radial section through the micropyle of an egg of *Esox*, 0.94 mm. in diameter, from the same series represented in Fig. 35. × 750.

" 37. Radial section of an egg of *Esox*, 1.5 mm. in diameter, from the same series. × 750.

" 39. Radial section of an egg of *Clupea vernalis*, 0.54 mm. in diameter. Preserved in Perenyi's fluid, and stained with picrocarminate of lithium. × 515.

" 40. Radial section through the egg of *Cyclogaster liparis*, 0.7 mm. in diameter. Ovary of April 26 preserved in Perenyi's fluid, and stained with picrocarminate of lithium. × 515.

" 41. Radial section through the egg of *Cyclogaster liparis*, from an ovary of May 7 preserved in Perenyi's fluid, and stained with picrocarminate of lithium. × 515.

" 42, 43, and 44. Radial sections through the micropyles of three eggs of *Cyclogaster*, about 0.25 mm. in diameter. Ovary of May 7 preserved in Perenyi's fluid. × 515.

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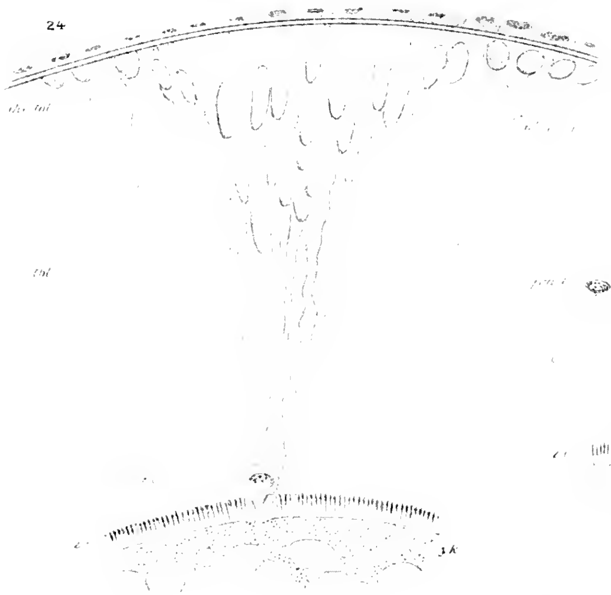

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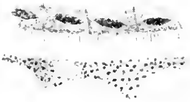

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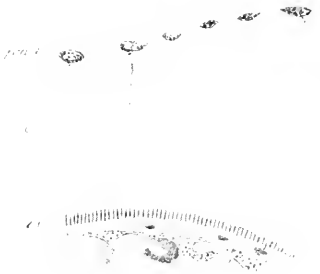

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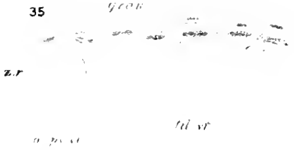

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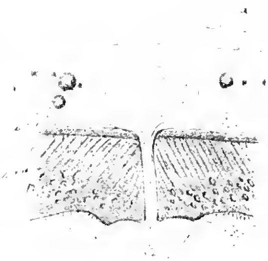

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No. 3. — *Report on the Results of Dredging, under the Supervision of* ALEXANDER AGASSIZ, *in the Gulf of Mexico* (1877–78), *and in the Caribbean Sea* (1879–80), *by the U. S. Coast Survey Steamer "Blake,"* LIEUT.-COMMANDER C. D. SIGSBEE, U. S. N., *and* COMMANDER J. R. BARTLETT, U. S. N., Commanding.

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XXXII.

Report on the Nudibranchs. By RUD. BERGH.

Während dieser Expedition wurden nur ganz wenige Formen von Nudibranchien gefischt, aber fast alle neu und darunter noch dazu ein Paar ziemlich interessante. Diese Formen waren die folgenden: —

1. *Tethys leporina*, L. var.
2. *Chromodoris scabriuscula*, Bgh., n. sp.
3. *Chromodoris punctilucens*, Bgh., n. sp.
4. *Chromodoris sycilla*, Bgh., n. sp.
5. *Phlegmodoris? anceps*, Bgh., n. sp.
6. *Nembrotha gratiosa*, Bgh., n. sp.
7. *Phyllidiopsis papilligera*, Bgh., n. sp.

Fam. TETHYMELIBIDÆ.

TETHYS, L.

R. Bergh, Malacolog Untersuch. (Semper, Philipp. II. ii.), Heft IX., 1875, pp. 346–362, Taf. XLV. Fig. 19–26, Taf. XLVI. Fig. 1–22, Taf. XLVII. Fig. 1, 2.

H. v. Hering, Tethys. Ein Beitrag zur Phylogenie der Gastropoden. Morpholog. Jahrb., II., 1876, pp. 27–62, Taf. II.

R. Bergh, Notizen üb. Tethys leporina. Jahrb. d. deutschen Malakolog. Ges., IV. 4, 1877, pp. 335–339.

R. Bergh, Beitr. zur Kenntn. d. Aeolidiaden. VII. Verh. d. k. k. zool. bot. Ges. in Wien, XXXII., 1882, pp. 67–68.

H. de Lacaze-Duthiers, Sur le Phænicurus. Comptes Rend., Cl. I., 1885, pp. 30–35.

R. Bergh, Sur la Nature du Phænicure. Arch. de Zool., 2 ser., III., 1886, pp. 73–76.

H. de Lacaze-Duthiers, Contrib. à l'Hist. du Phænicure. Arch. de zool., 2 ser., IV., 1887, pp. 77–108, Pl. IV.

List, Zur Kenntn. d. Drüsen im Fuss von Tethys fimbriata, L. Arb. aus dem zoolog. Institut zu Graz, I. 6, 1887, pp. 287–305.

Diese merkliche aberrante Nudibranchien-Gruppe ist erst durch die zwei ersten der obengenannten Arbeiten näher bekannt worden und diese Kenntniss ist nicht ohne wesentlichen Einfluss auf das Studium der ganzen Gruppe gewesen.

Die untenstehende Untersuchung hat wesentlich nur dadurch Interesse, dass sie das Vorkommen von einer *Tethys*, der altbekannten oder einer neuen Form, *im westlichen Theile des atlantischen Meeres* nachweisst.

T. leporina, L. var.

Tafel I. Fig. 1-3.

Hab. M. atlant. occ. (Dominica).

Von dieser Form wurde ein Individuum in der Nähe von Dominica aus einer Tiefe von 138 Faden hinaufgefischt.

Das in Alkohol ganz schlecht bewahrte, verdrehte, theilweise erhärtete und Papillenlose Individuum hatte eine *Länge* von 4.3 cm., von welchen die volle Hälfte auf dem Segel kamen, der Querdurchmesser des letzteren 3 cm.; die Höhe der Rhinophorscheide 7 mm., der Keule 2 mm.; die Länge der Randfäden bis 10 mm.; die Länge des Mundrohres 4 bei einem Durchmesser am Grunde von 3.5 mm.; die Breite des Rückens bis 13 mm.; die Höhe des Körpers bis 10 mm.; die Länge des Fusses 2.5 bei einer Breite bis fast 2 cm., der Vorderrand 7 mm. frei vortretend. — Die *Farbe* der Aussenseite des colossalen Segels ist gelblichweiss wegen dichtgedrängter ganz feiner gelblichweisser Püncktchen, die gegen den Rand hin zu unregelmässigen Fleckchen fast zusammenfliessen. Die Unterseite des Segels ist hinten kohlenschwarz so wie auch das grosse Mundrohr (aussen und innen), wird dann in der mittleren Strecke mehr braungrau, gegen den Rand hin schwärzlich und (theilweise fleckig) schwarz; die Randfäden des Segels meistens gelblichweiss, der Boden, auf dem sie sitzen, aber schwarz. Die Scheide der Rhinophorien schwarz, mit grossen gelblichweissen Flecken; die Keule am Grunde schwarz, sonst weisslichgelb. Der Rücken und die Körperseiten fast von der Farbe der Oberseite des Segels, aber mehr gelblich und im Genicke so wie in der Gegend des Rückenrandes starke, grosse, kohlenschwarze Flecken. Die Rückenpapillen fehlten ganz; die Kiemen (am Grunde der Papillenfacetten) weisslich, Die obere Seite des Fusses ringsum wie die Körperseiten gefärbt; der Fussrand weisslichgelb; die Fusssohle braungrau.

Der grosse *Segel* wesentlich wie bei der typischen Tethys des Mittelmeeres; an der Innenseite der Randparthie stehen die *Randfäden* in meistens 4–6 (8) sehr undeutlich geschiedenen Reihen; die äussersten sind ganz klein, die innersten von bedeutender Länge; die *Dorsalen Cirrhen* des Segelrandes kamen in gewöhnlicher etwas sparsamer Menge vor und von einer Höhe bis 2 mm. Das starke *Mundrohr* am Vorderende (Fig. 1 *a*) in gewöhnlicher Weise gekluftet; der gähnende Aussenmund bis an den Rand und bis in die Tiefe, bis an die schnürlochartige Pharynxöffnung mit starken Höckerchen, nur ausnahmsweise reihegeordnet, besetzt. Im Genick, dicht an der Gegend des hintersten Theils des Segels, ziemlich weit von einander stehend, die zusammengedrückten, oben etwas breiteren *Rhinophorien*, deren vorderer Theil oben eine Vertiefung mit umgeschlagenem Rande trägt, in welcher sich die zurückgebogene Keule fand; diese letztere etwas abgeplattet, mit 11 breiten Blättern. — Die Körperform wie in der typischen Tethys, der *Rücken* nur vielleicht etwas breiter. Am gerundeten Rückenrande, wie es schien, 7 rundliche *Papillenfacetten* gewöhnlicher Art, die Papillen selbst aber fehlend (wie so oft bei Exemplaren von Tethys) ; dicht neben jeder Facette zwei Kiemenbüschel, ein vorderer kleinerer, ein hinterer grösserer; die Kiemenbüschel wie gewöhnlich. Vor der zweiten rechten Papillenfacette die etwas hervorragende *Anal-Protuberanz*, neben derselben die *Nierenpore.* Die *Körperseiten* vorne ziemlich hoch ; aus der Genitalöffnung ragte ein Theil des Penis etwa 2 mm. hervor. Der grosse *Fuss* ganz wie bei der typischen Tethys; eine mediane Längsfurche fehlte nicht hinten an der Sohle.

Die Eingeweidemasse an die Körperwände durch Bindesubstanz geheftet.

Das weisslichgelbe *Centralnervensystem* zeigte die Hauptganglien von einander viel deutlicher geschieden, als ich es sonst bei Tethyden gesehen habe, nur zwischen den beiden pleuralen Ganglien war die Grenze undeutlich. Die buccalen (vorderen Eingeweide-) Ganglien zwischen dem hinteren Theile der Speicheldrüsen liegend (Fig. 1 *d*), oval, durch eine Commissur verbunden, die länger als der Querdurchmesser des Ganglions war ; oberhalb der Wurzel des nach vorne gehenden Nerven fanden sich mehrere Nervencellen eingelagert (Ganglion gastro-oesophagale). Der Riechknoten am Grunde der Keule des Rhinophors. Kleine (sympathische) Ganglien kamen an und zwischen den Eingeweiden zerstreut vor, besonders im Gebiete des Genitalsystems.

Die kleinen schwarzen *Augen* an der Oberfläche der Gehirnknoten nach aussen fast sessil, oval, von 0.12 mm. grösstem Diam., mit gelber Linse, reichlichem schwarzem Pigmente und ziemlich grossen Retinazellen. Die *Ohrblasen* als kalkweisse Punkten aussen an der oberen Seite der cerebralen Ganglien neben den pleuralen gelagert, kugelförmig, ganz kurzgestielt (Fig. 3), etwas kleiner als die grossen Nervenzellen, von 0.16 mm. Diam., mit zahlreichen runden und ovalen Otokonien von einem grössten Durchmesser von 0.016–0.02 mm. Die *Haut* mit Drüsencellen und Drüschen überall reichlichst ausgestattet.

Die Pharynxöffnung unten am Grunde der Mundröhre in die *Speiseröhre* übergehend; die etwas länger als die Mundröhre war; das vordere Ende (Fig. 1 *b*) derselben aussen schwärzlich, dann ringartig gelblichweiss (Fig. 1 *c*), dann wieder und in der übrigen Strecke schwärzlich. Die Innenseite vorne schwarz, mit etwa 15 starken Längsfalten, die sich vorne in den Pharynx hinein fortsetzen, hinten an dem erwähnten, nicht ganz schmalen, fast farblosen Ringe plötzlich anhalten; im vorderen Theile der folgenden Strecke kamen wieder etwa 15 starke Falten vor; diese Falten waren von einer schwach gelblichen Cuticula überzogen, die ganz fein und zierlich gefaltet war. In dem hinteren Theile der den Schlundkopf repräsentirenden (Fig. 1 *b*) vorderen Strecke der Speiseröhre mündet jederseits die langgestreckte, feinknotige (Fig. 1 *dd*, 2) gelblichweisse *Speicheldrüse* ein; der Ausführungsgang ganz (Fig. 2 *a*) kurz. — Der *eigentliche* aussen schmutzig schwarzblaue *Magen* 5.5 mm. lang, oval, von 3.5 mm. Durchmesser, von den gelblichen vorderen Lebern mit Ausnahme der Mitte der Rückenseite (und des Hinterendes) bedeckt (Fig. 1 *c*). Geöffnet zeigt der Magen feine Längsfalten der Innenseite; etwas nach vorne findet sich rechts die Oeffnung des Gallenganges der rechten Nebenleber; schräg gegenüber die Oeffnung für die mit einander verbundenen linke Neben- und Hauptleber. Hinten und rechts setzt sich der Magen in den Darm fort (Fig. 1 *g*); die schwarze Farbe hört plötzlich und scharf am Pylorus auf. Der Pylorustheil des Darmes ist gelblichweiss, und hier öffnet sich, dicht neben dem Pylorus, wie durch ein Schnürloch der sogenannte zweite Magen. Dieses ziemlich grosse *Diverticulum* (Fig. 1 *f*) ist gelblichweiss, fast kugelförmig, von 3 mm. Durchmesser; die Innenseite mit einem feinen pennaten Faltensystem. Der (Fig. 1 *g*) *Darm* erst nach unten und hinten, dann hinaufsteigend, kurz, ziemlich weit, nur in der letzten Strecke enger; aussen mit Ausnahme der ersten Strecke schwärzlich; die Innenseite schwarz, mit feinerer Längsfalten und einer stärkeren, die von der Oeffnung des Diverticulums anzufangen scheint. — Der Magen und der Darm von Nahrung vollgestopft; dieselbe bestand aus Massen von kleinen niederen Crustaceen (Copepoden, Ostracoden) und Stücken von kleinen Decapoden, mit Bruchstücken von kleineren Gasteropod-Schalen und Sandkörnern vermischt.

Die *rechte Nebenleber*, wie erwähnt (Fig. 1 *hi*), den rechten Theil des Magens mit einem dicken gelblichen Lager einhüllend; von derselben geht (wenigstens) ein Zweig an die erste rechte Papille (und wahrscheinlich an den (Fig. 1 *h*) Rhinophorstiel) ab; diese Lebermasse öffnet sich durch einen ganz kurzen Gallengang in den Magen. Die *linke*, der vorigen ganz ähnliche, *Nebenleber*, den linken Theil des Magens (Fig. 1 *kl*) einhüllend, sich nach hinten etwas verlängerend und sich mit (Fig. 1 *m*) dem Ausführungsgange der Hauptleber vereinigend; auch von dieser Leber geht ein Zweig an die Gegend der Facette der ersten Papille ab; diese Leber öffnet sich links in den Magen. Die *Hauptleber* viel grösser als die vorigen, an Länge etwa 1.8 cm. betragend bei einer Breite vorne von 11 und einer Höhe von fast 9.5 mm.; das Vorderende schief nach rechts- hinten- unten abgestutzt und (wegen der vordern Genitalmasse) vertieft; das Hinterende gerundet; nur central am Vorderende trat die graubraune Farbe der Leber hervor, sonst war sie von der gelblichen Zwitterdrüse gedeckt; das Organ bestand aus Lappen von verzweigten Läppchen, deren Ausführungsgänge sich allmählich traubenartig vereinigen und nach und nach den central verlaufenden Hauptgallengang bilden, welche links am Vorderende frei hervortritt (Fig. 1 *m*) und sich mit der linken Nebenleber vereinigt. An den Seitentheilen des Rückens der hinteren Eingeweidenmasse durchbrechen mehrere Leberzweige das Zwitterdrüsenlager und steigen an die Papillenfacetten auf.

Das Pericardium und das Herz wie gewöhnlich. — Die *Niere* mit ihrer baumartigen Veranstelung von schönen Kolben und Röhren den grössten Theil der hinteren Eingeweidemasse überziehend und die Längsfurche derselben auskleidend; in der Auskleidung von jenen viele horngelbe und braungelbe rundliche Concremente von einem Durchmesser von meistens 0.025–0.035 mm. Der Ureter wie gewöhnlich; in denselben öffnet sich der *Pericardialtrichter*, der kurz- birnförmig war, von 1 mm. Länge, gelblichweiss, mit stark durchschimmernden Längsfalten; der Gang kurz, fast ohne Vegetationen der Innenseite.

Die gelbliche, die Leber mit Ausnahme des grössten Theils ihres Vorderendes überziehende *Zwitterdrüse* wie gewöhnlich; in den Läppchen entwickelte Zoospermien. Der rechts am Vorderende der hinteren Eingeweidemasse entspringende *Zwitterdrüsengang* an die Hinterseite der *vorderen Genitalmasse* übertretend. Diese letztere 9 mm. lang bei einer Höhe von 7 und einer Dicke von 5 mm.; am oberen Rande vorne die Prostata, hinter derselben der Knäuel der Windungen der Ampulle des Zwitterdrüssenganges, unter dem letzteren die Samenblase; die Hauptmasse ist von der Schleimdrüse gebildet. Die gelbliche Ampulle durchgehends von beiläufig 0.5 mm. Diam.; aufgerollt hinter der Prostata einen Knäuel bildend, der ein wenig kleiner als die Prostata war; ausgerollt mass dieselbe 2.5 cm. Der aus der Theilungsstelle der Ampulle ausgehende *Samenleiter* etwa doppelt so lang wie der Durchmesser der Prostata. Diese letztere gelblich, fast kugelförmig, von 4 mm. Diam., mit einem kleinen Nabel der hinteren und einer tiefen Kluft der Vorderseite, aus welcher die Fortsetzung des Samenleiters hervortretet; die Oberfläche fein körnig, der Bau ganz der gewöhnliche. Die aus der tiefen Kluft vortretende Fortsetzung des Samenleiters gräulich, ziemlich dünn, etwa doppelt so lang wie der Durchmesser der Prostata, sich durch den Penis bis an seine Spitze windend. Der halb hervorgestreckte *Penis* gelblich, lang, kegelförmig; der gewöhnliche Nebensack konnte nicht gefunden werden. Der *Eiergang* geschlängelt an den Schleimdrüsengang gehend, ausgestreckt beiläufig 1.5 cm. messend, etwa so dick wie die Ampulle. Die gelbliche, sich in das Vestibulum genitale neben dem Schleimdrüsengange öffnende *Samenblase* birnförmig, von etwa 5 mm. Länge bei einem Durchmesser von etwa 2.3 mm., von Samen strotzend; der Ausführungsgang fast ebenso lang, mit starken Längefalten der Innenseite. Die *Schleimdrüse* gross, kalkweisslich; die Eiweissdrüse gelblich; der Schleimdrüsengang mit der gewöhnlichen starken Doppelfalte.

Ob diese Form nun eine (locale) Varietät der bisher nur im Mittelmeere und bei den canarischen Inseln gefundenen Tethys leporina darstellt oder eine eigene Art, muss vorläufig hingestellt werden. Das Erste ist wohl das wahrscheinlichste, obgleich die schwarze Farbe der Verdauungshöle und Abweichungen im Genitalsystem wohl auch die letzte Annahme ermöglichten. Die so träge und nie schwimmende Staurodoris verrucosa kommt doch auch im westlichsten Theile des atlantischen Meeres (unweit von Rio Janeiro) vor (Staurod. Januarii, Bgh.).[1]

Fam. DORIDIDÆ CRYPTOBRANCHIATÆ.

CHROMODORIS, Ald. et Hauc.

Vgl. R. Bergh, Report on the Nudibranchiata. Challenger Exped., Zoöl., X., 1884, pp. 64–78.

Vgl. R. Bergh, Malakolog. Unters., Heft XV. 2, 1884, pp. 64–78, pp. 347–350; Heft XVI. 2, 1889, pp. 831–837.

Die fast immer schlanken und meistens lebhaft gefärbten Chromodoriden haben *einfach gefiederte Kiemenblätter*, *starke Lippenplatten*, und *die Rhachisparthie der Radula* trägt höchstens nur Verdickungen, aber *keine Zahnplatten*.[2] Die Aphelodoriden,[3] die sonst sehr ähnlich sind, unterscheiden sich durch mehrfach gefiederte Kiemenblätter und durch Fehlen von Lippenplatten.

Die Gattung ist bisher nur aus den wärmeren (Mittelmeere) und den tropicalen Meeresgegenden bekannt. Sie scheint die Artenreichste Gruppe von Doriden zu sein; sie wird hier wieder durch mehrere neue Arten bereichert.

1. Chr. scabriuscula, Bgh., n. sp.

Tafel I. Fig. 11-19.

Hab. M. atlant. occidentale.

Von dieser form wurden am 24° 44′ Lon. und 83° 26′ Lat. (d. h. in der Nähe von Straits of Florida) aus einer Tiefe von 37 Faden 3 Individuen gefischt, die fast vollständig von derselben Grösse und Formverhältnissen waren.

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[1] Vgl. Ihering, Zur Kenntn. d. Nudibranchien d. brasilianischen Küste. Jahrb. f. d. Malacolog. Ges., XIII., 1886, pp. 230–233.

[2] Nur die Chr. Scabriuscula, B. macht hier eine Ausnahme.

[3] R. Bergh, Neue Chromodoriden. Malakolog. Bl. N. F. I., 1879, pp. 107–113.

R. Bergh, On the Nudibr. Gaster. Molls. of the North Pacific Oc. (Dall, Explor. of Alaska), II., 1880, Pl. VIII. (XVI.), Figs. 12–18.

Die in Alkohol gut bewahrten Individuum hatten eine *Länge* von 12 bei einer Breite bis 6 und einer Höhe bis 3.5 mm., die Länge des Fusses 10 bei einer Breite bis 2.5 mm.; die Breite des Mantelgebrämes 1.5 mm.; die Länge der Tentakel 0.6 mm.; die Höhe der (zurückgezogenen) Rhinophorien 1.8, der (zurückgezogenen) Kieme 1.5 mm. — Die *Farbe* war durchgehends gelblichweiss, die Keule der Rhinophorien und die Kieme mehr gelblich.

Die *Form* war länglich-oval, etwas niedergedrückt; die Rückenseite etwas gewölbt, überall bis an den Rand mit ziemlich zahlreichen kleinsten conischen Höckerchen besetzt, die am Mantelgebräme zahlreicher waren; die weit nach vorn stehenden Rhinophorlöcher, und die weit nach hinten stehende Kiemenspalte schnürlochartig zusammengezogen; die Keule der Rhinophorien stark, mit beiläufig 20 nicht dünnen Blättern; die Kieme aus 9, einem vorderen und jederseits 4, nach hinten an Grösse allmählich abnehmenden, einfach-pinnaten Blättern gebildet; die Analpapille niedrig. Der Kopf klein; die Tentakel kurz-cylindrisch, am Ende gleichsam eingestülpt. Die Unterseite des Mantelgebrämes eben, mit durchschimmernden, gegen den Rand senkrecht gehenden Spikelzügen. Der Fuss langgestrekt, mit parallelen Seitenrändern; der Vorderrand mit Furche und gerundeten Ecken; der Schwanz 2.2 mm. lang, etwas zugespitzt. Die Genitalpapille mit zwei Oeffnungen neben einander.

Die Eingeweide schimmerten nirgends durch, waren an der Körperwand angeheftet.

Das *Centralnervensystem* zeigte die cerebro-pleuralen Ganglien kurz-nierenförmig, die nach unten stehenden pedalen Ganglien grösser als die pleuralen; die gemeinschaftliche Commissur ziemlich kurz, kaum so lang wie der Querdurchmesser des Fussknoten. Die ganz kurzstieligen Ganglia olfactoria ungewöhnlich gross, fast halb so gross wie die Ganglia cerebralia; die buccalen und gastro-oesophagalen Ganglien wie gewöhnlich.

Die ganz kurzstieligen *Augen* ziemlich gross, mit schwarzem Pigment. Die *Ohrblasen* kleiner als die Augen; mit Otokonien gewöhnlicher Art prall gefüllt, unter denen ein kugelförmiger, der doppelt so gross wie die anderen war. In den Blättern der *Rhinophorien* zahlreiche, auf den Rand senkrecht stehende, gelbliche, harte Spikeln von einem Durchmesser bis 0.03 mm. Die Rückenhaut im Ganzen und besonders die Höckerchen derselben mit ähnlichen Spikeln stark ausgestattet.

Die *Mundröhre* stark, 1.5 mm. lang, wie gewöhnlich. Der kurze *Schlundkopf* 1.6 mm. lang; hinten an der Unterseite trat die Raspelscheide hervor. Die horngelbe ringartige Bewaffnung der Lippenscheibe unten viel breiter als oben, aus den gewöhnlichen, bis beiläufig 0.027 mm. langen, an der Spitze geklufteten (Fig. 11, 12), dicht zusammengedrängten Häkchen zusammengesetzt. Die *Zunge* von gewöhnlicher breiter Form mit tiefer Kluft; in der hellgelben Raspel 58 Zahnplattenreihen, weiter nach hinten in der starken Scheide 46 entwickelte und etwa 4 jüngere Reihen; die Gesammtzahl derselben somit 108. In der Raspel jederseits 25 Platten, und die Anzahl weiter nach hinten kaum 30 übersteigend. Die Zahnplatten schwach gelblich; die Breite der medianen Platten 0.01, die ersten lateralen 0.016 mm.; die Höhe der äussersten Platten meistens 0.028, die Höhe der Seitenplatten bis 0.04 mm. Es kamen wirkliche mediane, am Rande gezähnte Platten vor (Fig. 13 *a*, 14). Die innerste laterale Platte (Fig. 13) mit 8–10 Dentikeln des äusseren und 4–5 des inneren Hakenrandes; an den übrigen Seitenplatten fanden sich, wie gewöhnlich, nur Zähnchen am Aussenrande, aber in sehr variabler Menge, mitunter 5–6, mitunter nur 2–3 (Fig. 15–17); an den äussersten (Fig. 18) Platten war der Grundtheil kürzer, und unterhalb der Hakenspitze fanden sich nur 2–3 Zähnchen. — Die langen und weisslichen *Speicheldrüsen* wie gewöhnlich.

Die Speiseröhre etwa so lang wie der Schlundkopf; der Magen wie gewöhnlich; der Darm vor der Mitte der hinteren Eingeweidemasse hervortretend, sein Knie in gewöhnlicher Weise bildend, und in gewöhnlicher Weise verlaufend, gelb. — Die hintere Eingeweidemasse (*Leber*) 6.5 mm. lang bei einer Höhe und Breite von 3.2 und 3.5 mm., vorne sehr schief abgestutzt und hinten gerundet, (gelblich-) weiss. Die *Gallenblase* langgestreckt-birnförmig, weisslich, links am Pylorus erscheinend.

Das Pericardium mit dem Herzen, die weisslichen Blutdrüsen, die Niere und der Pericardialtrichter wie gewöhnlich.

In den gelblichen Lappen der *Zwitterdrüse* grosse Eierzellen. — Die *vordere Genitalmasse* gross, etwa 4 mm. lang, von ovaler Form, planconvex, gelblich; am Vorderende die starken Windungen des Samenleiters. Die Ampulle des Zwitterdrüsenganges weisslich, geschlängelt. Der Samenleiter lang; der weissliche prostatische Theil kürzer als der gelbliche muskelöse (Fig. 19 *a*); die kurzkegelförmige, ziemlich dicke glans penis am Boden des (Fig. 19 *b*) räumigen Praeputiums kaum vortretend. Die Spermatotheke kurz-birnförmig, die Spermatocyste wurstförmig und kleiner; der vaginale Gang lang, nach vorne weiter, mit einer starken gelben Cuticula ausgefuttert; die Vagina fast so lang wie das Praeputium, doppelt so dick wie der vaginale Gang, von einer dünneren Cuticula ausgekleidet. Die Schleimdrüse gross; die Eiweissdrüse etwas mehr gelblich.

Diese Form unterscheidet sich von den allermeisten Chromodoriden durch die harten Höckerchen des Rückens und damit durch die ziemlich starke Entwickelung der cutanen Spikeln, so wie besonders durch wirkliche mediane (rhachidiale) Zahnplatten. Auch die Auskleidung des vaginalen Ganges ist eigenthümlich.

2. **Chr. punctilucens,** Bgh., n. sp.

Tafel I. Fig. 4-10.

Hab. M. atlant. occid.

Ein einziges Individuum wurde aus einer Tiefe von 37 Faden auf 24° 44′ Lon. und 83° 26′ Lat. (d. h. in der Nähe der Straits of Florida) gefischt.

Das in Alkohol bewahrte Individuum hatte eine *Länge* von 3.5 bei einer Breite von 1.6 und einer Höhe von 1.5 cm.; die Breite des Mantelgebrämes 2 (vorne) bis 4.5 mm. ; die Höhe der (zurückgezogenen) Rhinophorien 3, der (zurückgezogenen) Kieme 5 mm.; die Länge des Fusses 3 bei einer Breite bis 1 cm. — Die *Farbe* der obern Seite war durchgehends olivenbraungrau mit ziemlich zahlreich zerstreuten gelben und weissen Punkten, die oft eine schwarze oder schwärzliche Areola zeigten ; am Mantelrande ein schmales, schwarzes, seiner Länge nach durch eine weisslichgelbe oder gelbe Linie getheiltes Band ; die Unterseite des Mantelgebrämes von der Grundfarbe des Rückens oder mehr grau, hier und da schwarzfleckig ; der Rand der Rhinophorlöcher so wie der Kiemenspalte schwarz mit gelben Punkten und Bruchstücken von gelben Linien ; die Keule der Rhinophorien schwarz, am Vorderrande und gegen die Spitze gelblich; die Kiemenblätter schwärzlich, die Rhachisparthien, die Spitze und theilweise die Ränder der Blätter gelbfleckig; die Analpapille schwarz mit gelblichem Rande. Die Körperseiten von der Farbe des Rückens, die gelben und weissen Punkte kommen aber sehr sparsam vor. Die Tentakel mit gelber Spitze; der Aussenmund schwarz. Die Fusssohle graulich; das Fussgebräme oben von der Farbe der Körperseiten, aber mit starken schwarzen Flecken ; der Fussrand gelb, hier und da mit schwarzen Fleckchen ; am Schwanzrücken zerstreute gelbe Punkte.

Im Aeusseren *simulirte* diese Form (in Alkohol bewahrt) ganz *eine Doriopse*, nur war der Mund wie bei den Doriden, und die Tentakel kurz kegelförmig (an der Spitze, wie bei so vielen Chromodoriden, gleichsam halb eingestülpt). Die Form länglich, die Consistenz weich. Der Rücken etwas gewölbt, das Mantelgebräme ziemlich breit, wellenförmig gebogen, an der Unterseite wie der ganze Rücken eben. Die Rhinophorlöcher fast glatrandig; die Keule der Rhinophorien kräftig, mit beiläufig 30 breiten Blättern. Die Kiemenspalte queroval, fein rundzackig. Die Kieme jederseits aus 7 einfach pinnaten Blättern gebildet, denen sich hinten eine Spirale von 13 Blättern anschliest ; diese letzteren etwas schmächtiger und unbedeutend niedriger als die andern, die alle fast von gleicher Grösse waren. Hinten zwischen den Spiralen die cylindrische, oben abgestutzte, etwa 3 mm. hohe Analpapille; rechts und vorne neben derselben die Nierenpore. Die Körperseiten ziemlich hoch; die Genitalpapille wie gewöhnlich. Der Fuss vorne gerundet-abgestutzt, mit feiner Randfurche; das Fussgebräme nicht schmal; der Schwanz stark, nicht kurz.

Das gelbe *Centralnervensystem* von den Blutdrüsen bedeckt, in reichliche, fest anhängende Bindesubstanz gehüllt; die Ganglien ziemlich dick. Die zwei Abtheilungen der cerebro-pleuralen Ganglien sehr ausgeprägt; die pedalen ausserhalb und unterhalb der vorigen liegend; die pleuralen grösser als die cerebralen, die pedalen wieder grösser als die pleuralen ; die gemeinschaftliche Commissur weit, doppelt so lang wie der Querdurchmesser des Centralnervensystems. Die Riechknoten, die buccalen und die gastro-oesophagalen Ganglien wie gewöhnlich.

Die *Augen* fast sessil, mit schwarzem Pigment. Die *Ohrblasen* so gross wie die Augen, mit Otokonien gewöhnlicher Art prall gefüllt. In den Blättern der Keule der *Rhinophorien* keine Spikel. In der *Haut* des Rückens kamen erhärtete Zellen sparsam vor.

Die *Mundröhre* sehr stark, etwa 6 mm. lang bei einem Diam. hinten von 6 mm.; aussen gelblich, innen vorne schwarz und hinten gelblich; die starken Retractoren wie gewöhnlich. — Der sehr kräftige *Schlundkopf* 5.5 mm. lang bei einer Breite von 4.5 und einer Höhe von 4.5 mm.; das abgeplattete Hinterende stark schräge; von der Unterseite ragt die starke (1.1 mm. in Diam. haltende) Raspelscheide 3 mm. nach oben und links empor. Die runde Lippenscheibe von 4 mm. Diam., von der schön dunkel ambergelben *Lippenplatte* (Fig. 4) überzogen, welche oben schmäler, unten (von vorn nach hinten) viel breiter (bis 2.5 mm.) war, unten continuirlich, oben durch einen ganz schmalen Zwischenraum in zwei Hälften geschieden. Die Lippenplatte in gewöhnlicher Weise von dicht zusammengedrängten gelblichen Stäbchen gebildet, welche (in gerader Linie gemessen) eine Länge bis zu fast 0.06 mm. erreichten, gebogen und in der Spitze gekluftet (Fig. 5, 6) waren. Die *Zunge* breit, abgeplattet, mit breiter Kluft; in der gelblichen Raspel 60 Zahnplatten, weiter nach hinten und in der ziemlich langen Raspelscheide 98 entwickelte und 12 jüngere Reihen; die Gesammtzahl derselben somit 170. Die vordersten 16–18 Reihen sehr incomplet. In den hintersten Reihen der Zunge fanden sich jederseits bis 53 Seitenzahnplatten, und die Anzahl stieg kaum wesentlich weiter nach hinten. Die Zahnplatten gelblich; die Höhe der äussersten Platten 0.04–0.05 mm. betragend, allmählig stieg die Höhe der Platten bis zu etwa 0.1 mm.; die Länge der medianen (Fig. 7 *a*) Verdickungen meistens 0.035 mm. Die Zahnplatten von der gewöhnlichsten Hakenform; an den äussersten ist der Körper in gewöhnlicher Weise reducirt, und die Platten mehr aufrecht. Die innerste (Fig. 7 *bb*) Zahnplatte an beiden Rändern des Hakens gezähnelt; alle die übrigen (Fig. 8, 9) nur am äusseren Rande mit 6–10 feinen Dentikeln; die 5–7 äussersten (Fig. 10) ohne Dentikel.

Die weissen *Speicheldrüsen* sehr langgestreckt, vorne etwas dicker, sich bis an die Unterseite der hinteren Eingeweidemasse hinab erstreckend.

Die *Speiseröhre* kaum länger wie der Schlundkopf bei einem Durchmesser von beiläufig 1 mm. Der in die hintere Eingeweidemasse eingeschlossene *Magen* rundlich, nicht klein. Der *Darm* vor der Mitte der oberen Seite die hintere Eingeweidemasse durchbrechend, in gewöhnlicher Weise verlaufend und sein Knie bildend; im ganzen 7 cm. lang bei einem Durchmesser von 1.5–2 mm. — Der Inhalt der Verdauungshöhle war ganz unbestimmbare thierische Masse, worin Stücke von Zahnplattenreihen des Thieres selbst.

Die hintere Eingeweidemasse (*Leber*) 2 cm. lang, bei einer Höhe und Breite von 1.2 cm.; nach hinten zugespitzt; die vordere Hälfte der rechten Seite (durch die vordere Genitalmasse) stark abgeplattet; die Substanz gelb. Die *Gallenblase* links neben dem Pylorus, sackförmig, von 4 mm. Länge, gräulich.

Das *Pericardium* gross, queroval, von 8 mm. kurzestem Diam. Die gelbe Herzkammer von 3.5 mm. Länge. Die *Blutdrüsen* in den Rändern etwas lappig, graugelb, abgeplattet; die vordere gestreckt-herzförmig mit der Spitze nach vorn, 6 mm. lang; die hintere breit, querliegend, 7 mm. breit. — Die Verbreitung der *Niere* über die hintere Eingeweidemasse sehr schön, die Urinkammer weit; der *Pericardialtrichter* stark, birnförmig, 2 mm. lang.

Die *Zwitterdrüse* mit einem 0.5–1 mm. dicken, mehr gelben Lager den grössten Theil der Leber überziehend; in ihren Läppchen grosse Eizellen. — Die (sehr stark erhärtete) *vordere Genitalmasse* gross, planconvex, 14 mm. lang bei einer Breite von 7 und einer Höhe von 11 mm. Am Vorderende die ziemlich dicke, geschlängelte, opak-weissliche Ampulle des Zwitterdrüsenganges. Der Samenleiter lang, gewunden, der gelbliche prostatische Theil kürzer als der muskulöse; die glans penis kegelförmig. Die Spermatotheke von ovaler Form, von 3.5 mm. Länge; die Spermatocyste wurstförmig, gebogen, ein wenig länger. Die Schleimdrüse gräulichgelb und kalkweiss, die Eiweissdrüse bräunlich; der äusserste Theil des Schleimdrüsenganges schwarz.

Dieses Thier repräsentiert gewiss eine neue Art. Unter den wenigen bisher[1] bekannten Arten aus dem westlichen atlantischen Ocean (Chr. Moerchii, B.; Chr. gonatophora, B.) giebt es keine zu welcher sie hingeführt werden könnte, und eben so wenig kann sie mit irgend einer der vielen im Mittelmeere vorkommenden identificirt werden.

3. Chr. sycilla, Bgh., n. sp.

Tafel III. Fig. 5-13.

Hab. M. atlant. occ. (Sin. Mexicanum).

Von dieser Form hat die Blake-Expedition 16 Meile gegen Nord von den Jolbos-Inseln (an der Küste von Yucatan) ein einziges Exemplar gefischt, aus einer Tiefe von etwa 14 Faden.

Das in Alkohol gut bewahrte, nur etwas zusammengezogene Individuum hatte eine *Länge* von 2.5 bei einer Breite von 1 und einer Höhe von 1.4 Cm.; die Höhe der (zurückgezogenen) Rhinophorien fast 4, der (zurückgezogenen) Kieme 4.25 mm.; die Breite der Fusssohle 4.5 mm. — Die *Grundfarbe* des Körpers war ein sehr schönes und lebhaftes Dunkelblau. Diese Farbe war am Rücken wie an den Seiten von zahlreichen, kalkweissen, dünnen, oft zerstückelten Längslinien durchzogen; die Stückchen mitunter an dem einen oder anderen Ende kurz- schlingen- oder ösenförmig oder mit einem kurzen Seitenaste; zwischen den Linien kamen noch hier und da einzelne rundliche oder ovale Fleckchen vor. Am Rücken fanden sich etwa 9–10 solche Linien vor, an den Körperseiten 5–6. Der Mantelrand (Fig. 13) so wie der Fussrand mit einer ganz ähnlichen, ebenso unterbrochenen, kalkweissen Linie geziert. Der Rand der Rhinophorlöcher weiss; die Rhinóphorien schmutzigblau. In dem theilweise weissen Rande der runden Kiemenspalte endigt die grösste Zahl der weissen Rückenlinien; die Kiemen-

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[1] Vgl. die von mir vor einigen Jahren gelieferte Liste in Challenger Exped., Zoöl., X., 1884, pp. 65–72.

blätter sehr schön blau; ihre an der Aussenseite ziemlich breite Rhachis weiss gerändert, der schmale innere Rhachisrand mitunter auch weiss. Die Fusssohle schmutzig gelblich.

Die *Formverhältnisse* wie bei den meisten Chromodoriden; das Stirngebräme, der Mantelrand und das Schwanzsegel schmal. Ringsum an der Unterseite des Mantelgebrämes fanden sich grössere und kleinere, durchsichtig-gelbliche, kugelförmige, sessile, ungleichgrosse Blasen (Fig. 13 *aa*) von einem Durchmesser von beilaüfig 0.3–2 mm; die grössten kamen am Schwanzsegel vor (Fig. 13 *aa*); jede zeigte am Scheitel eine meistens schon unter der Loupe sehr deutliche Oeffnung. Oberhalb und ausserhalb des Aussenmundes jederseits ein gleichsam eingestülpter Tentakel. Die etwas zusammengedrückte Keule der Rhinophorien mit etwa 40–45 breiten Blättern. Die Kieme weit nach hinten stehend, mit 12 schönen Blättern, von welchen das hinterste Paar kleiner, die übrigen fast gleichgross. Im Centrum des Kiemenkreises die niedrige (oben weisse) Analpapille, rechts und vorn neben derselben die Nierenpore. Der Fuss wie gewöhnlich ziemlich schmal.

Das Peritonaeum farblos oder hier und da bläulich.

Die das *Centralnervensystem* eng einhüllende starke Bindesubstanzcapsel mit der Unterseite der vorderen und mit dem Vorderende der hinteren Blutdrüse innig verwachsen. Die Ganglien an der Unterseite der ganzen Ganglienmasse deutlich geschieden. Die cerebro-pleuralen Ganglien länglich-nierenförmig, die cerebrale grösser als die pleurale Abtheilung; die rundlichen pedalen Ganglien etwas grösser als die cerebralen. Die grosse gemeinschaftliche Commissur ziemlich weit, doppelt so lang wie der Querdurchmesser des Centralnervensystems. Die proximalen und distalen Riechknoten wie gewöhnlich. Die buccalen Ganglien oval, fast unmittelbar mit einander verbunden; die gastro-oesophagalen sehr kurzstielig, etwa $\frac{1}{8}$ der vorigen betragend.

Die *Augen* mit schwarzem Pigment und schwach gelblicher Linse, durch einen kurzen N. opticus mit dem kleinen Gangl. opticum verbunden. Die *Ohrblasen* wie gewöhnlich, mit zahlreichen Otokonien gewöhnlicher Art. In den dünnen und breiten Blättern der Keule der *Rhinophorien* kamen zerstreute erhärtete Zellen, aber keine Spikel vor.

Die *Mundröhre* aussen blaugrau, innen gelblichweiss, kurz und weit; der Diam. und die Länge etwa 5 mm. betragend. — Der *Schlundkopf* sehr stark, 6 mm. lang bei einer Breite von 5 und einer Höhe von 4.75 mm., von gewöhnlichen Formverhältnissen; die 3.5 mm. lange, starke Raspelscheide längs des Hinterendes des Schlundkopfes hinaufgekrümmt; die Lippenscheibe gross, gewölbt, mit sehr starker, grünlich- olivenfarbiger *Lippenplatte.* Diese letztere einen etwa 3 mm. breiten Ring bildend oder eigentlich zwei Halbringe, die in der Mittellinie oben und unten durch ein schmaleres Zwischenstück vereinigt sind. Die Platte in gewöhnlicher Weise von ganz dicht gedrängten Stäbchen mit gebogenem hakenartigem Kopf gebildet (Fig. 5); sie erreichten eine Höhe bis zu beiläufig 0.04 mm.; die Stäbchen der erwähnten Zwischenstücke ganz klein. Die *Zunge* breit; in der grünlich- olivenfarbigen Raspel 39 Zahnplattenreihen, weiter nach hinten kamen dazu 41 entwickelte und 4 jüngere Reihen, die Gesammtzahl derselben somit 84 Die 8 vordersten Reihen mehr oder weniger incomplet. Die hintersten Reihen der Zunge enthielten (jederseits) etwa 290 Zahnplatten, und die Anzahl stieg kaum wesentlich weiter nach hinten. Die Zahnplatten schwach gelblich mit etwas grünlichem Anfluge; die Seitenzahnplatten erreichten eine Höhe bis zu 0.14 mm., die der äussersten betrug etwa 0.06–0.08 mm. Die Rhachisparthie sehr schmal, meistens mit einer seichten medianen Längsfalte. Die Zahnplatten von der allergewöhnlichsten Form (Fig. 6–9); die Haken gabelig, der obere Ast länger und mehr gebogen als der untere; unterhalb dieses *letzteren eine Andeutung von feinen* Rundzacken, die nach aussen in den Reihen besonders etwas deutlicher wurden und selbst in feine Dentikelbildungen übergehen können (Fig. 6). Die (meistens) 5–6 äussersten Platten sind von etwas abweichender Form (Fig. 10, 11), zeigen den Haken reducirt und mit gerundetem Ende; die 1–2 alleräussersten haben keine Auskerbung oben (Fig. 12).

Die *Speicheldrüse* sehr lang, sich über die Unterseite der vorderen Genitalmasse erstreckend, kalkweiss, dünn; vorne etwa 1.25 mm. breit, in der hinteren Hälfte kaum halb so dick; die ganz kurzen Ausführungsgänge in die Wurzel der Speiseröhre einmündend.

Die *Speiseröhre* dünn, etwa 14 mm. lang (bei einem Durchmesser von 0.8 mm.), ganz unten am Vorderende der hinteren Eingeweidemasse eintretend und sich in die weite Leber- Magenhöhle öffnend. Der *Darm* die Leber vor der Mitte ihrer oberen Seite durchbrechend, vorwärts gehend; sein Knie über die vordere Genitalmasse legend und dann nach hinten verlaufend; die Länge des Darmes im Ganzen etwa 5 cm. betragend, bei einem wechselnden Durchmesser von 1.5–4 mm. Der weissliche Inhalt des Darmes (und der Leberhöhle) war unbestimmbare thierische Masse, mit langen und spitzen Spikeln vermischt.

Die hintere Eingeweidemasse (*Leber*) war 15 mm. lang bei einer Höhe von 12 und einer Breite von 9 mm. (stark zusammengezogen), hinten gerundet, vorne schief abgestutzt; ihre Substanz hell gelblichgrau. Die *Gallenblase* horizontal an der linken Seite des Pylorus liegend, 4 mm. lang bei einem Durchmesser von 1 mm., gelblichweiss.

Das Pericardium blaugrau. Das Herz wie gewöhnlich. Die grünlich-gelbgrauen *Blutdrüsen* an der oberen Seite mit hell grünlichblauem Ueberzuge, die vordere kleiner, 3 mm. breit bei einer Länge von 2.5 mm.; die hintere grösser, gerundet-dreieckig, die Spitze nach hinten kehrend, 5.5 mm. breit bei einer Länge von 3.5 und einer Dicke von 0.8 mm. — Die Niere wie gewöhnlich; das pericardio-renale Organ birnförmig, 1.8 mm. lang.

Die gelbliche *Zwitterdrüse* als ein dünnes Lager die Leber fast vollständig überziehend; in den Läppchen der Drüse kamen reife Zoospermien vor. — Die *vordere Genitalmasse* 8 mm. lang bei einer Höhe von 6 und einer Breite von 4 mm.; die dunkelblauen Hauptausführungsgänge noch 4 mm. lang; das Hinterende der Masse wird zum grössten Theile von der grossen Samenblase gebildet, die aber oben und an der äusseren (rechten) Seite von den Windungen des Samenleiters gedeckt wird. Die Ampulle des Zwitterdrüsenganges opak-gelblichweiss, wurstartig, etwas zusammengebogen, ausgestreckt an Länge 6 mm. bei einem Durchmesser von beiläufig 0.75 mm. messend. Der lange, viele längere und kürzere Windungen machende, weissliche prostatische Theil des Samenleiters ausgestreckt etwa 5–6 cm. lang bei einem fast durchgehenden Diam. von 0.5 mm.; der mehr gelbliche muskulöse Theil nur beiläufig 12 mm. lang und etwas dünner. Der letztere geht in den sich nach und nach verdickenden, am Ende blauen *Penis* über, der eine Länge von 4.5 bei einem Diam. (vorne) bis zu 1.5 mm. hatte ; nur der unterste Theil desselben ist hohl (Praeputium), auch an der Innenseite blau, am Boden der Höhle die gewöhnliche, wenig vortretende papilläre glans. Die *Spermatotheke* gross, kugelförmig, von 5 mm. Diam., die Ausführungsgänge nicht lang ; die *Spermatocyste* birnförmig, 2.5 mm. lang, ziemlich kurzstielig. Die *Schleim-* und *Eiweissdrüse* kaum die Hälfte der ganzen Genitalmasse betragend, 5.2 mm. lang bei einer Höhe von 4.8 und einer Breite von 3 mm., gelblichweiss und weiss ; der weite Schleimdrüsengang aussen und innen blau.

Ringsum die Gegend der Cardia, an die Leber (Niere?) angeheftet, fanden sich vier, 1.5–2 mm. lange Individuen eines mit dem *Distoma glauci*[1] wenigstens ganz nahe verwandten Thieres.

Man kennt jetzt eine kleine Reihe von *Chromodoriden* (Chr. runcinata, pantharella, sannio (Fig. 15), picturata, camoena, elegans (Fig. 16), glauca, californiensis (Fig. 14), Marenzelleri, gonatophora, sycilla (Fig. 13)), *mit* eigenthümlichen *blasenartigen Drüsenbildungen am Mantelgebräme*, wozu jetzt auch die hier untersuchte Form gehört. — Sie scheint von den schon bekannten Chromodoriden specifisch verschieden.

PHLEGMODORIS, Bgh.

R. Bergh, Malacolog. Unters., Heft XIII., 1878, pp. 593–597.

Corpus molle quasi subgelatinosum, dorso tuberculoso. Tentacula pro majore parte affixa, applanata. Branchia e foliolis tripinnatis paucis formata. Podarium sat latum, sulco marginali anteriori non profundo, labio superiore capite affixo.

Armatura labialis nulla. Radula rhachide nuda, pleuris multidentatis ; dentes intimi forma simpliciori, reliqui hamati. — Penis inermis.

Die Phlegmodoriden sind von weicher Körperbeschaffenheit, der Rücken mit Knoten und Knötchen bedeckt. Die Tentakel etwas applanirt, zum grössten Theile angeheftet. Die (retractile) Kieme aus wenigen (5) tripinnaten Federn

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[1] Vgl. meinen: Report on the Nudibranchiata. Challenger Exped., Zoöl., X., 1884, p. 18, Pl. X., Figs. 5–17.

gebildet. Der Fuss ziemlich breit, mit nicht tiefer vorderer Randfurche, die obere Lippe derselben an die Seiten des Kopfes angeheftet. — *Keine Lippenplatte.* Die Raspel ohne Mittelzahnplatten; die Seitenzahnplatten ziemlich zahlreich, die innersten von einfacherer Form, die anderen hakenförmig. — Der Penis unbewaffnet.

Die Phlegmodoriden gehören den tropischen, hauptsächlich den indischen Meeresgegenden.

1. *Phl. mephitica*, Bgh. *M. philippin.*
2. *Phl. areolata* (Ald. et Hanc.). *M. indic.*
3. *Phl. spongiosa* (Kelaart). *M. indic.*
4. *Phl.? anceps*, Bgh. *M. mexican.*

Phlegmod.? anceps, Bgh., n. sp.

Tafel I. Fig. 20-26. Tafel II. Fig. 6.

Hab. M. atlant. occ.

Von dieser Form lag ein einziges, in Alkohol mittelmässig conservirtes Individuum vor, an der Long. 89° 16′ und Lat. 23° 13′ (d. h. im mexicanischen Golfe) aus einer Tiefe von 84 Faden gefischt.

Die *Länge* des Individuums betrug 10 mm. bei einer Breite bis 5 und einer Höhe bis 2 mm.; die Länge des Fusses 7 bei einer Breite bis 2.2 mm.; die Breite des Mantelgebrämes 2 mm.; die Höhe der Rhinophorscheide 0.8, des Kiemenhügels 1 mm. — Die *Farbe* war durchgehends hell schmutzig gelblich, am Rücken mit dunklen erhabenen Punkten (Höckerchen). Die Consistenz des Körpers ziemlich weich.

Die *Form* länglich-oval, abgeplattet, mit breitem und ziemlich dünnem Mantelgebräme. Der Rücken mit Andeutung von einem medianen und jederseits einem, der Grenze des eigentlichen Rückens folgenden, lateralen Kamme; der Rücken übrigens überall mit zerstreuten spitzen Höckerchen bedeckt, die, besonders am Mantelgebräme, durch Ausläufer oft mit einander verbunden waren; am medianen Kamm so wie an den hohen Rhinophorscheiden (Fig. 20), und am hohen Kiemenhügel waren die Hökerchen höher und dichter stehend, besonders am Rande von jenen und diesem. Die Keule der Rhinophorien beiläufig so hoch wie die Rhinophorscheide, mit etwa 25 dünnen Blättern; die Kieme aus 5, bis 1.2 mm. hohen, einfach- hier und da doppelt- gefiederten Blättern gebildet, von denen die 3 vorderen höher; die Analpapille niedrig. Die Unterseite des Mantelgebrämes eben. Die Körperseiten ganz niedrig; die Genitalpapille wie gewöhnlich. Der Fuss nicht schmal, vorn gerundet und mit Randfurche; die obere Lippe stark vorspringend, in der Mitte ausgerandet; der Schwanz nicht ganz kurz. Die Tentakel fingerförmig.

Das *Centralnervensystem* abgeplattet; die cerebro-pleuralen Ganglien ziemlich rundlich, die Grenze zwischen den zwei Abtheilungen derselben wenig ausgeprägt; die pedalen Ganglien rundlich, grösser als die pleuralen, ausserhalb derselben liegend. Die proximalen Riechknoten fast sessil, ziemlich gross; die einander fast berührenden buccalen und die gastro-oesophagalen Ganglien wie gewöhnlich; die kugelförmigen sessilen Ganglia optica kleiner als die Augen.

Die *Augen* ziemlich gross, fast sessil, mit reichlichem schwarzem Pigment. Die *Ohrblasen* etwas kleiner als die Augen, von beiläufig 0.08 mm. Diam., von Otokonien gewöhnlicher Art strotzend, die einen Durchmesser bis 0.009 mm. erreichten. In den Blättern der *Rhinophorien* lange, aber nicht stark erhärtete, auf dem Blattrand senkrecht und schiefstehende Spikel. In der *Rückenhaut* sehr zahlreiche, lange, mehr oder weniger erhärtete Spikel, die auch, und zum Theile bündelweise, in den Höckerchen vorkommen, hier aber weniger erhärtet und meistens mit den Spitzen am Scheitel der (Fig. 6, Fig. 21) Höckerchen hervorragend; eben derselben Art war das Verhältniss an den Rhinophorscheiden und am Kiemenhügel.

Die aussen weisslich, innen gelbliche (Fig. 22) *Mundröhre* stark, etwa 1.5 mm. lang; hinten scheinen mehrere drüsenartige Körper einzumünden (Fig. 22). — Der kräftige *Schlundkopf* etwa so lang wie die Mundröhre, hinten an der Unterseite trat die Raspelscheide als eine dicke Papille hervor; die kräftige, rundliche, gelblichgraue Lippenscheibe zeigte sich von einer starken gelben Cuticula überzogen. Die *Zunge* breit und etwas abgeplattet; in der breiten gelben Raspel 7 Zahnplattenreihen, von denen die erste sehr incomplet; weiter nach hinten 8 entwickelte und zwei jüngere Reihen; die Gesammtzahl derselben somit 17. Die Rhachis ziemlich breit, nackt; von lateralen Platten jederseits 17–18 hinten an der Zunge und weiter nach hinten 19–20. Die Zahnplatten horngelb. Die Länge der 4 innersten betrug meistens 0.06–0.08–0.1–0.11 mm.; die Höhe des Hakens der Platten übrigens bis 0.11 steigend, die der äussersten nur 0.04–0.06 mm. betragend. Die innersten (Fig. 23, 24) 4 Platten sind wenig gebogen, schlanker, mehr aufrecht; danach entwickelt sich schnell die durch die Reihe bleibende Form (Fig. 25), die allergewöhnlichste Hakenform; die äusserste oder die zwei äussersten Platten mit verkürztem Körper, mehr aufrecht stehend (Fig. 26 *aa*); die äusserste schlanker als die nächst stehenden.

Die weisslichen *Speicheldrüsen* langgestreckt.

Die *Speiseröhre* beiläufig so lang wie der Schlundkopf, ziemlich weit. Der 1.5 mm. lange, freie *Magen* und der Darm wie gewöhnlich. Die Verdauungshöhle leer. — Die hintere Eingeweidemasse (*Leber*) kurz-kegelförmig, vorne schief abgestutzt, hinten gerundet, schmutzig-weisslich.

Das Pericardium mit dem Herzen wie gewöhnlich; ebenso die abgeplatteten, gräulich-weisslichen Blutdrüsen.

Die *Zwitterdrüse* schien den grössten Theil der Leber zu überziehen. kaum etwas heller als diese; in den Läppchen Massen von Zoospermien. — Die *vordere Genitalmasse* beiläufig 1.5 mm. lang, etwas zusammengedrückt; die Ampulle des Zwitterdrüsenganges ziemlich dick, wurstförmig, gebogen, ausgestreckt ein wenig länger als die Genitalmasse, opak gelblichweiss. Der Samenleiter nicht lang, der kurze Penis schien unbewaffnet. Die Spermatotheke kugelförmig; die Spermatocyste sackförmig, gebogen, etwas kleiner. Die den grössten Theil der Genitalmasse bildende Schleimdrüse weisslich, die Eiweissdrüse gelblich.

Ob diese Form nun wirklich zur Gattung Phlegmodoris gehört, ist sehr zweifelhaft. Diese Thierform zeigt wie die letztere Gattung die inneren Seitenzahnplatten von einfacherer Form, hat auch eigenthümliche drüsenartige Körper hinten am Mundrohre, so wie stark vortretende Rhincphorscheiden. Die Kieme ist hier aber nicht tripinnat wie bei den Plegmodoriden, und das Vorderende des Fusses scheint von anderer Beschaffenheit.

Fam. DORIDIDÆ PHANEROBRANCHIATÆ.

NEMBROTHA, Bgh.

R. Bergh, Malacolog. Unters., Heft XI., 1877, pp. 450–461.
R. Bergh, Beitr. zu einer Monogr. d. Polyceraden, II. Verh. d. k. k. zool. bot. Ges. in Wien, XXX., 1880, pp. 658–663; III. Ib., XXXIII., 1883, pp. 164, 165.

Corpus limaciforme, fere laeve; tentacula breviora, lobiformia; rhinophoria retractilia clavo perfoliato; branchia paucifoliata, foliolis bi- vel tripinnatis; podarium angustius.

Armatura labialis inconspicua vel nulla. Radula sat angusta; rhachis dentibus depressis subquadratis vel arcuatis; pleurae dente laterali majori falciformi singulo et dentibus externis depressis compluribus.

Glandula hermaphrodisiaca hepate connata; prostata discreta nulla; glans penis armata.

In den Formverhältnissen stehen diese Thiere *den Trevelyanen sehr nahe*, zeigen auch den Körper Limax-artig, eben, und den Fussrand von den Körperseiten fast nicht oder nur wenig vortretend. Die Tentakel sind auch kurz, lappenförmig; die Rhinophorien retractil, mit durchblätterter Keule. Die (nicht retractile) *Kieme* auch an etwa der Mitte der Länge des Rückens stehend, aber *aus wenigen* (3–5) *Federn gebildet.* — *An der Lippenscheibe keine Bewaffnung* oder eine ganz schwache (N. nigerrima). Die Zungenbewaffnung gewissermassen an die der Polyceren erinnernd. An der Rhachis kommen (im Gegensatze zu der nackten Rhachis der Trevelyanen) subquadratische oder bogenförmige, niedergedrückte *Mittelzahnplatten* vor; neben der Mittelzahnplatte eine grosse unregelmässig sichelförmige Seitenzahnplatte; die äusseren Platten niedergedrückt, ohne entwickelten Haken. Die *Zwitterdrüse* ist (im Gegensatze zu dem Verhältnisse der Trevelyanen) *von der Leber nicht gesondert.* Der Penis ist in gewöhnlicher Weise mit Hakenreihen bewaffnet.

Die Nembrothen sind bisher nur aus den tropischen Meeresgegenden bekannt und zwar fast nur aus dem philippinischen und dem Stillen Meere.

Der kleinen Reihe von Arten wird die untenstehende neue aus dem mexicanischen Golfe hinzufügen sein.

1. *N. nigerrima*, Bgh. *M. philippin., pacific.*
2. *N. Kubaryana*, Bgh. *M. pacific.*
3. *N. gracilis*, Bgh. *M. philippin.*
4. *N. cristata*, Bgh. *M philippin.*
5. *N. morosa*, Bgh. *M. philippin.*
6. *N. diaphana*, Bgh. *M. philippin.*
7. *N. gratiosa*, Bgh., n. sp. *M. mexican.*
8. *N.? Edwardsi* (*Angas*). *M. pacific.*

N. gratiosa, Bgh. n. sp.

Tafel II. Fig. 1-5. Tafel III. Fig. 1-4.

Hab. Sinum Mexicanum.

Es fand sich nur ein einziges Individuum vor, an der Breite von 24° 26′ und Länge von 83° 16′ aus einer Tiefe von beiläufig 36 Faden gefischt.

Das *in Alcohol bewahrte* Individuum hatte eine *Länge* von 22 bei einer Höhe von 6 und einer Dicke von 4 mm., die Höhe der Kieme noch 4 mm. betragend; die Höhe der Rhinophorien 2.5, des Schwanzkammes so wie der Rhinophorkämme 1.5 mm.; die Breite des Fusses 2.5 mm. — Die *Farbe* des Thieres wird im Leben prachtvoll gewesen sein; die Grundfarbe des Körpers war jetzt hell gelblich, am Rücken wie an den Körperseiten mit zahlreich zerstreuten, runden und ovalen, grüngrauen und graugrünen Flecken von einem Diam. von meistens 0.6–0.8 mm.; die Rhinophorkämme an ihrem Grunde aussen von einer Linie von ähnlicher Farbe eingefasst, ihr oberer Rand schwarzblau, ebenso der Stirn; die Rhinophorien schwarzblau oben, gelb unten; der Rand der Becherartigen Tentakel schwarzblau, ebenso der Scheitel und der Grund der Höcker des Schwanzkammes und des Fussrandes oben die Rhachis-Parthien der Kiemenblätter hell gelblich, das Laub schwarzblau; die Fusssohle gelb.

Das Thier war von etwas mehr zusammengedrückter *Form* und länger als andere bekannte Nembrothen. Die Tentakel wegen einer sich ihrer Länge nach erstreckenden Furche fast ohrenförmig, am äusseren Ende etwas gelöst (Fig. 2 *a*). Zwischen den Tentakeln der rundliche Aussenmund. Oberhalb des Mundes tritt der ziemlich schmale, im Vorderrande ein wenig ausgekerbte *Stirn* etwa 1.5 mm. hervor. Hinter dem Stirne erhebt sich jederseits ein starker *Rhinophorkamm* (Fig. 1 *a*) mit gebogenem, ebenem Rande; innen am Grunde des Kammes die rundliche Oeffnung der Rhinophorhöhle, der Rand derselben hinten mit einem vortretenden Zipfel (Fig. 1 *c*); die Rhinophorien kurzstielig, ihre Keule mit etwa 35–40 Blättern. Der *Rücken* schmal, gerundet in die Körperseiten übergehend; ein wenig vor seiner Mitte stand die *Kieme*, von drei doppelt-fiederigen Blättern gebildet, von denen das hinderste an seinem Grunde noch ein kleines Blatt trug. Dicht hinter der Kieme die wenig vortretende Analpapille, an ihrem Grunde rechts die feine Nierenpore. Die Mitte des *Schwanzes* (des hinter der Kieme liegenden Körpertheils) trug (in einer Länge von 5 mm.) einen *Kamm*, der sich in mehrere, grössere und kleinere, zusammengedrückte, oben gerundete Höcker erhebt. Die *Körperseiten* ziemlich hoch; die (zusammengezogene) Genitalöffnung in der Mitte zwischen dem Hinterrande des Rhinophorkammes und der Kieme, etwas nach oben liegend. Der *Fuss* wie gewöhnlich schmal; der Vorderrand mit tiefer (Fig. 2 *b*) Furche; das Fussgebräme schmal.

Die Eingeweide schimmerten am Vorderkörper undeutlich (weisslich) durch. — Das Peritonaeum farblos. Die Eingeweidehöhle sich nur bis etwa dicht hinter der Gegend der Analpapille erstreckend.

Das *Centralnervensystem* in eine dünne Bindesubstanzhülle eingeschlossen; die Ganglien ziemlich dick. Die cerebro-pleuralen Ganglien je ein fast 8-Zahl-ähnliche Masse bildend; die beiden Abtheilungen derselben fast gleichgross; die rundlichen, von vorne nach hinten nur ein wenig zusammengedrückten, pedalen Ganglien etwas grösser als die pleuralen; die gemeinschaftliche Commissur ziemlich kurz, nur noch ein halbes Mal so lang wie der Querdurchmesser des pleuralen Ganglions. Die proximalen Riechknoten fast sessil, zwiebelförmig; die distalen ein wenig grösser, kugelförmig. Die buccalen Ganglien abgeplattet-rundlich, fast unmittelbar mit einander verbunden, etwa so gross wie die proximalen Riechknoten; gastro-oesophagale Ganglien wurden nicht gesehen.

Die *Augen* kurzstielig, mit schwarzem Pigment, hellgelblicher Linse. Die *Ohrblasen* etwas kleiner, mit runden und ovalen Otokonien gewöhnlicher Art gefüllt. Die Blätter der Keule der Rhinophorien ohne Spikel. In der *interstitiellen Bindesubstanz* kamen erhärtete Zellen nur sparsam vor.

Die *Mundröhre* kurz und weit, an Länge und in Durchmesser 1.5 mm. messend. — Der *Schlundkopf* von gewöhnlicher Form, 2.6 mm. lang bei einer Höhe und Breite von beiläufig 2 mm.; vom hintersten Theil der Unterseite ragt die Raspelscheide 0.75 mm. hinab; die Lippenscheibe ziemlich gross, nur von einer, besonders im Innenmunde und oben, ziemlich starken gelblichen Cuticula überzogen. Die *Zunge* stark, etwas abgeplattet. In der hell horngelben, in der Randparthie (wegen der Aussenplatten) braungelben Raspel 10 Zahnplattenreihen; weiter nach hinten fanden sich deren 4 entwickelte und 2 jüngere; die Gesammtzahl der Reihen somit 16. Die vorderste Reihe war auf die mediane Platte und die letzte Aussenplatte reducirt. Die Reihen sonst an jeder Seite der medianen eine laterale und drei Aussenplatten enthaltend. Die medianen und die Aussenplatten stark horngelb, die lateralen fast farblos. Die Breite der vordersten medianen Platten 0.24, der hintersten 0.29 mm.; die Länge der lateralen Platten hinten an der Zunge 0.56, die Länge der Aussenplatten von innen nach aussen meistens 0.2–0.18–0.14 mm. Die *medianen Platten* (Taf. II. Fig. 3) flach, mehr breit als lang; der Vorderrand nicht umgebogen, convex, nicht oder kaum in der Mittellinie ausgerandet, mit etwas vortretendem gerundetem Ecken; der Hinterrand mit dem Vorderrande parallel, etwas dünner als dieser (Fig. 3 *a*); die Seitenränder fast gerade, mit einander parallel. Die *lateralen Platten* (Taf. III. Fig. 1 *aa*, 2) gross, unregelmässig, sichelförmig oder eigentlich gleichsam unregelmässige, ein wenig zusammengebogene, zum Theil am Rücken ausgehöhlte, in den Rändern theilweise verdickte und oben kurz-gekluftete (Fig. 1, 2) Blätter bildend; von dem Doppelthaken der Spitze ist der untere Theil der kleinste. Von den drei *Aussenplatten*, die alle vorne breiter waren, war die innerste fast doppelt so gross wie die folgende, subquadratisch, mit einem ziemlich starken, nach innen gerichteten Kamm (Fig. 1 *bb*). Die folgende Platte war ziemlich convex (Fig. 1 *cc*, 3 *aa*), mit Andeutung einer Längsleiste. Die äusserste Platte (Fig. 1 *dd*, 3 *bb*) war auch convex, nicht halb so gross wie die vorige.

Die gelblichweissen, nicht recht dicken *Speicheldrüsen* begleiteten den über den Schlundkopf verlaufenden Theil der Speiseröhre; die Ausführungsgänge kurz.

Die *Speiseröhre* etwa 3.5 mm. lang, vorne weiter, hinten schmäler, sich oben am Vorderende der hinteren Eingeweidemasse in die Leberhöhle (den Magen) öffnend. Der *Darm* aus der letzteren an der linken Seite der Cardia ausgehend; in seinem Verlaufe erst links, dann quer, dann rechts und nach hinten gehend, mehrere grosse Biegungen machend; ausgestreckt beiläufig 16 mm. messend bei einem Durchmesser von 1–1.5 mm., in seiner ganzen Länge (wegen seines Inhalts) kalkweiss. Der *Inhalt des Darmes* und der weiten Leberhöhle war thierische Masse, theilweise von Bryozoen herrührend, und parenchymatöse-pflanzliche.

Die hintere Eingeweidemasse (*Leber*) 11 mm. lang bei einer Höhe und Breite von 4; sie war fast cylindrisch, hinten gerundet, vorne schief nach unten und vorne abgestutzt; ihre Farbe war aussen schwärzlichgrau, dieselbe aber zum grössten Theil von der Zwitterdrüse verdeckt; die Substanz der Leber und die Wand der weiten Höhle schwarz oder schwarzbraun.

Das Herz wie gewöhnlich. Die *Blutdrüse* gelblichweiss, queroval, ziemlich abgeplattet, hinter dem Centralnervensystem liegend und etwa so breit wie dieses. — Die Niere wie gewöhnlich, der *Nierentrichter* birnförmig, etwa 0.55 mm. lang, mit etwa 10 Hauptfalten.

Die gelbliche *Zwitterdrüse* mit einem fast einfachen Lager von dichtstehenden meistens an einander stossenden Läppchen (Taf. II. Fig. 4), die Leber fast überall überziehend. In den Ovarialfollikeln der Läppchen grosse Eierzellen, in der nicht sehr abgeplatteten Testicularplatte keine reife Zoospermien. Der dünne weissliche *Zwitterdrüsengang* frei an der rechten Seite der Cardia vortretend und längs der Speiseröhre an die *vordere Genitalmasse* verlaufend. Diese letztere, etwa 2.5 mm. lang bei einer Breite und Dicke von 2.2 mm.; die Ausführungsgänge noch 1.6 mm. vortretend; das Vorderende der Masse wird von der Schlinge der Ampulle des Zwitterdrüsenganges gebildet; hinten an der oberen Seite liegt die grosse Samenblase, und an der äusseren (rechten) Seite schlängelte sich der Samenleiter. Die erwähnte Ampulle wurstförmig, stark zusammengebogen, ausgestreckt 3 mm. lang bei einem Durchmesser von 0.8. Der stark geschlängelte prostatische

Theil des Samenleiters etwa 5 mm. lang; der muskulöse beiläufig 4 mm. lang, eine grosse Schlinge bildend, unten endigte derselbe als eine kleine Glans am Boden des etwas dickeren, etwa 0.7 mm. langen *Praeputiums*. In fast dem unteren Viertel des musculösen Samenleiters findet sich eine sich bis in die Glans fortsetzende *Bewaffnung*. Dieselbe besteht aus etwa 10–12 Quincunx-Reihen von kleinen gelblichen Haken, die eine Höhe bis zu beiläufig 0.02 mm. erreichen (Fig. 5). Die *Spermatotheke* (Taf. III. Fig. 4 *a*) kugelförmig, von etwa 1 mm. Durchmesser. Die (von dem Samenleiter verdeckte *Spermatocyste* ein wenig kleiner, auch (Fig. 4 *d*) kugelförmig; ihr Ausführungsgang etwas länger als die Blase, in den uterinen Ausführungsgang der Spermatotheke (Fig. 4 *c*) übergehend. Die Schleim- und Eiweissdrüse (wie alle die übrigen der vorderen Genitalmasse gehörenden Organe) weiss und gelblichweiss. Das Vestibulum genitale mit starken Längefalten.

Diese unzweifelhaft neue Form der Gattung Nembrotha scheint der N. diaphana am Nächsten zu stehen, unterscheidet sich aber schon im Aeusseren deutlich genug durch die starken Rhinophorkämme und durch die ganz verschiedene Farbenzeichnung, noch dazu durch die etwas verschiedene Beschaffenheit der Raspel.

Fam. PHYLLIDIADÆ.

PHYLLIDIOPSIS, Bgh.

R. Bergh, Neue Beitr. zur Kenntn. d. Phyllidiaden. Verh. d. k. k. zool. bot. Ges. in Wien, XXV., 1875, pp. 661, 670–673, Taf. XVI. Fig. 11–15.
R. Bergh, Malacolog. Unters. (Semper, Philipp. II. ii.), Heft XVI., 2, 1889, pp. 859, 866–867, Taf. LXXXIV. Fig. 23–27.

Dorsum ut in Phyllidiis propriis. Apertura analis dorsalis.
Tubus oralis ut in Doriopsidibus; glandula ptyalina discreta (?).

Die Phyllidiopsen bilden gewissermassen ein interessantes Zwischenglied zwischen den Phyllidien und den Doriopsen. Im Ganzen sehen sie den ächten Phyllidien ähnlich aus und haben dieselbe Lage der Analöffnung. Die Tentakel sind sehr klein und wie bei den Doriopsen ihrer ganzen Länge nach angeheftet. Die Mundröhre ist wie bei den Doriopsen; es scheint, auch wie bei den Doriopsen, eine gesonderte Mundspeicheldrüse (Gland. ptyalina) vorzukommen.

Die Gruppe ist, wie andere Phyllidiaden, nur aus den tropischen Meeresgegenden bekannt, und umfasst bisher nur die unterstehenden Arten.

1. *Ph. cardinalis*, Bgh. *M. pacific.* (Ins. Tonga).
2. *Ph. striata*, Bgh. *M. africano-indic.* (Maurit.).
3. *Ph. papilligera*, Bgh., n. sp. *M. mexicanum.*

Phyllidiopsis papilligera, Bgh., n. sp.

Tafel II. Fig. 7-14.

Hab. M. mexicanum.

Von der Form lag nur ein einziges Individuum vor, aus einer Tiefe von 101 Faden an 25° 33′ Br. und 84° 21′ L. (d. h. im mexicanischen Golfe) hinauf gefischt.

Das in Alkohol bewahrte Individuum hatte eine *Länge* von 12 bei einer Breite bis 11 und einer Höhe bis 4.5 mm.; die Breite des Mantelgebrämes 3 mm., die Höhe der (zurückgezogenen) Rhinophorien 1.5 mm.; die Länge des Fusses 7.5 bei einer Breite bis 6 mm. — Die *Grundfarbe* des Rückens weisslich, an derselben viele runde und ovale, grosse und kleine, sammetschwarze (bis 2.5 mm. breiten) Flecken, die meistens Papillen tragen, welche theilweise auch schwarz sind; an der weisslichen Unterseite des Mantelgebrämes schimmerten die schwarzen Rückenflecke durch; die übrige Unterseite (gelblich-) weisslich. Die Rhinophorien und der Aussenmund gelblich.

Die *Form* fast rundlich, etwas gewölbt (Fig. 7), mit breitem dünnem Mantelgebräme. Die Consistenz des Thieres nicht hart, nicht recht steif. Der Rücken eben, aber mit ziemlich zahlreichen, bis etwa 1.6 mm. hohen, zusammengedrückten, mehr oder weniger, besonders an der einen (meistens vorderen) Seite, schwarzfarbigen Papillen bedeckt. Die Rhinophoröffnungen (Fig. 7 *a*) ziemlich weit von einander liegend, die starke Rhinophorkeule mit etwa 20–25 Blättern. Die Analpore median hinten am Rücken (Fig. 7 *b*). Der innerste Theil des Mantelgebrämes ist dicht mit quergehenden, meistens an der Mitte höheren, bis 1.5 langen dünnen Blättern bedeckt; hinten begegnen sich die Blätterreihen über den Schwanzgrund, vorn erstrecken sie sich bis an den Aussenmund; die Anzahl der Blätter jederseits 45–50. Keine Spur von Tentakeln wurde gesehen; der Aussenmund fand sich als eine starke durchbohrte Papille vor dem Vorderrande des Fusses. Die Genitalpore an gewöhnlicher Stelle der niedrigen (rechten) Körperseite. Der Fuss gross, breit, vorne abgestutzt-gerundet und mit Randfurche, das Fussgebräme nicht schmal, der Schwanz nicht kurz.

Das *Centralnervensystem* (Fig. 9) zeigte die cerebro-pleuralen Ganglien nierenförmig, schräge gegen einander liegend, nach vorne convergirend (Fig. 9 *ab*); die pedalen Ganglien an der Unterseite der pleuralen liegend, grösser als diese, rundlich (Fig. 9 *cc*); die gemeinschaftliche Commissur doppelt, dünn (Fig. 9 *d*). Die proximalen Riechknoten fast sessil, zwiebelförmig (Fig. 9); die distalen kugelförmig. Die buccalen Ganglien (Fig. 13 *c*) an gewöhnlicher Stelle, rundlich, einander berührend.

Die *Augen* fast sessil, von 0.1 mm. Diam., mit reichlichem schwarzem Pigment (Fig. 9). Die *Ohrblasen* weit von den vorigen an der Unterseite (Fig. 9) der Gehirnknoten liegend, von beiläufig 0.06 mm. Diam.; etwa 50–100 ovalen Otokonien von einem Durchmesser bis 0.013 mm.. enthaltend, unter denen ein grösserer rundlicher (Fig. 12). In den Blättern der Keule der *Rhinophorien*, wie gewöhnlich, dünne, mehr oder weniger erhärtete, kürzere und längere Spikel, die letzteren zum grossen Theile auf dem freien Rande senkrecht stehend (Fig. 10). In der *Haut* des Rückens eine Unmasse von grösseren und kleineren Spikeln und Bündel von solchen, welche auch unter der Loupe schon durchschimmerten (Fig. 7); im Mantelgebräme waren dieselben zum grossen Theile senkrecht und schräg (Fig. 11) gegen den Rand geordnet; sonst lagen sie mehr ungeordnet. Die Spikel waren von den gewöhnlichen bei diesen Thieren vorkommenden Formverhältnissen (Fig. 10, 11), meistens stark erhärtet, oft glasartig; von einem Durchmesser bis 0.16 mm., von sehr wechselnder Länge, die oft bis über 0.4 mm. stieg. Bündel von ähnlichen Spikel stiegen in die Papillen bis an ihre Spitze auf (Fig. 8). In der *interstitiellen Bindesubstanz* kamen überall Massen von grösseren und kleineren meistens stark erhärteten Spikel vor, so wie verkalkte Klumpen und Kugeln.

Durch den Aussenmund war das Ende des Mundrohres etwas hervorgestülpt; unter jenem fand sich die Oeffnung der Mundröhrendrüse (Fig. 13 *g*). Die gelblichweisse *Mundröhre* (Fig. 13 *a*, 14 *a*) weit, nicht kurz, 2 mm. lang, hinten mit (Fig. 13) einer kreisartigen Einschnürung ; die Innenseite (Fig. 14 *a*) mit Längsfalten; in das vertiefte Hinterende derselben senkt sich der gelbliche Schlundkopf, der am Boden der Mundröhrenhöhle stark vorspringt (Fig. 14 *b*). Dieser *Schlundkopf* (Fig. 13 *b*) von gewöhnlicher Form, fast cylindrisch, von starker gelblicher Cuticula an der Innenseite überzogen, etwa 2 mm. lang; am etwas engeren Hinterende des Schlundkopfes (Fig. 13 *c*) die buccalen Ganglien. Hinter den letzteren finden sich (Fig. 13 *d*) die gewöhnlichen, hier fast kugelförmigen eigentlichen (*hinteren*) *Speicheldrüsen* (Gl. salivales) (Fig. 13 *d*). Es kommt aber jederseits (?) noch eine längliche, etwas lappige, weissliche *vordere Speicheldrüse* (Gl. saliv. access.) vor, (Fig. 13 *f*), die neben dem Schlundkopf das Hinterende der Mundröhre durchbohrt; sein Hinterende geht in einen bindegewebigen Strang über. Unter dem Schlundkopf liegt die lappige, weissliche *Mundröhrendrüse* (Gl. ptyalina), welche in einen starken Ausführungsgang übergeht, die sich hier nicht in die Mundröhre, sondern unmittelbar unter dem Aussenmunde öffnete (Fig. 13 *gg*).

Das Hinterende des Schlundkopfs geht etwas enger in die gestreckt-schlauchförmige *Speiseröhre* (Fig. 13 *e*) über, welche ein wenig kürzer als der Schlundkopf ist und die obere Seite der hinteren Eingeweidemasse durchbohrt. Die in dieser letzteren eingeschlossene *Magenhöhle* nicht weit. Der *Darm* die Eingeweidemasse am Anfang des letzten Drittels durchbrechend und in gewöhnlicher Weise verlaufend. — Die Verdauungshöhle war leer.

Die hintere Eingeweidemasse (*Leber*) 5.5 mm. lang bei einer Breite von 4 und einer Höhe von 3 mm., vorne schräg abgestutzt, hinten gerundet ; die Substanz gelblichweiss.

Das querliegende Pericardium ziemlich gross; das Herz wie gewöhnlich. Die *Blutdrüse* gerundet-viereckig, gräulichweiss. Die *Niere* in gewöhnlicher Weise die obere Seite der hinteren Eingeweidemasse überziehend; die Urinkammer wie gewöhnlich.

Die *Zwitterdrüse* durch mehr gelbliche Farbe von der Leber hier und da unterscheidbar; in den Läppchen Eierzellen und reife Zoospermien. — Die *vordere Genitalmasse* gerundet-viereckig, beiläufig 3 mm. lang. Die weissliche Ampulle des Zwitterdrüssenganges wurstförmig gebogen. Die Samenblasen weisslich; die Spermatotheke kugelförmig, die Spermatocyste eiförmig. Der Samenleiter nicht lang; das Dasein einer Penis-Bewaffnung konnte nich nachgewiesen werden. Die Schleimdrüse weisslich, die Eiweissdrüse mehr gelb.

Bisher war keine am Rücken Papillen-tragende Form von Phyllidiaden bekannt worden. Diese nimmt in dieser Beziehung eine ähnliche Stellung unter den Phyllidiaden wie die Echinodoris[1] unter den Doriden ein.

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[1] R. Bergh, Neue Nacktschnecken der Südsee, II. Journ. d. Mus. Godeffroy, Heft VI., 1874, pp. 19-22, Taf. III. Fig. 4-20.

TAFEL-ERKLÄRUNG.

TAFEL I.

Tethys leporina (L.).

Fig. 1. Verdauungssystem; *a* das (an der Unterseite gekluftete) Mundrohr; *b* vorderer, *c* hinterer Theil der Speiseröhre; *dd* die in den ganz rudimentären Schlundkopf einmündenden Speicheldrüsen, zwischen den Hinterenden derselben die buccalen Ganglien; *e* Hinterende des (ersten) Magens, *f* zweiter Magen, *g* Darm; *h* Zweig der rechten Nebenleber in das Rhinophor, *i* in die vorderste rechte Papille; *kl* linke Nebenleber mit ihren Zweigen, *m* Hauptausführungsgang der Hauptleber.

Fig. 2. Speicheldrüse (linke), mit Cam. gezeichnet (Vergr. 55).

Fig. 3. Otocyste, mit Cam. gezeichnet (Vergr. 350); *a* Stiel.

Chromodoris punctilucens, Bgh.

Fig. 4. Lippenscheibe mit Mundöffnung und Lippenplatte.

Fig. 5. Stück der Lippenplatte.

Fig. 6. Grösste Elemente derselben.

Fig. 7. Von der Rhachisparthie der Raspel; *a* rhachidiale Verdickung, *bb* innerste Seitenzahnplatte.

Fig. 8. Zahnplatte aus dem inneren Drittel einer Reihe.

Fig. 9. Eine der grössten Platten.

Fig. 10. Aeusserer Theil zweier Zahnplattenreihen; *a* äusserste Platte der Reihen.

Fig. 5–10 mit Cam. gezeichnet (Vergr. 350).

Chromodoris scabriuscula, Bgh.

Fig. 11. Elemente der Lippenplatte, von vorne.

Fig. 12. Aehnliche, von der Seite.

Fig. 13. Stück der Raspel; *a* mediane Platte.

Fig. 11–13 mit Cam. gezeichnet (Vergr. 350).

Fig. 14. Mediane Platte, von oben.

Fig. 15, 16. Zahnplatten vom inneren Drittel einer Reihe.

Fig. 17. Eine der grössten Platten.

Fig. 18. Aeusserste Platte einer Reihe.
Fig. 14–18 mit Cam. gezeichnet (Vergr. 750).
Fig. 19. *a* muskulöser Theil des Samengangs, *b* Praeputium mit zurückgezogener Glans; mit Cam. gezeichnet (Vergr. 55).

Phlegmodoris? anceps, Bgh.

Fig. 20. Rhinophorscheide, *a* Grund; mit Cam. gezeichnet (Vergr. 55).
Fig. 21. Höckerchen des Rückens, mit Cam. gezeichnet (Vergr. 200).
Fig. 22. Mundröhre, *aa* Retractoren, *b* Drüsen am Hinterrande des Mundrohres.
Fig. 23. Innerster Theil einer Zahnplattenreihe; *a* erste Platte.
Fig. 24. Aehnlicher von zwei Reihen, *ab* erste Platte derselben.
Fig. 25. Eine der grössten Platten.
Fig. 26. Aeusserster Theil zweier Zahnplattenreihen mit 8 und 9 Platten, *aa* äusserste.
Fig. 23–26 mit Cam. gezeichnet (Vergr. 350).

TAFEL II.

Nembrotha gratiosa, Bgh.

Fig. 1. Rhinophorkamm, in *b* den Rücken übergehend, *c* Rhinophoröffnung.
Fig. 2. *a* Tentakel, *b* Vorderrand des Fusses.
Fig. 3. Mediane Zahnplatte, mit Cam. gezeichnet (Vergr. 200), *a* Hinterrand.
Fig. 4. Läppchen der Zwitterdrüse.
Fig. 5. Haken der Penis-Bewaffnung, mit Cam. gezeichnet (Vergr. 750).

Phlegmodoris? anceps, Bgh.

Fig. 6. Stück der Rückenhaut, vom Rande gesehen; mit Cam. gezeichnet (Vergr. 200).

Phyllidiopsis papilligera, Bgh.

Fig. 7. Das Thier, von der Rückenseite; *a* Gegend der Rhinophor-Oeffnungen, *b* Gegend der Analpore.
Fig. 8. Eine der kleineren Rückenpapillen.
Fig. 9. Das Centralnervensystem, mit Cam. gezeichnet (Vergr. 55); *ab* cerebro-pleurale, *cc* pedale Ganglien; *d* gemeinschaftliche Commissur.
Fig. 10. Rand eines Rhinophor-Blattes, mit Cam. gezeichnet (Vergr. 350).
Fig. 11. Vom Mantelrande; mit Cam. gezeichnet (Vergr. 55).
Fig. 12. Otocyste, mit Cam. gezeichnet (Vergr. 350).
Fig. 13. *a* Mundröhre, *b* Schlundkopf, *c* buccale Ganglien, *d* Speicheldrüsen (Gl. salivales), *e* Speiseröhre, *f* Accessorische Speicheldrüsen (Gl. access.), *gg* Ausführungsgang der Mundröhrendrüse (Gl. ptyalina).
Fig. 14. *a* geöffnete Mundröhre, *b* Vorderende des Schlundkopfs.

TAFEL III.

Nembrotha gratiosa, Bgh.

Fig. 1. Zwei Reihen von pleuralen Zahnplatten (linker Seite) von oben; *aaa* laterale Platten; *bb* innerste, *cc* mittlere, *dd* äusserste Aussenplatte.

Fig. 2. Laterale Platte von der Rückenseite.

Fig. 3. Aeusserster Theil dreier Zahnplattenreihen mit den je zwei äussersten Aussenplatten.

Fig. 1-3 mit Cam. gezeichnet (Vergr. 200).

Fig. 4. *a* Spermatotheke, *b* vaginaler und *c* uteriner Gang; *d* Spermatocyste, *e* Diverticulum des Ausführungsganges der Spermatocyste.

Chromodoris sycilla, Bgh.

Fig. 5. Elemente der Lippenplatte.

Fig. 6. Zwei Zahnplatten aus der Mitte einer Reihe der Zunge.

Fig. 7. Zahnplatte vom inneren Zehntel einer Reihe.

Fig. 8. Zwanzigste Zahnplatte von aussen *ab*.

Fig. 9. Eine der innersten Seitenplatten.

Fig. 10. Vierte Zahnplatte, von aussen *ab*.

Fig. 11. Dritte Zahnplatte, von aussen *ab*.

Fig. 12. Die drei äussersten Zahnplatten, von innen; *a* äusserste.

Fig. 5-12 mit Cam. gezeichnet (Vergr. 350).

Fig. 13. Hinterende des Körpers, von oben (Mantelgebräme), mit den weissen Flecken; *aa* Drüsenbildungen der Unterseite des Mantelgebrämes.

Chromod. Californiensis, Bgh.

(Vgl. R. Bergh, On the Nudibr. Gaster. Moll. of the North-Pacific Ocean. II., 1880, Pl. XIV. Fig. 5. Scientific Results of the Explor. of Alaska, Vol. I. Art. vi., 2.)

Fig. 14. Hinterende des Mantelrandes, von der Unterseite, mit 6 Drüsen beutel; *a* Fuss.

Chromod. sannio, Bgh.

(Vgl. R. Bergh, Malacolog. Unters. (Semper, Philipp. II. ii.), Heft XVII., 1890, Taf. LXXXVII. Fig. 1.)

Fig. 15. Hinterende des Mantelrandes, von der Unterseite, mit 4 grossen Drüsenbeuteln; *a* Fuss.

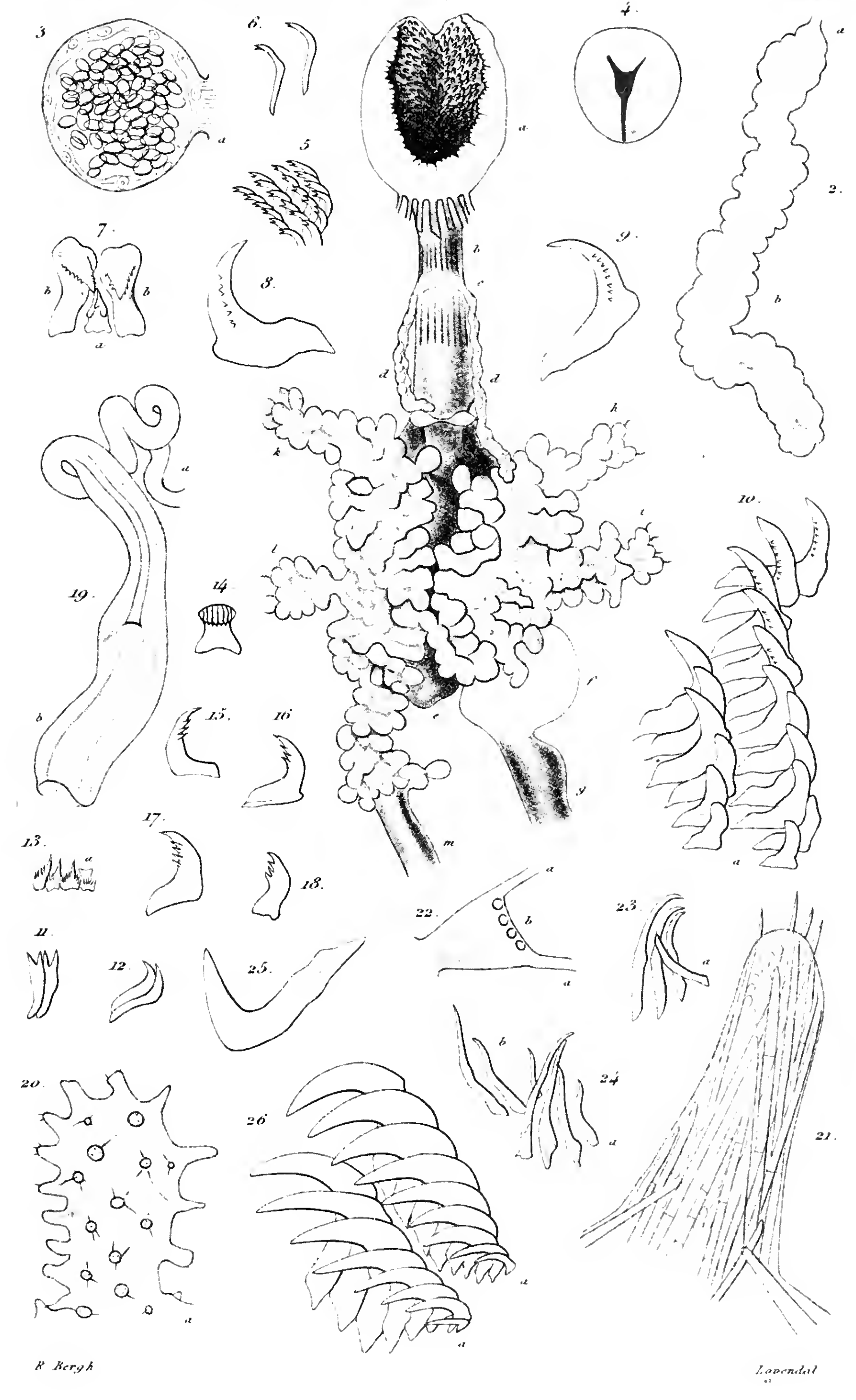
Chromod. elegans (Cantr.).

(Vgl. R. Bergh, Untersuch. d. Chromod. elegans und villafranca. Malakozool. Blätter, XXV., 1878, Taf. I. Fig. 4.)

Fig. 16. Drüsenbeutel von der Unterseite des Mantelgebrämes.

Blake Mollusca . Nudibranchiata

Plate I



R. Bergh

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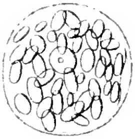

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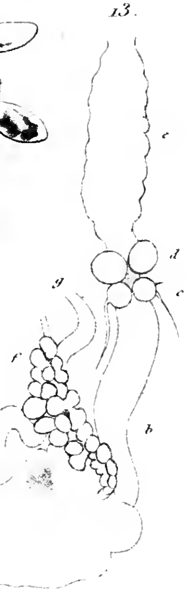

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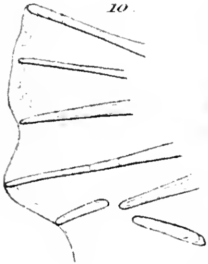

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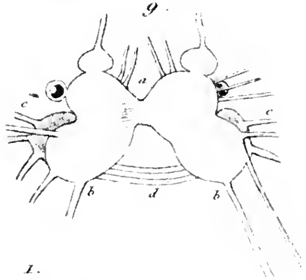

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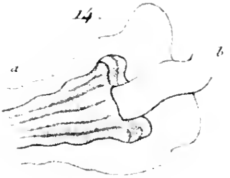

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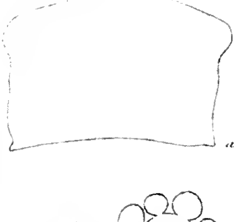

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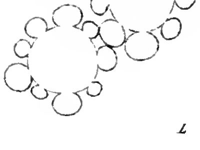

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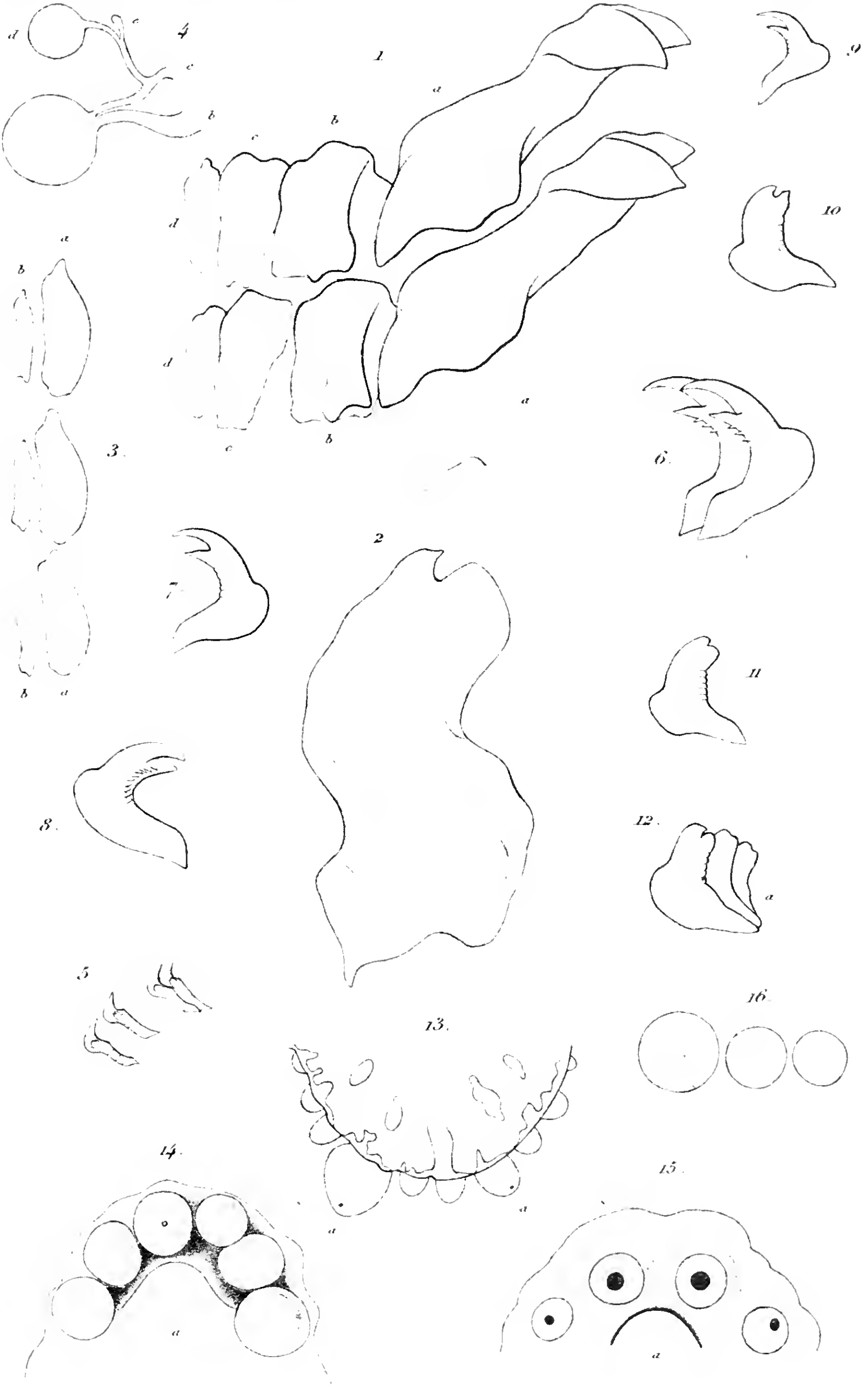

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No. 4. — *A Third Supplement to the Fifth Volume of the Terrestrial Air-Breathing Mollusks of the United States and adjacent Territories.* By W. G. Binney.[1]

As promised in the Second Supplement, the Eastern Province Species are here given, with addenda to those of the other Provinces. My purpose is to bring the subject down to this date. The "Manual of American Land Shells," published subsequently to Vol. V., must also be used in connection with the present paper. I have added figures of many species to replace those of Volume V.

Burlington, New Jersey, January 1, 1890.

SPECIES OF THE NORTHERN REGION.

It must be borne in mind that the Universally Distributed Species are also found here. They are: —

Patula striatella, Anthony.

Microphysa pygmæa, Drap.

Placed in this genus on account of the similarity of its jaw and lingual dentition to those of other species of *Microphysa*. See 2d Suppl., p. 35.

Helicodiscus lineatus, Say.

Vallonia pulchella, Müll.

Pupa muscorum, Linn.

See below, p. 186, for vars. *bigranata* and *Lundstromi*.

It may readily be doubted whether this species is not rather confined to the Northern Region.

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[1] The Terrestrial Air-Breathing Mollusks of the United States and the adjacent Territories of North America, described and illustrated by Amos Binney. Edited by A. A. Gould. Boston, Little and Brown, Vols. I., II., 1851; III., 1857. Vol. IV., by W. G. Binney, New York, B. Westermann, 1859 (from Boston Journ. Nat. Hist.). Vol. V., forming Bull. Mus. Comp. Zoöl., Vol. IV., 1878. Supplement to same, in same, Vol. IX. No. 8, 1883. Second Supplement, in same, Vol. XIII. No. 2, 1886.

Zonites nitidus, Müll.
arboreus, Say.
indentatus, Say.

See Suppl., p. 139.

Zonites minusculus, Binn.

Dall thus describes a var. *Alachuana* (Pr. U. S. Nat. Mus., 1855, 270): —

> A form of it which, at first sight, looks different from *minuscula* is rather larger than usual, and above shows no differences. On the base in the type the junction of the inner lip with the body whorl takes place, following the course of the whorl, inward from the middle line of the base of the whorl and generally about the inner third. This gives a peculiarly thimble-shaped umbilicus. In the variety under consideration, the above mentioned junction takes place outside of the middle line, or even at the outer third, while the aperture is a little dilated. The result of this is to show a much larger portion of the base of the penultimate whorl, and to alter the facies of the umbilicus. For this form, found in Alachua County, Florida, I would suggest the varietal name *Alachuana.*

Zonites viridulus, Mke.
milium, Morse.
fulvus, Drap.

These will not be repeated in the lists of the various Regions into which the Province may be divided. (See Vol. V., p. 17.)

The following are Northern Region Species: —

Vitrina limpida, Gld.
Angelicæ, Beck.

Vitrina exilis, Morelet.

The distinction between the Eastern, Central, and Pacific Provinces not being marked in these high latitudes, this species is given here. It might, perhaps, with *Patula pauper* and *Pupa borealis,* rather be considered a species of the Pacific Province.

Zonites Fabricii, Beck.
Binneyanus, Morse.
ferreus, Morse.

Zonites exiguus, Stimpson.

Plate III. Fig. 4.

The figures are copies of original drawings of Dr. Stimpson.

Zonites multidentatus, Binney.

See Suppl., p. 144.

Acanthinula harpa, Say.
Patula asteriscus, Morse.

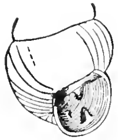
Patula pauper, Gould.

See remarks under *Vitrina exilis*, above.

Pupa Blandi, Morse.
borealis, Morelet.

See remark under *Vitrina exilis.*

The figure was drawn by me from a specimen collected at the original locality.



Pupa borealis, enlarged.

Pupa decora, Gould.
Höppii, Möller.
Vertigo Gouldi, Binney.
Bollesiana, Morse.

A variety *Arthuri*, from Dakota, is mentioned by Von Martens, Gesell. Nat. Freunde zu Berlin, 21 Nov., 1882, p. 140.

Very near, if not identical with, *V. milium.*

Vertigo simplex, Gould.
ventricosa, Morse.

Very near, if not identical with, *V. Gouldi.*

Ferussacia subcylindrica, Linn.

In the mountains of McDonnel Co., North Carolina, a colony of this species was found by Mr. Hemphill. He found no colony of *Vitrina*, which might be expected to exist at those high elevations.

Succinea Haydeni, W. G. B
Verrilli, Bland.
Grönlandica, Beck.
Higginsi, Bland.
Totteniana, Lea.

Dr. Westerlund, in the " Land- och Söttvatten-Mollusker " of the Vega Expedition, quoted in the Manual of American Land Shells, pp. 473, 474, also catalogues from Arctic America the following species: —

Limax hyperboreus, Westerlund. (See below, p. 205.)
Pupa arctica, Wall.
columella, Benz.
Succinea chrysis, Westerlund. (See p. 186.)
turgida, Westerlund.
annexa, Westerlund. (See p. 186.)
Vallonia Asiatica, Nevin.
Pupa edentula, Drap. ?
signata, Ms.
Vertigo Bollesiana, var. **Arthuri.**

Pupa muscorum, var. **bigranata**, Ross.
  muscorum, var. **Lundstromi**, Westerlund.
  columella, Benz., var. **Gredleri**, Clessin.
  Krausseana, Reinh.

Of the above, descriptions and figures are given of two only, *Succinea chrysis* and *S. annexa*, which are copied here.

Succinea chrysis, Westerlund.

(Figures copied on my Plate I. Fig. 14.)

Testa oblongo-ovata, solida, irregulariter transversim striata vel sæpe costulato-plicata, colore varia, sæpissime spira pallidiore, apice rubro, anfractu ultimo antice rotuntiore, subviolaceo-rufescente, postice pallidiore, ubique strigis transversis numerosis albidis; spira elevata, acuta, anfr. 3½, convexi, ultimus deorsum lente attenuatus, penultimus subtus tumidulus, antepenultimus transversalis, extus depressus, sutura forte excisa, anfr. ultimo minutissimo; sutura perimpressa, apertura ovata, intus aureo-micans, pariete arcuatula, obliqua; peristoma obscure marginatum, marginibus æqualiter arcuatis (exteriore superne ad insertionem forte curvato), in pariete callo tenuissimo albido conjunctis. Long. 11½, diam. 7½, ap. 7½ mm. l., 5 mm. d.; long. 13, diam. 7½, ap. long. 9, diam. 7½ mm.; long. 10, diam. 6, ap. long. 6½, diam. 5 mm.

Asia: America, Port Clarence, Alaska.

I figure also a specimen from St. Michael's, Alaska (Dall), which has usually been referred to a form of *S. lineata*.

Succinea chrysis.

Succinea annexa.

(Figures copied on my Plate I. Fig. 15.)

Testa elongato-ovata, fragilis, intus rugas incrementales fuscas (in spec. max.) validas et extus abruptas dense striata, anfr. penultimo dense distincte spiraliter lineata, anfr. ultimo transversim irregulariter alternatim rufo- et albido-strigata; sutura impressa; spira exserta, apice mamillata; afr. 4, ultimus convexus, penultimus tumidus, antepenultimus altus, exitus convexus, sutura tenui a præcedente sejunctus, summus (subtus visus) globosus; apertura ovata, pariete obliqua, columella arcuata, marginibus linea tenui alba junctis. Long. 11, diam. 8, apert. long. 8, diam. 6 mm.; long. 10, diam. 6½, apert. long. 6, diam. 4½ mm.

Fort Clarence, Alaska.

INTERIOR REGION SPECIES.

Macrocylis concava, Say.
Zonites capnodes, W. G. B.
  fuliginosus, Griffith.
  friabilis, W. G. B.

Zonites lævigatus, Pfeiffer.
Rugeli, W. G. B.

See Suppl., p. 138.

Zonites demissus, Binney.

The variety *acerrus* has been found near Fort Gibson, Indian Territory, by Mr. Simpson.

Zonites ligerus, Say.

A variety *Stonei* is thus described by Mr. Pilsbry: "From Mr. Witmer Stone I have received a form of *Z. ligerus* differing from the type in having a concave, broadly excavated base, with comparatively wide umbilicus, collected by him in New Castle Co., Del. The axis in the type is barely perforated; but in this form it is a millimeter or more wide, and the base around it broadly concave." (Nautilus, III. 4, p. 46, Aug., 1889.)

Zonites intertextus, Binney.
subplanus, Binney.

See Suppl., p. 139.

Zonites inornatus, Say.
sculptilis, Bland.
Elliotti, Redfield.
limatulus, Ward.
capsella, Gould.

Lawæ, W. G. B.

See Suppl., p. 142, Plate II., Fig. E. The name is suggested for the shell figured by me in Vol. V. (Fig. 44) as *Z. placentula*.

Zonites placentula, Shuttleworth.

See Suppl., p. 142.

Zonites Wheatleyi, Bland.

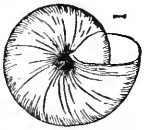
See Suppl., p. 141. Clingham's Peak, N. C. (Hemphill).

Zonites petrophilus, Bland.

Habersham Co., Ga.; Clarkesville, N. C. (Hemphill). See Suppl., p. 140.

Zonites Sterkii, Dall.

Shell minute, thin, yellowish translucent, brilliant, lines of growth hardly noticeable, spire depressed, four-whorled; whorls rounded, base flattened, somewhat excavated about the centre, which is imperforate; aperture wide, hardly oblique, not very high, semilunate, sharp-edged, the upper part of the columella slightly reflected; upper surface of the whorls roundish, though the spire as a whole is depressed. Greater diam. 1.1, height 0.52 mm.



Zonites Sterkii, enlarged.

New Philadelphia, Ohio. Collected on a grassy slope, inclining to the northward, and covered with grass, moss, and small bushes, and so far has not been found elsewhere. Clearly not young of a *Pupilla* or *Zonites*. It is probably one of the smallest species known, and remarkable for its imperforate umbilicus.

The above forms a portion of the description by Dall of *Hyalina Sterkii*, from Proc. U. S. Nat. Mus., XI., p. 214, Figs. 1, 2, 3, 1888. The figure given by me is drawn from an authentic specimen.

Zonites gularis, Say.
suppressus, Say.
cuspidatus, Lewis.

See Suppl., p. 143.

Miss Law thus wrote from Philadelphia, Tenn., of this species: "Unlike *gularis* it seems to be a rare shell, and I find it only by scraping off the surface of the ground in the vicinity of damp mossy rocks. Its habits are more like *placentula* than *gularis*. Neither Miss Clara Bacome nor I ever mistake one for a *gularis*, even before picking it up; the thickened yellow splotch near the lip, and the thinner spot behind, showing the dark animal through it, as well as its more globular form, particularly on the base, make it look very different when alive."

Zonites lasmodon, Phillips.

Plate III. Fig. 5.

Enlarged drawings by Miss Lawson are given of this species.

Zonites macilentus, Shuttl.

See Suppl., p. 143.

Zonites significans, Bland.

See Suppl., p. 144.

Zonites Andrewsi, W. G. B.

See Suppl., p. 144.

Zonites internus, Say.
Vitrinizonites latissimus, Lewis.

See Suppl., p. 145; for other localities, see Man. of Am. Land Sh., p. 231, Also in Washington Co., N. C., and in Watauga Co. at Banner's Elk (Hemphill).

Limax campestris, Binney.

Limax montanus, castaneus, occidentalis, hyperboreus, and *Hemphilli* are probably identical with this.

Tebennophorus Caroliniensis, Bosc.
Tebennophorus dorsalis, Binney.
Tebennophorus Wetherbyi, W. G. B.

See Plate VI. Fig. F.

Tebennophorus Hemphilli, W. G. B.

Plate VI. Fig. H.

See Man. of Amer. Land Sh., p. 247.

The animal is long, narrow, cylindrical, with pointed tail. Its color is black. The jaw is strongly arched, with median projection, and four or five ribs converging to the centre, all crowded on the middle third, the outer thirds being ribless. The lingual membrane has 24–14–1–14–24 teeth, all of same types as figured by Morse for that of *T. dorsalis*. Length of largest individual contracted in spirit 25 mm.

The penis sac is long, cylindrical, receiving retractor muscle and vas deferens at its summit.

Patula solitaria, Say.
alternata, Say.
Cumberlandiana, Lea.
perspectiva, Say.
Bryanti, Harper.

See Suppl., p. 147.

Helicodiscus fimbriatus, Wetherby.

See Suppl., p. 148.

A curious form, wanting the epidermal fringe and most of the revolving ridges, was found in great numbers near Fort Gibson, Indian Territory, by Mr. C. T. Simpson. The same form has been found by Mr. Hemphill on Salmon River, Idaho. He proposes for it the name *Salmonacea.*

Strobila labyrinthica, Say.

A form from Venezuela, without the costæ, is noticed by Dall as var. *Morsei* (U. S. Nat. Mus. Proc., 1855, p. 263).

Polygyra leporina, Gould.
Hazardi, Bland.
Troostiana, Lea.
fastigans, Say.
Stenotrema spinosum, Lea.
labiosum, Gould.
Edgarianum, Lea.
Edvardsi, Bland.
barbigerum, Redfield.
stenotremum, Ferussac.
hirsutum, Say.

A widely separated locality is the bank of the Yaqui River, near Guaymas (Palmer).

Stenotrema maxillatum, Gould.
monodon, Rackett.
Triodopsis palliata, Say.

Triodopsis obstricta, Say.
appressa, Say.

It is quoted by Von Martens from the banks of the Columbia River, but from drawings and description of the single specimen found by Kraus, kindly sent me by Dr. Von Martens, it appears that the species was confounded with flattened forms of *Mullani* or *devius*.

Triodopsis inflecta, Say.

A depauperated form of this species is about being described and figured as *T. edentula* by Mr. F. A. Sampson.

Triodopsis Rugeli, Shuttleworth.
tridentata, Say.

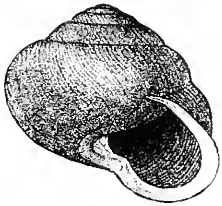
The deformed specimen figured is one of *appressa*, not of this species.

Triodopsis fallax, Say.
introferens, Bland.
Van Nostrandi, Bland.

Also, Jacksonville, Florida.

Mesodon major, Binney.

On Plate I. Fig. 2, I have figured the dentition of an individual of this species differing from that figured in Vol. V. Plate VIII. Fig. G, by wanting the side cusps and cutting points of the central and first lateral teeth. The individual from which the lingual was extracted is labelled B in the collection given by me to the United States National Museum. Fig. 3 gives an outer lateral of the same membrane, on which the side cusp and cutting point are present. Fig. 1 gives a central tooth with side cusps and cutting points from the membrane of the specimen labelled A.



Mesodon major.

The figures show a larger range of variation in the dentition of individuals of the same species than would have been anticipated. (See also *M. Andrewsi.*)

Mesodon albolabris, Say.
Andrewsi, W. G. B.

In the Manual of American Land Shells, p. 302, I have described and figured specimens of a larger form of this species, which would be called *major* by most collectors, but which has the genitalia and lingual dentition of *Andrewsi*. (See figure above.)

The penis sac of *Andrewsi* was described by me as constricted in the middle. Further study has convinced me that it is rather twisted than constricted. On Plate I. Fig. 4, I give a figure of the genitalia to show this; and in Fig. 5, the penis sac of still another individual

In studying the lingual membrane of many individuals of *M. Andrewsi*, I have found some variation. I give here notes on membranes of specimens labelled as specified in the Binney collection in the United States National Museum.

AA. 60–1–60 teeth, with about 14 laterals on each side.

N. 51–1–51 teeth, with 11 laterals; some extreme marginals have decidedly multifid cusps.

Q, from Hayesville, N. C., has also about 11 laterals.

V has 9 laterals, 60–1–60 teeth.

M. 60–1–60 teeth, with about 14 laterals. Some outer laterals have side cusps: one is figured on Plate I. Fig. 12.

G has same count as M; no side cusps to outer laterals.

N has 64–1–64 teeth, with 14 laterals. The extreme laterals have side cusps.

L has 61–1–61 teeth, with 11 laterals; no side cusps on outer laterals.

J same. 64–1–64 teeth, with 14 laterals.

B. 60–1–60 teeth, with 16 laterals, none with side cusps.

F. All laterals, even first, have decided side cusps (see Plate I. Fig. 10) and cutting points: and marginals also (Fig. 11). 50–1–50 teeth, with 15 laterals.

K. 53–1–53 teeth, with 14 laterals.

I. 50–1–50 teeth, outer laterals with side cusps.

O. 68–1–68 teeth, with 14 laterals.

As remarked above, most collectors will refer this large form of *Andrewsi* to *major*. It differs from that species as hitherto understood very decidedly in its lingual dentition and genitalia. In its shell, also, the species differs from the generally known *major* in so marked a manner, that from it alone I could say, before examination, what were the characters of the dentition and genitalia of every specimen collected by Mr. Hemphill in the mountains of North Carolina. One of the puzzling questions to be left to future solution is the limitation of *albolabris*, *major*, and *Andrewsi*. It must be studied from the lingual dentition and genitalia, as well as from the shell. The student must also consider whether the *Helix major* of the Boston Journal and of the Terrestrial Mollusks are the same species.

Practically, the simplest way of treating specimens in collections is to refer to a variety of *albolabris* all forms more resembling that species than they do the *major* of the Terrestrial Mollusks, and to call *major* all specimens most nearly conforming to the figure and description of that species in Terrestrial Mollusks of U. S., Vols. II. and III. In the former category would be placed the *major* of the Boston Journal; in the latter, the large forms I have referred to *Andrewsi* in Manual of American Land Shells, such, for instance, as are figured in Fig. 322½, repeated here, *ante*, page 190. This variety of *albolabris* and this *major*, as above identified, would be found to differ widely in dentition and genitalia, the former in these respects resembling *albolabris*, the latter *Andrewsi.* The latter species must also be recognized as subject to variation, rendering it in some cases difficult to separate from *major,* — never from the large variety of *albolabris.*

The original specimen of *major* of the Terrestrial Mollusks was included in the collection given by Mr. J. S. Phillips to the Philadelphia Academy of Sciences. The points in which it differs from the large form of *albolabris* are pointed out in Terrestrial Mollusks, Vol. II. p. 98.

Mesodon multilineatus, Say.
Pennsylvanicus, Green.
Mitchellianus, Lea.
elevatus, Say.
Clarki, Lea.
Christyi, Bland.
exoletus, Binney
Wheatleyi, Bland.
dentiferus, Binney.

In a specimen collected by Mr. Hemphill, at Banner's Elk, N. C., I found the etractor muscle of the penis sac near its junction with the vas deferens, not at half the length of the latter. There was no constriction to the penis sac.

Mesodon Wetherbyi, Bland.
thyroides, Say.
clausus, Say.
Downieanus, Bland.
Lawæ, Lewis.
profundus, Say.
Sayi, Binney.

Pupa pentodon, Say.

The enlarged view of the aperture gives on the left *P. Tappaniana,* on the right *P. curvidens.*

Under the name of *Pupilla Floridana,* Mr. Dall has described what I consider as a form of this species in Proc. U. S. Nat. Mus., 1885, p. 251, Plate XVII. Fig. 11.

Shell greenish spermaceti-white; when living, the tissues of the animal show with pale salmon-color through the shell in the apical whorls; surface smooth or lightly striated, with a tendency to retain dirt upon itself; form subcylindrical, with a rather obtuse apex, the last whorl forming nearly half the shell; suture evident; whorls five, neatly rounded; aperture longer than wide; lip white, thin, reflected; teeth about nine, of which there are generally three larger than the rest, their tips nearly meeting, and their bases mutually nearly equidistant; one is on the pillar, one on the body whorl, and one on the anterior margin; on either side of the latter are two generally subequal much smaller denticles. Lon. 1.60, lat. 0.75 mm.

Habitat. — Under loose oak bark, oak hamak, Archer, Alachua County, Florida, April, 1885, W. H. Dall, sixteen specimens.

This is one of our smallest species, and is related to *P. pentodon* and *P. pellucida.* It is about half the size of the former and much more slender. Its teeth recall those of *P. curvidens*, Gould, in their arrangement, but the shell is more cylindrical and smaller than it is in *P. pellucida* (*servilis*) as figured by Gould. The teeth are more numerous than in the latter shell, and set, as in *P. pentodon*, in one series; not, as in *pellucida*, partly deeper in the throat.

I describe this with some hesitation, for the condition in which the Pupidæ and Vertigos of North America are is most unsatisfactory, and offers an excellent field to some careful student who shall be able to examine and figure large series of authentic specimens. Still, as there is absolutely no other form with which I feel able to unite this one, it is better to give it a name than to leave it erroneously with some other species.

The above description is copied from that of Dall, while the figure, Plate XVII Fig. 11, is copied in my Plate III. Fig. 2. I have seen no specimen of it.

Pupa fallax, Say.
armifera, Say.
contracta, Say.

Pupa Holzingeri, Sterki.

Shell narrowly perforated, turrited-cylindrical, vitreous (or whitish), very minutely striate, shining; apex rather pointed; whorls 5, regularly increasing, well rounded, especially the upper ones, the last somewhat narrowed and a little ascending towards the aperture, compressed at the base but not carinated, at some distance from the outer margin provided with an oblique, rather prominent, acute crest corresponding in direction to the lines of growth, extending from the base to the suture, formed by a whitish callosity; behind the crest the whorl is flattened, and corresponding to the lower palatal lamella, impressed; aperture lateral, scarcely oblique, relatively small, inverted subovate, with a slight sinus at the upper part of the outer wall, margins approximated; peristome moderately reflected; lamellæ 6; one parietal, rather long, very high, in its middle part curved outward, towards the aperture bifurcated, the outer branch reaching the parietal wall; one columellar, longitudinal, rather high, its upper end turning in nearly a right angle towards the aperture, but not reaching the margin; basal exactly at the base, short, high, dentiform; 3 in the outer wall, viz.: the lower palatal long, ending in the callus, highest at about its middle; the upper short, rather high on the callus; above the upper, one supra-palatal, quite small, dentiform, nearer the margin.

Length 1.7 mm., diam. 0.8 mm. = .068 × .032 inch.

As already stated, our species ranges beside *P. armifera* and *P. contracta*, Say, standing nearer the latter. Yet it is different from this species by the shape of the aperture, the wanting callus[1] connecting the margins on the

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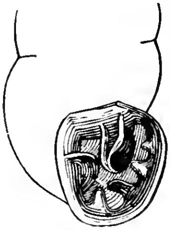
[1] In many specimens of *P. contracta* so strongly developed that the peristome is rendered continuous.

body whorl, by the longer crest behind the aperture, which in *contracta* disappears in about the middle of the (height of the) whorl, and by the wanting constriction, especially in the columellar wall, not to speak of the size and shape of the whole shell. The lamellæ also show some marked differences, such as the presence of a high basal, the shorter columella not reaching the base, but with relatively larger horizontal part, the bifurcation of the parietal and the presence of a supra-palatal, the last just as it is in *P. armifera.*

It must be added here that the specimen first obtained from Minnesota in several respects differs from those found in Illinois and Iowa, which I consider as typical; by its size which is one third smaller, by the basal lamella developed in a peculiar way, being rather longer at the truncated top than at its foot, and by the stronger, thicker palatal lamellæ. Yet, as there was only one specimen, it was liable to be an individual peculiarity, — even then of interest. Should, however, more specimens be found with the same configuration, they would represent a distinct and well characterized variety; possibly it is a peculiar northern form.



Pupa Holzingeri, enlarged

New Philadelphia, Ohio, June, 1889.

The above is a description by Dr. V. Sterki[1] of a Pupa received by him from Winona, Minn., and Northern Illinois. He kindly furnished me the above figure.

Pupa rupicola, Say.
corticaria, Say.
Vertigo milium, Gould.
ovata, Say.
Succinea retusa, Lea.
ovalis, Say.
avara, Say.
aurea, Lea.
obliqua, Say.

SOUTHERN REGION SPECIES.

Glandina Vanuxemensis, Lea.
truncata, Say.
bullata, Gould.
decussata, Pfeiffer.
Texasiana, Pfeiffer.

Lingual membrane as usual in the genus. Teeth 35-1-35. Central small, narrow, with a single blunt rounded cutting point. See Plate IX. Fig. G.

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[1] The Nautilus, Vol. III., No. 4, p. 37, August, 1889.

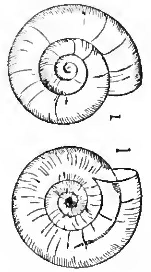
Zonites caducus, Pfeiffer.

cerinoideus, Anthony.

Gundlachi, Pfeiffer.

Found also in Texas, at Hidalgo, by Dr. Singley.

Zonites Singleyanus, Pilsbry.


Zonites Singleyanus, enlarged.

Shell minute, broadly umbilicate, planorboid, the spire scarcely perceptibly exserted; subtranslucent, waxen white, shining, smooth, under a strong lens seen to be slightly wrinkled by growth-lines; whorls three, rather rapidly increasing, separated by well impressed sutures, convex, the apex rather large; body whorl depressed, slightly descending, indented below around the umbilicus; aperture small, semilunar, oblique; peristome simple, acute. Umbilicus nearly one third the diameter of the shell, wide, showing all the whorls.

Alt. 1, diam. 2 mm.

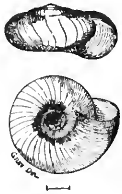
New Braunfels, Comal Co., Texas.

Allied to *Z. minusculus*, but much more depressed, more shining, smoother, smaller, with broader umbilicus and a complete whorl less than *minusculus*.

This species, one of the most distinct of the smaller forms of *Hyalina*, was communicated to me by Mr. J. A. Singley, in whose honor it is named. I have also found a few specimens among the shells collected by myself in Central Texas, during the winter of 1885–86. With *Z. Singleyanus* at New Braunfels are found quantities of *Z. minusculus*. The latter species exhibits some variation, being often more depressed than more northern specimens. This depressed form has been noticed in Mexico by Strebel, who proposes for *Z. minusculus* the new generic title of *Chanomphalus*, which is, of course, completely synonymous with *Pseudohyalina*, Morse, 1864, and this, again, is not different enough from *Hyalina* to warrant the erection of a new genus or subgenus. There is some variation in the width of the umbilicus in Texan specimens of *Z. minusculus*, but I have not seen specimens with it so wide as Dr. Dall indicates for his var. *Alachuana* from Florida. *H. elegantulus*, Pfr., is about the size and form of my *Zonites Singleyanus*, but it is a strongly sculptured species.

The above description was published by Pilsbry, Proc. Phil. Acad., N. S., 1889, p. 84, Plate XVII. Figs. 6, 7, 8. A specimen kindly furnished me by Dr. Singley for the purpose is drawn in my figure.

Zonites Dallianus, Simpson.


Zonites Dallianus, enlarged.

Shell minute, depressed, narrowly umbilicated, fragile, pale straw-colored, somewhat shining; under a lens seen to be marked with delicate growth-lines above, smoother beneath. Spire a little convex; apex subacute; sutures scarcely impressed. Whorls three and one half, scarcely convex, the last wide. Aperture oblong-lunate, oblique, upper and lower margins sub-parallel, slightly converging; peristome acute. Alt. 1½, diam. maj. 3, min. 2½ mm.

West Florida, at Shaw's Point, Manatee Co., and Little Sarasota Bay.

Differs from *Z. arboreus*, Say, in the smaller spire and wider last whorl; fewer whorls; differently shaped aperture. It is about half the size of *Z. arboreus*, and the sculpture is the same as in that species. The *Helix Ottonis* of Pfeiffer, of which specimens from Cuba and Hayti are before me, has no special relationship to this species, but is undoubtedly a synonym of *Z. arboreus*, as Pfeiffer himself concluded. *H. Ottonis* differs from *arboreus* in nothing but the lighter color; the form and dimensions are precisely as in *arboreus*. (See Pfr. in Wiegm. Archiv für Naturgeschichte, 1840, p. 251: the species was never described in the Monographia Heliceorum.)

The aperture in *Z. Dallianus* is less lunate than in *Z. arboreus*, embracing less of the penultimate whorl; seen from beneath, the greater portion of the aperture lies outside of the periphery of the penultimate whorl; whilst in *Z. arboreus* the reverse is the case. The much smaller size of *Dallianus* also separates it from *Z. arboreus*.

This species was sent me under the above name by Mr. Charles T. Simpson, the well known student of Floridian shells. The same form I find in the museum of the Academy, collected by Mr. Henry Hemphill.

The above description was published by Mr. Pilsbry in Proc. Phil. Acad., N. S., 1889, p. 83, Plate III. Figs. 9, 10, 11. A specimen kindly furnished me for the purpose by Mr. Pilsbry is also figured above.

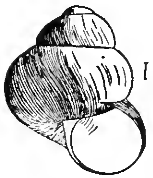
Microphysa incrustata, Poey.

vortex, Pfeiffer.

All the specimens received from West Florida collected by Mr. Hemphill, and from East Florida by Mr. G. W. Webster, are heavily incrusted with dirt.

Microphysa (?) dioscoricola, C. B. Adams.

Shell minute, subperforate, conic globose, thin, very delicately striate, horn-colored; spire elevated, obtuse; whorls 3–3½, convex, the last medially subimpressed; aperture lunately rounded; peristome simple, acute, the columellar margin subvertically descending, very slightly reflected, diam. greater 1¾, lesser 1⅔, height 1½ mm. (Pfr.).



Microphysa dioscoricola, enlarged.

This species is placed by Von Martens (Die Heliceen, p. 73) in *Conulus*, a subgenus of *Hyalina*, with *fulvus*, *Gundlachi*, and others. Mr. Dall tells us (Nautilus, III. 25) that it belongs to *Microconus*. This last is synonymous with *Microphysa*, a subgenus of *Zonites*, according to Tryon, Syst. Conch., III. 24. Mr. Dall says also that the species was originally described from Jamaica by Adams, and subsequently from Trinidad by Guppy as *cæca*. In its jaw and lingual dentition it seems to agree with most of the other species of *Microphysa* which I have examined. I retain it, therefore, in that genus.

The species seems widely distributed in Florida. St. Augustine; Blue Spring, St. John's River; Lake Worth to Hawk's Park along the east coast; Hilo River, emptying into Mosquito Inlet, east coast, not Hillsborough River, emptying into Tampa Bay, as stated by Dall. The specimens examined by me were collected by G. W. Webster at Hawk's Park, "widely distributed in dry places, where other species are not found." Also at Hidalgo, Texas (Singley).

The shell is figured on preceding page.

The jaw (Plate III. Fig. 6) is high, strongly arched, with acuminated ends; it is very thin, membranous, light horn-colored and transparent; there are numerous — some fifteen on each side the median line — narrow, delicate ribs, running obliquely to this line, denticulating either margin; on the upper median portion the ribs meet before reaching the lower margin, leaving upper, median, triangular plates as in *Orthalicus*. The jaw is quite such as I have described and figured for *Macroceramus* in Terr. Moll., V. 384. It also resembles that of *Microphysa turbiniformis* (Ann. N. Y. Acad. Sci., III., Plate XV. Fig. C), excepting that the latter wants the upper median triangular plates. A greatly magnified view of the central portion of the jaw is given.

The lingual membrane is long and narrow. Owing to its small size, it was very difficult to determine the shape of any but the lateral teeth. Three of these last are figured on Plate II., Fig. 5, drawn by camera lucida. They have wide, square bases of attachment, bearing, as usual, two cusps, both stout and blunt, and bearing short, stout cutting points; the centrals appear of the same shape and tricuspid, but I failed to distinguish them clearly enough to draw by camera; the laterals are separated, low, wide, quadrate, with long irregularly serrated cusp. I failed also to distinguish these clearly enough to draw by camera. I have represented them in the figure as they appeared to me. The laterals seem like the teeth of *Pupa*, the marginals much like those of *Cionella subcylindrica*. The dentition is somewhat similar to what I have figured of *vortex* on page 356 of the Manual of American Land Shells. There are about 15–1–15 teeth, with six perfect laterals on each side the median line.

Mr. Dall says of this species that the shell is much smaller than that of *granum*, olive-greenish, with a silky lustre and few inflated whorls, the first of which is usually finely punctate. The suture is very deep, and the umbilicus is proportionally larger than in *granum*.

The figure of the dentition of an undetermined species found by Dr. W. M. Gabb, in Costa Rica, published by me in the Annals of the New York Academy of Science, Vol. III. p. 261, Plate XI. Fig. G, is said by Mr. Pilsbry to represent that of this species, — he having identified the shell from which the lingual was extracted to be *H. cæca*, Guppy.

Hemitrochus varians, Menke.

Strobila Hubbardi, Brown.

Polygyra auriculata, Say.

Dall (U. S. Nat. Mus. Proc., 1855, p. 263) thus characterizes a variety *microforis*: —

> This form is quite well marked, and when fully adult shows as a rule little variation from the form figured by the Binneys, and generally regarded as typical. A quite uniformly characterized variety was found, however, by me at Johnson's

Sink, Alachua County, Florida, where it was abundant. Some twenty specimens were picked up in a few moments during a hurried visit made with other ends in view, and a quart could easily have been gathered in half an hour. This form is distinguished by its generally smaller size (max. diam. 12.0, min. diam. 10.0, alt. 6.0 mm.) as compared with the type (15.0, 12.0, and 7.9 mm.), and by being more closely rolled, thus having not only an actually smaller umbilicus, but one in which one third less of the preceding whorl is visible. The specimens were uniform in this, and in all other respects were like the typical *auriculata*.

Polygira uvulifera, Shuttleworth.
auriformis, Bland.
Postelliana, Bland.
espiloca, Bland.
avara, Say.
ventrosula, Pfeiffer.
Hindsi, Pfeiffer.
Texasiana, Moricand.
triodontoides, Bland.
Mooreana, W. G. B.
hippocrepis, Pfeiffer.

Through the kindness of Mr. Singerly, I have the opportunity of examining the jaw and lingual membrane.

Jaw long, low, ends blunt; anterior surface with over 14 ribs denticulating either margin.

Lingual membrane long and narrow (Plate III. Fig. 8, *a*, *b*). Centrals tricuspid, laterals bicuspid, marginals low, wide, irregularly denticulate. Teeth 30–1–30, the ninth lateral having its inner cutting point bifid.

Polygyra Jacksoni, Bland.

A form was found abundantly near Fort Gibson, Indian Territory, by Mr. C. T. Simpson, who thus describes it in Proc. U. S. Nat. Mus., 1888, p. 449.

> Instead of the bicrural tooth on the body whorl, at the aperture there is a heavy elevated deltoid callus, which is joined to the upper and lower margins of the peristome, and which occupies about the same area as the tooth in the type. The number of whorls is 5; greater diam. 7, lesser 6, height 3 mm. In examining several hundred specimens, I have found none which approach the type, and I would therefore propose for it the varietal name of *deltoidea*.

Polygyra oppilata, Moricand.
Dorfeuilleana, Lea.
Ariadnæ, Pfeiffer.
septemvolva, Say.
cereolus, Muhlfeldt.
Carpenteriana, Bland.

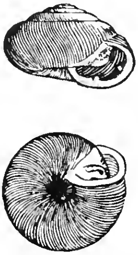
Polygyra Febigeri, Bland.
pustula, Férussac.
pustuloides, Bland.

Triodopsis Hopetonensis, Shuttleworth.
Levettei, Bland.

See 2d Suppl. This species may perhaps be considered one of the Central Province. A variety, however, approaches very nearly the Indian Territory shell lately described as *Mesodon Kiowaensis*. This variety is toothless. It is smooth, like *Levettei*, and has six whorls.

Triodopsis vultuosa, Gould.
Copeï, Wetherby.

See 2d Suppl. To the synonymy add *Triodopsis Cragini*, Call, Bull. Washburne Coll. Library, I., No. 7, p. 202, Fig. 5, Dec., 1888, Topeka, Kansas. I have seen an authentic specimen, given by Mr. Call to the National Museum. It is figured here.


Triodopsis Cragini, enlarged.

Mesodon Romeri, Pfeiffer.
divestus, Gould.

The typical form has few separated, very stout ribs ; a variety from Eufala, Indian Territory, sent me by Mr. C. T. Simpson, has numerous fine ribs and revolving microscopic lines. One individual is 24 mm. in greater diameter.

Mesodon jejunus, Say.

See Manual of American Land Shells, p. 390.

Mesodon Kiowaensis, Simpson.

Shell umbilicated, orbicularly depressed, solid, dark brown in color ; whorls 5, with rather coarse striæ, and fine revolving impressed lines, which are much more conspicuous on the last whorl. Suture deeply impressed, leaving the whorls well rounded ; aperture oblique, somewhat transversely rounded, forming fully three fourths of a circle ; peristome thick and solid, whitish or purplish, evenly reflected, with a slight constriction behind it ; umbilicus moderate, deep, exhibiting but little more than one of the whorls. Greater diam. 15, lesser 13, height 7 mm.

Kiowa Station, about thirty specimens, mostly dead. Limestone Gap, two dead specimens. Another badly bleached shell was obtained not far from Eufaula (Indian Territory).

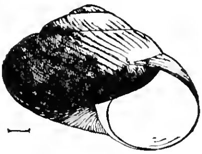

Mesodon Kiowaensis.

Jaw with 9 ribs; teeth with fewer laterals than *Sayii*, and the inner cusp is bifid on the marginals, while in *Sayii* it is entire (Simpson).

The foregoing description is copied from the Proceedings of the U. S. National Museum, 1888, p. 449, while the figure is drawn from a specimen kindly furnished by Mr. Simpson.

The shell appears to me to be a toothless form of some *Triodopsis*, rather than a *Mesodon* (see above, under *Triodopsis Levettei*). It also resembles nearly some of the toothless forms of *Triodopsis Mullani.*

Acanthinula granum, Strebel and Pfeiffer.



Acanthinula granum, enlarged.

Shell small, umbilicated, thin, scarcely shining, light horn-colored, with rib-like striæ of growth, crossed obliquely with rib-like folds, in fresh specimens hirsute or with punctate epidermis. Whorls 4½, four of them broad, rounded, regularly increasing in size, rapidly in elevation, the last descending, impressed at the umbilicus. Peristome simple, broadly reflected at its columellar margin, partially covering the deep umbilicus, within with whitish, light thickening. Greater diam. 2.8, lesser 2.6, height 2.8 mm.; of aperture, height 1.2, breadth 1 mm. (Strebel and Pfeiffer.)

Acanthinula granum, Strebel and Pfeiffer, Beitrag zur Kennt. der F. Mex. L. und S. W. Conch., IV., 1880, p. 31, Plate IV. Fig. 13, not Plate IX., as quoted in text.

A Mexican species, found also in Florida; Archer, Alachua Co.; Evans Plantation, Rogers River; Lake Worth (Dall).

Mr. Dall says the shell, when perfect, is nearly the size of *labyrinthica*, very thin, reddish brown, with very deep sutures and a rather deep, small tubular umbilicus. It is covered with beautiful deep oblique epidermal ridges, which are easily lost, and do not agree with the lines of growth.

The figure is drawn from a specimen kindly furnished by G. W. Webster.

Dorcasia Berlandieriana, Moricand.
griseola, Pfeiffer.

Bulimulus patriarcha, W. G. B.
alternatus, Say.

I am assured by Dr. Singerly and Mr. Simpson that the form known as *alternatus* does not always have a dark aperture, and the intermingling of the forms leads an observer on the spot to believe *alternatus, Schiedeanus, Mooreanus*, and *dealbatus* varieties of one and the same species. They were so treated by my father in Vol. II.

Bulimulus Schiedeanus, Pfeiffer,
var. Mooreanus, W. G. B.
dealbatus, Say.

Bulimulus serperastrus, Say.
multilineatus, Say.
Dormani, W. G. B.

Bulimulus Floridanus, Pfeiffer.

I have already in Terr. Moll., IV., Plate LXXIX. Fig. 3, figured the front view of the typical specimen in Mr. Cumings's collection, drawn by Mr. G. B. Sowerby. The back view is now offered (Plate III. Fig. 7), received from the same source.

A comparison of the front view of Mr. Sowerby's drawing referred to above, with the figure of *Bulimulus Hemphilli* (Plate III. Fig. 9), recently received from Mr. George W. Webster, will lead one to believe the two to be identical. I so suggested in Manual of American Land Shells (p. 408), when treating the variegated shell figured in Fig. 449 of that work, here repeated. There appear to be two varieties of coloring, one corresponding to Pfeiffer's description, and one to Sowerby's figure.

Bulimulus Floridanus.

I give the description of *B. Hemphilli* in full, though I believe it to be identical with *Floridanus*.

Shell imperforate, very thin, transparent, amber-colored and marked by coarse lines of growth; body whorl with six revolving and slightly interrupted brownish red bands, the lower two being close together and upon the rounded base, spire obtuse, whorls five, slightly convex, the body whorl constituting two thirds of the entire length of the shell. Suture slight, base uniformly and gracefully rounded. Aperture direct and oval, peristome thin. Length, 19 mm.; diameter, 8 mm. Hab. both coasts of South Florida.

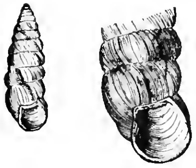
Remarks. Mr. Henry Hemphill, of San Diego, Cal., first found a few dead and badly preserved specimens of this shell in 1884, at Marco, west coast of Florida. These Mr. Binney thought identical with *B. Floridanus*, Pf. (See Manual of American Land Shells, 1885.) Numerous specimens collected during the past summer by the author and Mr. G. W. Webster and son, prove beyond a doubt that this is not identical with the shell figured and described on page 407 of Mr. Binney's Manual. The *B. Hemphilli* is more ventricose, not angular at base, imperforate, differs in color, and in fact there is a general difference.

Mr. Berlin H. Wright describes the above species in the West American Scientist, San Diego, April, 1889, p. 8. He found also a variety of uniform light brown or russet color, bandless, which I have figured on Plate III. Fig. 9. This form had a jaw and lingual membrane the same as in *B. Marielinus* and *Dormani*

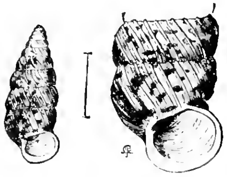
Bulimulus Marielinus, Poey.
Cylindrella Poeyana, D'Orbigny.
jejuna, Say.

Macroceramus pontificus, Gould.

I give here, for comparison, a figure of the true *M. Kieneri*, from a type in Dr. Pfeiffer's collection, from Honduras.



Macroceramus Kieneri.



Macroceramus Gossei.

Macroceramus Gossei, Pfeiffer.

The figure given represents the species.

Pupa variolosa, Gould.
modica, Gould.
pellucida, Pfeiffer.
Strophia incana, Binney.
Holospira Römeri, Pfeiffer.

Pfeiffer says "allied to *Goldfussi*, but from all species easily recognized by the basal carina of the last whorl, and its singular twist, which at first sight gives a sinistral appearance to the shell."

Holospira Goldfussi, Menke.
Stenogyra octonoides, D'Orb.
subula, Pfeiffer.
gracillima, Pfeiffer.
Cæcilianella acicula, Müller.
Liguus fasciatus, Müller.

See p. 435 of Manual of American Land Shells for still another variety of coloring of this species.

Orthalicus undatus, Bruguière.
Succinea Concordialis, Gould.
luteola, Gould.
effusa, Shuttleworth.
Salleana, Pfeiffer.
campestris, Say.
Veronicella Floridana, Binney.

Onchidium Floridanum, Dall.

See Plate VI. Figs. B, C, for a drawing of an original specimen, enlarged three times.

To Mr. Hemphill is due the credit of adding this genus to the fauna of Eastern North America. The specimens arrived as this paper is going through the press, and a detailed description must be deferred. The following notes, however, will indicate its external characters: —

When living, the creature is of a uniform slaty blue, the under parts bluish white, with a greenish tinge to the veil. The surface appears beautifully smooth and velvety without dorsal tubercles; just within the slaty margin of the mantle is a single row of about (in all) one hundred whitish elongated tubercles. When crawling, it is of an oval shape, about an inch long, and two tentacles extend forward beyond the mantle margin, resembling the oculiferous ones of *Vaginulus Floridanus*. In spirits the surface is still smooth, but numerous circular hardly elevated domelets cover the back, each appearing to contain one of the dorsal eyes described by Semper. The tentacles are entirely retracted; a narrow veil, with lightly escalloped edge, precedes the head; the muzzle is not prominent, is indented in the middle, and puckered at the edges. The foot is about one third wider than the mantle at each side of it. There is no jaw. The penis resembles that of *Siphonaria* in form and position. The animal exudes very little mucus. It was found on rocks between tides associated with *Chiton piceus*. Fifteen specimens were found at Knight's Key by Hemphill.

Onchidium indolens of Couthouy (Rio) and *O. armadillo* of Mörch differ from the above in coloring. The latter, described from St. Thomas, has a very different dorsal surface. No others are known from East America. It would seem as if the small Northern species, possessing a jaw like *O. boreale*, Dall, and *O. Celticum*, Cuvier, might appropriately be separated from the agnathous tropical forms as a subgenus, for which the name of *Onchidella* might be revived in a restricted sense.

The above description is by Dall (Proc. U. S. Nat. Mus., 1885, p. 288). Specimens received by him have the lingual dentition of the genus. (See my Plate III. Fig. 10, where a central tooth and adjacent lateral are given.) There are numerous rows of over 97–1–97 teeth.

The following are to be added to the species treated in the Second Supplement.

PACIFIC PROVINCE SPECIES.

Microphysa Stearnsi, Bland.
Lansingi, Bland.

It must be borne in mind that the other species of *Microphysa* examined by me have quadrate marginal teeth, while *Stearnsi* and *Lansingi* have the aculeate marginal teeth of the *Vitrininæ*. Thus they can hardly be classed in *Microphysa*. The name *Pristina* has been suggested by Ancey (Conchologists'

Exchange, I. 5, p. 20, Nov., 1886). As a substitute for this preoccupied name, Mr. Pilsbry suggests *Anceyia*. (See same, I. 6, p. 26, Dec., 1886.) Mr. Ancey's description is: —

Pristina, Anc. (nov. subg. Hyalinæ). Testa parvula, imperforata, cornea, nitens, multispirata; spira depresse conica. Apertura interdum lamellis radiantibus subserratis in palato sitis insignis.

Geographical Distribution: Western and Arctic North America.

Types: *Hyalina Stearnsi*, Bland, and *Lansingi*, Bland.

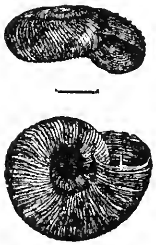
Mr. W. G. Binney put these species, but with doubt, in Microphysa, while other authors consider them as Hyalinæ; they differ from the latter by anatomic features, and from the former by the form of the shell. Altogether I am inclined to place the group in Hyalina, as a series nearly allied to *Conulopolita*, Boettger (type, *C. Raddei*, Boettg.); I am confident the presence or absence of internal laminæ or tooth-like processes within the aperture of *Helices* are not generic characters; in some instances they are either present or absent in closely allied species. I established this fact when at work (Le Naturaliste, 1882) on the New Caledonian forms, and I now repeat this as my opinion in regard to *Pristina* and *Gastrodonta*. In the latter the teeth are frequently absorbed by the animal when growing larger.

Macrocyclis Duranti, Newc.

To the synonymy add: —

Selenites cœlatura, Mazyck, Proc. U. S. Nat. Mus., 1886, p. 460, with figures of that form and of typical *Duranti*. Also, Proc. Elliott Soc., Feb., 1886, p. 114, same figures.

Mr. Mazyck's description and figures are repeated here: —



Macrocyclis Duranti, var. cœlata, enlarged.

Shell small, depressed, brownish horn-color, with very coarse, rough, crowded, sub-equidistant, irregular ribs, which are obsolete at the apex; whorls four, rounded, somewhat inflated below, gradually increasing, the last not descending at the aperture; suture impressed; umbilicus wide, clearly exhibiting all of the volutions; aperture almost circular, slightly oblique; peristome simple, its ends approaching and joined by a very thin, transparent, whitish callus, through which the ribs are distinctly seen. Greater diameter, 4 mm.; height, 1¾ mm.

Santa Barbara, California, Dr. L. G. Yates. Hayward's, Alameda County, California, W. H. Dall, U. S. National Museum.

Newcomb's description of this little shell (*M. Duranti*) is as follows: —

"Shell depressed, discoidal, pale corneous, under the lens minutely striated, opaque, broadly and perspectively umbilicated; whorls 4, the last shelving but not descending (at the aperture); suture linear; aperture rounded, lunate, lip simple, the external and internal approaching.

"*Habitat.* — Santa Barbara Island."

Mr. Binney's description, which is repeated in each of his works above named, differs in this important particular. For Newcomb's "Under the lens minutely striated," he substitutes the contradictory words "with very coarse, rough striæ"

In a note written in answer to an inquiry addressed to him regarding this singular discrepancy, he says, "My description and figure are from an individual, not from the species. I am absolutely sure my specimen was one of the original find." His figure, drawn by Morse, rather represents a comparatively smooth, semitransparent shell.

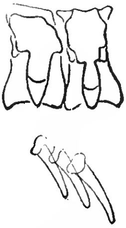
Limax hyperboreus.

See Manual of Amer. Land Shells, p. 473. I have figured on Plate VIII. Fig. F, an individual from British Columbia. Here I give the dentition.

Jaw arched, smooth, with blunt median projection. Lingual membrane with 42–1–42 teeth; centrals tricuspid; laterals bicuspid, 12 in number on each side; marginals about 30 on each side, aculeate, simple, without bifurcation or side spur.

The figure shows a central tooth with its adjacent lateral, and three extreme marginals.

Limax montanus, L. castaneus, L. occidentalis, and *L. campestris* all have side spurs to their marginal teeth. Otherwise, their dentition shows no specific distinction from that of *hyperboreus.* Until the genitalia of the last is shown to vary, I am inclined to believe all four to be one and the same species.


Limax hyperboreus

Limax Hemphilli.

Mr. Henry Hemphill has sent me in spirits from Julian City, California, a small, slender, smooth, dark species of *Limax,* 20 mm. long in its contracted state. It does not outwardly resemble *Limax agrestis,* nor does it seem probable that that species would have been accidentally introduced from the Eastern cities.[1] The dentition, however, agrees with that of *agrestis* by its having the peculiar side spur to the larger cutting point of all the lateral teeth. I venture to propose a specific name for it, in hopes of having an opportunity later to fix its specific position by an examination of the genitalia. It is figured on Plate VIII. Fig. E.

The jaw is as usual in the genus.

There are 50–1–50 teeth to the lingual membrane, of which ten on each side are laterals. Centrals tricuspid; laterals bicuspid, the larger cutting point having a well developed side cutting point on its inner side; the laterals have also an inner, slightly developed, horizontal side cusp, bearing a small, stout cutting point (see Plate I. Fig. 13); marginals simple, without side spur.

The figure on Plate II. Fig. 3, shows one central with its adjacent laterals, an outer lateral, and several extreme marginals.

A specimen, apparently of the same species, from British Columbia, has 53–1–53 teeth, of which 13 on each side are laterals.

I have the same species, with similar dentition, from San Tomas, Lower California (Hemphill).

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[1] It is, however, found in San Francisco

Limax Hewstoni, J. G. Cooper.

On Plate II. Fig. 4, will be found a better figure of the dentition of this species than is given in Terr. Moll., V. It will be seen that the inner side cusp of the lateral teeth is quite distinct from the side spur found in *Limax Hemphilli* and *agrestis*. (See line third of p. 223.)

I have figured (Plate VIII. Figs. D and I) individuals received from Dr. Cooper, drawn by Mr. Theo. D. A. Cockerell.

Limax campestris, var. occidentalis.

The specimen figured on Plate VIII. Fig. H, was kindly furnished by Dr. Cooper. I have already expressed my belief in the identity of this with the Eastern form.

Arion foliolatus, Gould.

It is with the greatest pleasure that I announce the rediscovery by Mr. Henry Hemphill of this species, which has hitherto escaped all search by recent collectors. It has till now been known to us only by the description and figure of the specimen collected by the Wilkes Exploring Expedition, almost fifty years ago, and given in Vols. II. and III. of Terrestrial Mollusks. A single individual was found in December, 1889, at Olympia, Washington, and sent to me living by Mr. Hemphill. It can thus be described. (See Fig. A of Plate VIII.)

Animal in motion fully extended over 100 millimeters. Color a reddish fawn, darkest on the upper surface of the body, mantle, top of head, and eye-peduncles, gradually shaded off to a dirty white on the edge of the animal, side of foot, back of neck, and lower edge of mantle, and with a similar light line down the centre of back ; foot dirty white, without any distinct locomotive disk ; edge of foot with numerous perpendicular fuscous lines, alternating broad and narrow ; mantle minutely tuberculated, showing the form of the internal aggregated particles of lime, the substitute of a shell plate, reddish fawn color with a central longitudinal interrupted darker band and a circular marginal similar band, broken in front, where it is replaced by small, irregularly disposed dots of same color ; these dots occur also in the submarginal band of light color. Body reticulated with darker colored lines, running almost longitudinally, scarcely obliquely, toward the end of the tail, and connected by obliquely transverse lines of similar color, the areas included in the meshes of this network covered with crowded tubercles, as in *Prophysaon Andersoni*, shown in Plate IX. Figs. I, J. Tail cut off by the animal. (See page 207.)

What appears to be the same species, or a very nearly allied one, was found by Mr. Hemphill at Gray's Harbor, Washington, on the banks of the Chehalis River, near its mouth. This form is figured on Plate VIII. Fig. C. When extended fully, it is 70 millimeters long. It is more slender and more pointed at the tail than the large form. The body is a bright yellow, with bluish black reticulations. The edge of the foot and the foot itself are almost black; shield irregularly mottled with fuscous; the body also is irregularly mottled with fuscous, and has one broad fuscous band down the centre of the back, spreading as it joins the mantle, with a narrower band on each side of the body. The other characters, external and internal, are given below. This smaller form loses its colors on being placed in spirits, becoming a uniform dull slate color.

The large Olympia form is surely *Arion foliolatus*, Gould, agreeing perfectly with his description in Vol. II., and with his figure in Vol. III., excepting that the latter is colored with a deeper red.

Mr. Hemphill writes of it: "I have to record a peculiar habit that is quite remarkable for this class of animals. When I found the specimen, I noticed a constriction about one third of the distance between the end of the tail and the mantle. I placed the specimen in a box with wet moss and leaves, where it remained for twenty-four hours. When I opened the box to examine the specimen, I found I had two specimens instead of one. Upon examination of both I found my large slug had cut off his own tail at the place where I noticed the constriction, and I was further surprised to find the severed tail piece possessed as much vitality as the other part of the animal. The ends of both parts at the point of separation were drawn in as if they were undergoing a healing process. On account of the vitality of the tail piece, I felt greatly interested to know if a head would be produced from it, and that thus it would become a separate and distinct individual." The animal on reaching me still plainly showed the point of separation from its tail. (See Plate VIII. Fig. A.) The tail piece was in an advanced stage of decomposition. I noticed the constriction towards the tail in one of five individuals of *Prophysaon cœruleum* from Olympia. (See page 209.) Another individual of the same lot had a truncated tail, having undergone the operation. The edges of the cut were drawn in like the fingers of a glove.

The tail of the *Arion foliolatus* having been cut off, I was unable to verify the presence of a caudal pore from this individual. On the only living one of the lot from Gray's Harbor, the pore was distinctly visible, and is figured on Plate VIII. Fig. C. Usually, it seemed more "a conspicuous pit" than a longitudinal slit, as in *Zonites*. At one time I distinctly saw a bubble of mucus exuding from it. It opened and shut, and is still plainly visible on the same individual, which I have preserved in alcohol and added to the Binney Collection of American Land Shells in the National Museum at Washington. Another individual from Seattle plainly shows the pore.

Five specimens of the Gray's Harbor lot had, concealed in the mantle, a group of particles of white limy matter which it was impossible to remove as one shell plate. In the large Olympia individual these irregularly disposed particles of lime, of unequal size, seemed attached to a transparent membranous plate. With care, I removed this entire, and figure it. It is suboctagonal in shape (Plate VIII. Fig. B). Under the microscope it appears that the particles of lime do not cover the whole plate ; at many points they are widely separated. This aggregation of separate particles is the distinctive character of the subgenus *Prolepis*, to which *A. foliolatus* belongs.[1]

The genitalia of the large individual from Olympia is figured on Plate IX. Fig. D. The ovary is tongue-shaped, white, very long and narrow; the oviduct is greatly convoluted ; the testicle is black in several groups of cœca ; the vagina is very broad, square at the top with the terminus of the oviduct, and the duct of the genital bladder entering it side by side ; the genital bladder is small, oval, on a short narrow duct ; the penis sac is of a shining white color, apparently without retractor muscle; it is short, very stout, blunt at the upper end where the extremely long vas deferens enters, and gradually narrowing to the lower end. There are no accessory organs. The external orifice of the generative organs is behind the right tentacle.

The form from Gray's Harbor (Plate IX. Fig. H) has its generative system very much the same as described above. The ovary is much shorter and tipped with brown, and is less tongue-shaped. The penis sac tapers to its upper end. The vagina is not squarely truncated above. The system much more nearly resembles that of *Prophysaon Andersoni* (see Terr. Moll., V.) than that of the Olympia *foliolatus*.

The jaw of both forms is very low, wide, slightly arcuate, with ends attenuated and both surfaces closely covered with stout, broad separated ribs, whose ends squarely denticulate either margin. There are about 16 of these ribs in one specimen from Gray's Harbor, and over 20 in that of the true *foliolatus* from Olympia (see Plate IX. Fig. B). The lingual membrane in each form is long and narrow, composed of numerous longitudinal rows of about 50–1–50 teeth, of which about 16 on each side in the true *foliolatus* (Plate IX. Fig. C), and 19 in the other form, may be called laterals. Centrals tricuspid, laterals bicuspid, marginals with one long inner stout cutting point, and one outer short side cutting point. The figure shows a central tooth with its adjacent first lateral, and four extreme marginals.

I have figured both the true *foliolatus* from Olympia (Plate VIII. Fig. A) and the smaller form from Gray's Harbor (Plate VIII. Fig. C) of natural size. Should the latter prove a distinct species or variety, I would suggest for it the name of *Hemphilli*, in honor of the discoverer of it and the long lost *foliolatus*.

Prophysaon Hemphilli.

See Plate VII. Fig. D, drawn by Cockerell from the living animal.

Prophysaon Andersoni, J. G. Cooper.

Figure 1 of Plate III. was drawn from a specimen received from Dr. Cooper. It represents the true *Andersoni*, distinguished by a light dorsal band, and by genitalia such as I have described for *P. Hemphilli*. The same form, also re-

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[1] Mr. Theo. D. A. Cockerell, finding the slug not to be a true *Arion*, is about to suggest for it the generic name of *Phenacarion*.

ceived from Dr. Cooper, is drawn by Mr. Cockerell on Plate VII. Fig. C. Mr. Cockerell has shown me that I have confounded with it another species, which he proposes to call *P. fasciatum.* See next species.

Prophysaon fasciatum, Cockerell.

This species is described by Mr. Cockerell as distinct from *Andersoni,* with which I have formerly confounded it. (2d Suppl. to Vol. V., p. 42.) It has a dark band on each side of the body, running from the mouth to the foot. To this must be referred the descriptions of animal, dentition, jaw, and genitalia formerly published by me as of *Andersoni.*

I am indebted to Mr. Theo. D. A. Cockerell for a figure and description of this species. The former is given on Plate VII. Fig. A, while the latter is given here in the words of Mr. Cockerell, whose name must consequently be associated with it as authority : —

Length (in alcohol), 19 mm. Mantle black, with indistinct pale subdorsal bands, — an effect due to the excessive development of the three dark bands of the mantle. Body with a blackish dorsal band, commencing broadly behind the mantle and tapering to tail, and blackish subdorsal bands. No pale dorsal line. Reticulations on body squarer, smaller, more regular, and more subdivided than in *P. Andersoni,* Cooper. Penis sac tapering, slender. Testicle large. Jaw ribbed.

Prophysaon cœruleum, Cockerell.

Plate VIII. Fig. I, J.

In the Nautilus, 1890, p. 112, it is thus described : —

Length (in alcohol), 22½ mm.; in motion, 43 mm. Body and mantle clear blue-gray, paler at sides, sole white. Mantle finely granulated, broad, without markings. Length of mantle, 7 mm ; breadth, 5 mm. Respiratory orifice, 2½ mm. from anterior border. Body subcylindrical, tapering, pointed. (In one specimen eaten off at the end.) Distance from posterior end of mantle to end of body, 10¾ mm.

The reticulations take the form of longitudinal equidistant lines, occasionally joined by transverse lines, or coalescing. Sole not differentiated into tracts. Jaw pale, strongly ribbed. Liver white.

Mr. Binney sends me colored drawings of the living animal; the neck is long and white, or very pale. Mr. Binney has examined the jaw and lingual, and finds them as usual in the genus.

Several specimens were sent from Olympia, Washington, by Mr. Hemphill to Mr. Binney.

P. cœruleum is an exceedingly distinct species, distinguished at once by its color and the character of its reticulations.

Prophysaon cœruleum, var. dubium, n. var., Cockerell.

Length (in alcohol), 8 mm. Length of mantle, 4 mm. Distance from posterior end of mantle to end of body, 3½ mm. Mantle broad, with four bands composed of coalesced black marbling, very irregular in shape, and running together anteriorly. Body dark, tapering. Sole pale, its edges gray. Liver white.

With the *P. cœruleum* is a small dark slug, probably a variety of it, but differing as described above. It will easily be distinguished by its blackish color and the peculiar markings on the mantle.

Prophysaon Pacificum, Cockerell.

Plate VII. Figs. B, E, F, H.

Mr. Theo. D. A. Cockerell gives the following in the Nautilus of February, 1890, pp. 111–113 : —

Length (in alcohol), 17½ mm. Body and mantle ochrey brown, head and neck gray. Mantle granulated, rather broad, with a black band on each side not reaching the anterior border ; these bands are farthest (2¼ mm.) apart near the respiratory orifice, from which point they converge posteriorly, and anteriorly by the bending of the band on the right side. Length of mantle, 7¾ mm. ; breadth, 4 mm. Respiratory orifice 3¼ mm. from anterior border. Body cylindrical, rounded and very blunt at end, not conspicuously tapering. Distance from posterior end of mantle to end of body, 8 mm. Body dark grayish-ochre above, with an indistinct pale dorsal line ; sides paler. Reticulation distinct, with indistinct "foliations." Sole somewhat transversely wrinkled, but not differentiated into tracts.

Jaw dark, strongly curved, blunt at ends, with about ten well-marked ribs (Plate VII. Fig F). Lingual membrane with about 35–1–35 teeth ; centrals tricuspid, the side cusps very small, laterals bicuspid, marginals with a large sharp straight inner point and a small outer one. Compared with *P. humile* the centrals are slightly shorter and broader. Liver dark gray-brown.

Found by Mr. H. F. Wickham under logs in ditches by the roadside and damp places at Victoria, Vancouver Island, 1889.

This is a very distinct species, easily recognized by its color, the absence of dark bands on the body, the pale dorsal line, and the blunt posterior extremity.

Prophysaon flavum, Cockerell.

Plate VII. Fig. K.

From the Nautilus, 1890, p. 111 : —

Length (in alcohol), 25 mm. Body and mantle dull ochreous, head and neck ochreous. Mantle tuberculate-granulose, grayish ochre, pale at edges, and with black marbling or spots in place of the bands of *P. Pacificum*. Length of mantle, 11 mm. ; breadth, 5½ mm. Respiratory orifice 5 mm. from anterior border. Body cylindrical, hardly tapering, and blunt at end. Distance from posterior end of mantle to end of body, 14 mm. Body dark grayish-ochre above, with a pale ochreous dorsal line not reaching much more than half its length ; sides paler. Reticulations distinct, "foliated." Sole with well marked transverse lines or grooves, those of either side meeting in a longitudinal median groove, which divides the foot into two portions. Liver pale grayish.

Uniform tawny, as is *Limax flavus*. It stretches itself out in a worm-like shape unlike other species. Internal shell plate, jaw, and tongue as in *Andersoni*.

Gray's Harbor, Washington. (Hemphill, 1889.)

This is probably a variety of *P. Pacificum*.

Prophysaon humile, Cockerell.

Plate VII. Figs. F, G, L, M.

From Nautilus, 1890, p. 112.

Length (in alcohol), 16½ mm. Body above and mantle smoke-color, obscured by bands. Mantle wrinkled, and having a broad dorsal and two lateral blackish bands, reducing the ground-color to two obscure pale subdorsal bands. Length of mantle, 7 mm.; breadth, 5½ mm. Respiratory orifice 2¾ mm. from anterior border. Body subcylindrical, somewhat tapering, rather blunt at end. Distance from posterior end of mantle to end of body, 8 mm. Back with a blackish band reaching a little more than half its length, and lateral darker blackish bands reaching its whole length. Reticulations distinct, "foliated." Sole strongly transversely striate-grooved, but not differentiated into tracts.

Jaw pale, strongly striate, moderately curved, not ribbed. (See Fig. F.) Lingual membrane long and narrow. Teeth about 35–1–35. Centrals tricuspid, laterals bicuspid, marginals with a large inner point, and one (sometimes two) small outer points. Liver pale chocolate.

Found by Mr. H. F. Wickham under the bark of rotten logs in the woods around Lake Cœur d'Alene, Idaho, 1889.

In its reticulations, and general external characters, this species resembles *P. Andersoni*, of which it is possibly a variety.

Hemphillia glandulosa.

(See also p. 216.)

From Olympia and Gray's Harbor, Washington, Mr. Hemphill sent me living specimens of this species, both young and mature. Several of the young had the horn-shaped process to the tail noticed in the original description of the genus. The shell in these young individuals is very slightly attached, apparently simply by having its posterior margin lightly covered by the mantle. It often becomes detached. In these young, the mantle is proportionally smaller, and the neck much longer. I have figured an enlarged view of a young individual, Plate IV. Fig. D.

Ariolimax[1] Columbianus, Gould.

Found also by Mr. Hemphill on Santa Cruz Island.

Plate VI. Fig. A, represents the mottled variety, found recently by Mr. Hemphill in the State of Washington. Mr. Cockerell suggests for it the varietal name *maculatus*. This form shares with the type the peculiar penis sac (Fig. G) distinguishing it from the next species.

Ariolimax Californicus, Cooper.

See Plate V. Fig. E, for the animal in motion, and a portion of the genital system (Fig. H), showing variation from that of *A. Columbianus*.

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[1] The name should be *Arionilimax*.

Ariolimax Andersoni.

See Plate V. Fig. F, showing the typical specimen in spirits restored.

Ariolimax Hemphilli.

Plate V. Fig. B, G.

A variety *maculatus*, Cockerell, is figured in B. The Figure G is drawn from a typical specimen, with the tail, the pore, and the locomotive disk.

Ariolimax niger, J. G. Cooper.

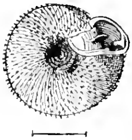
Plate V. Fig. A, gives a lighter-colored form ; Fig. I, the typical form; Figs. C and D, the caudal pore.

Triodopsis inflecta, Say.

This has erroneously been quoted from the Pacific Province, at the mouth of Columbia River. It is difficult to decide what species Middendorff had in view. His words are thus translated : —

> Let it not be objected that *Helix clausa* up to this time has not been discovered west of the Rocky Mountains. The Northwest Coast of America is almost wholly unexplored conchologically, and I do not doubt that *H. clausa* will be there found, just as I can now assert with reference to *H. planorboides*. Even the American authors know this hitherto only from the Ohio and Missouri. Its distribution nevertheless appears to extend over the whole of North America, since I have received a great number of specimens of the same through Mr. ——, from Sitka, whereby it becomes incorporated with our Russian Fauna. Southwards it extends to the west coast of America, at least to Upper California, where they were likewise collected by Mr. ——. It appears to have undergone no alteration whatsoever, and presents in Sitka a considerable size, as the ordinary representations show (up to 22, etc.). Moreover, Binney in the Boston Journal, III., Plate XIV., has them copied equally large.

Polygyra Roperi, Pilsbry.



Polygyra Roperi, enlarged.

Shell umbilicated, plane above, slightly inflated below, shining, pellucid, light horn-color, with delicate wrinkles of growth ; spire flattened ; whorls 5½, scarcely rounded, very regularly increasing, the last flattened above, abruptly deflected at the aperture, deeply constricted behind the peristome ; aperture transversely lunar, gaping, much contracted, tridentate ; peristome thickened, broad, white, gradually thinning and scarcely reflected at its edge, and not extending beyond the surface of the whorl, its ends approached, joined by a light callus, on which is a heavy white callus bearing a stout, white, broad, blunt, transverse tooth, slightly curving inward, its basal margin with an erect conical, short tooth, separated by a small circular sinus from another rather more deeply seated similar tooth on its upper margin. Umbilicus broad, showing the volutions clearly. Greater diameter, 9 mm.; lesser, 7 mm.; height, 2½ mm.

Helix (Triodopsis) Roperi, PILSBRY. The Nautilus, Vol. III. No. 2, June, 1889, p. 14.

Redding, Shasta Co., California, in drift of the Sacramento River, three dead shells were collected by Mr. Edward W. Roper, of Chelsea, Mass.

The above description is drawn from one of the original specimens, kindly lent me by Mr. Roper, while another in the collection of the Academy of Natural Sciences of Philadelphia, from which Mr. Pilsbry drew his description, is figured above. The third specimen was given by Mr. Roper to Mr. Henry E. Dore of Portland, Oregon.

Never having seen a specimen of *P. Harfordiana*, I cannot say if this species is identical with it. At least, it must be nearly allied.

Aglaja fidelis, GRAY.

New figures of several forms of this species are given. Plate X. Fig. A represents the black elevated form approaching *infumata.* Its sculpturing is given in Fig. B. The small, black, elevated form is given in Fig. C, with its sculpturing in D; the small, depressed form, in E.

Aglaja infumata, GOULD.

Plate X. Fig. F, gives an enlarged view of the hirsute surface.

Arionta arrosa, GOULD.

Plate XI. Fig. A gives this species. A form of *arrosa* nearly approaching *A. exarata* is given in Fig. B, its sculpturing in Fig. C.

Arionta exarata, PFEIFFER.

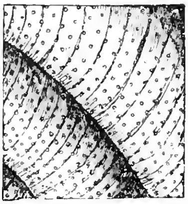
The typical form and its sculpturing are given on Plate XI. Figs. D and E.

Arionta Mormonum, PFEIFFER.

The typical form is given on Plate XI. Fig. F. The variety (Vol. V. p. 141) approaching *Aglaja Hillebrandi*, is given in Figs. G and H; the sculpturing of the same form, on Plate X. Fig. G. The genitalia of this form are the same as of the type.

Arionta sequoicola, J. G. COOPER.

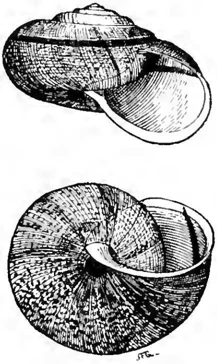
A figure of the sculpturing of this species is here given, greatly enlarged.



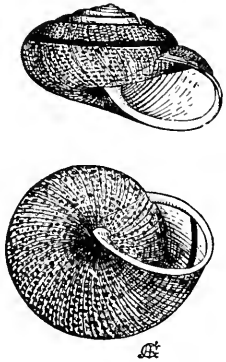
Enlarged sculpturing of Arionta sequoicola.

Arionta Californiensis, Lea.

I give here new figures of two forms of this species, *Arionta Diabloensis* and the depressed variety of *A. Bridgesi*, the former drawn from a shell received from Dr. Cooper.



Arionta Diabloensis.



Arionta Bridgesi, depressed.

Onchidella Carpenteri, Dall.

An alcoholic specimen received from Mr. Dall is figured on Plate VI. Figs. D, E, enlarged twice.

Veronicella olivacea, Stearns.

I have failed to receive Californian specimens. That figured on Plate IX. Figs. E, F, is one of the original lot from Folvon, Central America.

CENTRAL PROVINCE SPECIES.

Limax montanus, Ingersoll.

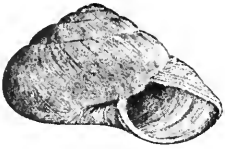
A specimen is figured on Plate VIII. Fig. G.

The species is surely identical with *L. campestris.*

Patula solitaria, Say.

Mr. Hemphill found this species very abundant at Old Mission, Cœur d'Alene, Idaho. There was an albino variety, a depressed form, and one very much more elevated than that which I figured in the Second Supplement, Plate I. Fig. 10.

Patula strigosa.

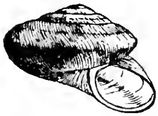


P. strigosa, var. subcarinata, Hemphill.

Among the shells recently collected by Mr. Hemphill at Old Mission, Cœur d'Alene, Idaho, was a marked variety of this species, for which Mr. Hemphill suggests the name *subcarinata*. The specimens vary greatly in elevation of the spire, and in the number and disposition of the revolving bands, often quite wanting. All have a very heavy shell, the body whorl of which has an obsolete carina which is well marked at the aperture, modifying the peristome very decidedly. See the figure.

In examining the genitalia I find the base of the duct of the genital bladder greatly swollen along a fifth of the total length of the duct.

On the banks of the Salmon River, Idaho, Mr. Hemphill found a form like var. *Gouldi*, but distinctly carinated. None of the Utah individuals of this form are so characterized.



Patula strigosa, var jugalis, Hemphill.

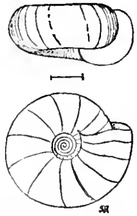
Another form of *strigosa* from the same locality is very large, flat, with a transversely oval aperture, the ends of the peristome so nearly approached as almost to touch, and often joined by a heavy callus, which forms a continuous rim around the aperture. Mr. Hemphill has called this var. *jugalis*.

Microphysa pygmæa.

Found by Mr. Hemphill at Old Mission, Cœur d'Alene, Idaho.

Microphysa Ingersolli, BLAND.

A better figure of this species is here given.



Microphysa Ingersolli, enlarged.

Triodopsis Hemphilli.

Mr. Tryon has suggested the name *binominata* for this species, though *Hemphilli* is not preoccupied in *Triodopsis*.

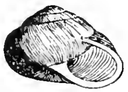
Triodopsis Sanburni.

Triodopsis Sanburni, enlarged.

The cut is drawn from one of the original specimens.

Mesodon ptychophorus.

At Old Mission, Cœur d'Alene, Idaho, Mr. Hemphill found a form of this species characterized by a heavy, dead white shell with scarcely any trace of ribs or wrinkles of growth which are usually so characteristic of the species. On the banks of the Salmon River he found a small form, the lesser diameter of which is only 12 mm. See figure.



Mesodon ptychophorus, var.

Triodopsis Harfordiana.

Ancey suggests *commutanda*, and Tryon *Salmonensis*, as a substitute for the name *Harfordiana*. I retain the last name, it not being preoccupied in the genus *Triodopsis*.

Prophysaon Andersoni?

Specimens collected by Mr. Hemphill at Old Mission, Cœur d'Alene, Idaho, appear to agree with specimens of this species received from Dr. Cooper. The jaw is low, wide, slightly arcuate, with over 12 broad, stout ribs, denticulating either margin. The lingual membrane is given in Plate II. Fig. 2. The central and lateral teeth are slender and graceful. The latter have, apparently, a second inner cutting point, as is found in *Limax agrestis*. I have so figured it, hoping to draw attention to it, and thus settle the question of its being there.

Hemphillia.

Plate IV.

From Old Mission, Cœur d'Alene, Idaho, Mr. Henry Hemphill has sent me fine large specimens of *Hemphillia* alive. From these I am able to give the outward characteristics of the animal in drawings by Mr. Arthur F. Gray.

The animals are larger and much lighter in color than those originally found at Astoria. They do not while in motion differ from other slugs, though my former figure of the animal in spirits shows a very great difference, owing to the contraction being resisted by the internal shell. The rear end of the mantle seems swollen and blunt, separated from the back, however, and thus alone does there seem to me any difference in its appearance from *Limax*, whose mantle lies flat upon the back. The slit in the mantle is sometimes open, sometimes closed, and the slit seems to extend quite to the rear of the mantle. There is a profuse flow of mucus from over the slit. There seem on the mantle to be little protuberances, rather than the elongated reticulation of the rest of the animal. The caudal pore opens and shuts, and exudes mucus in bubbles sometimes, which occasionally form a solid lump of mucus on the tail. The horn-like process of the tail so prominent in the first specimens from Astoria — contracted in alcohol — does not exist in these living specimens, though occasionally there is a kind of hump above the pore. (See Plate IV. Fig. D.)

Mr. Hemphill writes: "*Hemphillia* has a peculiar habit when removed from its resting place of switching its tail, so to speak, quite rapidly, — a habit I never noticed in any of our other slugs. I find them hibernating in old rotten logs."

The viscera are enclosed under the mantle.

Mr. Gray in drawing the animal called my attention thus to the characters of the outward markings of the slug: —

"You are right in saying that the slit in the mantle extends to the back margin. The central pit seems flooded with mucus at all times, but does not change its form; the slit, however, seems to widen and show a little ridge on either margin when the animal is fully expanded. The little tubercles, or small pimples as it were, seem to cover the posterior portion of the mantle, while the elongated tubercles seem to cover the anterior half, though these at times disappear and the anterior portion runs into folds, which break up the surface, and starting from the margin of the mantle run to its centre in parallel lines like miniature waves. They move steadily inward from both margins, disappearing before reaching the little mucous pit in the centre of the mantle, little wavelets rising at the margins and keeping up a constant rhythmic motion toward the centre."

The jaw of this specimen has about 25 ribs, denticulating either margin. It is low, wide, slightly arcuate, with slightly attenuated ends. (See Plate IX. Fig. A.)

The lingual membrane is as described and figured by me in Vol. V.; there are, however, in this form, 57–1–57 teeth, with some eleven true laterals.

The genitalia I have figured in Plate III. Fig. 3. It agrees with my figures in Vol. V. of the genitalia of the original specimens, excepting that the penis sac, as represented there in Plate XII. Fig. K, is here doubled on itself.

Pupa hordeacea, Gabb.

An authentic specimen of this species is figured in the Second Supplement, Plate III. Fig. 10, referred by mistake to *P. Arizonensis* in the explanation of Plate III.

Pupa Arizonensis, Gabb.

The reference to *hebes* in Second Supplement should be Fig. 12, not Fig. 10.

LOCALLY INTRODUCED SPECIES.

Tachea nemoralis, Linn.

Fine large specimens of this species have been sent me by Prof. James H. Morrison, found by him living during the last three years at Lexington, Virginia. They form part, no doubt, of a colony descended from living individuals introduced from Europe around plants.

Zonites cellarius, Müller.

Also at San Francisco (Cooper).

Limax maximus, Linn.

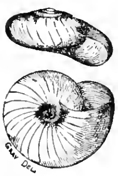
Also at New Braunfels, Texas (Singerly).

A drawing of the lingual dentition on Plate II. Fig. 1, shows the cutting points of central and lateral teeth to be trifid. This is not shown in my figure in Vol. V.

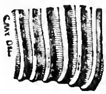
SINCE the foregoing was written, the following species have been described: —

Zonites selenitoides, PILSBRY.

This species is similar in form and general appearance to *Z. minusculus*, Binn., though decidedly larger. The umbilicus is broad, as in the latter species. The shell is thin, light yellowish horn-color, almost white. Surface shining, covered with close strong oblique rib-striæ, like *Patula striatella;* these striæ, while generally regular, sometimes bifurcate, or separate to give room for another to be intercalated. The spire is flatter than *minusculus*, nearly plane. The earlier 1¾ to 2 whorls are smooth, polished, not striate; the sutures are well impressed. There are 3½ whorls in all, convex, gradually widening, the last proportionately wider than in *Z. minusculus*. Aperture slightly oblique, lunate, narrower than in *Z. minusculus*, its margins thin, acute, scarcely converging, the columellar shortly subreflexed.



Zonites selenitoides, enlarged.



Sculpturing, enlarged.

Alt. 1.2 mm., diam. 3 mm.

The specimens were presented to me by Mr. W. G. Binney, who, regarding them as new, kindly permitted me to describe them. They were gathered by Hemphill, prince of collectors! at Mariposa Big Trees, California. The name *selenitoides* is given because of a certain resemblance to the little *Selenites Duranti* of Southern California.

The above description was published by Pilsbry in Proceedings of Academy of Natural Sciences of Philadelphia, 1889, p. 413, Plate XII. Figs. 13–15.

I give a figure of the original specimen, and of its sculpturing.

Zonites Simpsoni, PILSBRY.

This species belongs to that group of *Hyalina* comprising *capsella*, Gld., *Lauræ*, W. G. Binn., and *placentula*, Shutt., — species with narrow umbilicus, numerous closely coiled narrow whorls, and without a callus or thickening within the base of the last whorl. *Z. Simpsoni* differs from *placentula* in its much smaller size, nearly straight, instead of arcuate, basal lip, seen from beneath, proportionately wider last whorl, and the more trigonal, wider aperture. With *Z. Lauræ* I need not compare it, as that species is much larger and more elevated. *Z. capsella* is about the same size, color, and texture as *Simpsoni*, but has a narrow umbilicus and very much narrower aperture, narrowly semilunar instead of trigonal in outline. *Z. Simpsoni* has 5 whorls. Alt. 2, diam. maj. 4½, min. 4 mm.

The specimens before me were collected by Mr. C. T. Simpson, at Limestone Gap, Indian Territory. The trigonal form of the aperture is so peculiar that the species may be separated from *Z. capsella* at a glance. My comparisons were made with specimens of *capsella* received from Gould, and *placentula* from W. G. Binney. The figures are camera lucida drawings.

From Proc. Acad. Nat. Sci. Phila., 1889, p. 412, Plate XII. Figs. 8–10.

Pupa calamitosa, Pilsbry.

Shell minute, cylindrical, very blunt at apex, chestnut-colored; whorls 4½, the first one and a half smooth, the following regularly costulate striate, the costulæ separated by spaces wider than themselves; last whorl abruptly turning forward, rounded beneath, encircled by a slight central constriction or furrow; aperture about one third the total length of shell, rounded, truncated above, contracted within; peristome thin, expanded, without crest or callous thickening behind; columellar margin rather dilated; parietal wall bearing two entering lamellæ, one arising near the termination of the outer lip, the other more deep seated, elevated, entering less obliquely; columella with a strong white deep-seated obliquely entering fold; outer lip with two short white lamellæ.

Alt. 1.70, diam. 0.80 mm.

Two trays of this tiny species are before me. One received from Henry Hemphill, collected near the mouth of San Tomas River, Lower California, the other collected by Orcutt near San Diego, California. Most specimens show the widening inward of the outer lip shown in the figure. Several specimens have only one lamella on the outer lip, and are rather larger than the typical form described, measuring 1.90 mm. alt. The second parietal lamella is usually much larger than the first, but in one or two specimens before me this is not the case. The umbilical rimation terminates in a tiny depression, perhaps minutely perforated at the axis. The formula of denticles or folds (according to Dr. Sterki's scheme[1]) AA B D E or AA B E. The species is of a decidedly different type from any known American *Pupa*. *P. hordacea*, *Californica*, and *Rowelli*, abundant Western forms, belong in quite diverse groups; the first being allied to *P. corticaria* and *pellucida*, the last two grouping with *P. decora*, *Rowelli*, and *corpulenta*.

From the *Pupæ* of the Mexican fauna, *leucodon*, *pellucida*, and *chordata*, the present species is quite distinct in every respect.

The inward continuation of the parietal and columellar folds is shown in Figure 17. They are white, regularly veined with darker, like polished plates of agate.

From Proc. Acad. Nat. Sci. Phila., 1889, p. 411, Plate XII. Figs. 16, 17.

Mr. Hemphill sends me the following descriptions, which must be fully credited to him : —

Helix tudiculata, var. Binneyi.

This beautiful variety belongs to the globosely depressed forms of *H. tudiculata*, Binn. It is of a uniform greenish yellow color, without blotches or markings, except a very faint trace of a band at the periphery. *H. tudiculata* is very variable in form, size, and sculpture, and with the umbilicus either open or closed, but it is very constant in its dark chestnut-color in Southern California. North of Merced County, however, it becomes a shade lighter, and passes towards the light, thin form of *H. arrosa*, which I regard as the

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[1] See Proc. U. S. Nat. Mus., 1888, p. 369. I have repeated the letter presenting the parietal fold, as the two seem to be of equal importance.

progenitor of *tudiculata*, *arrosa* in turn having evolved from its northern neighbor, *H. Townsendiana*, Lea, and *Townsendiana* from the form we now call *H. ptychophorus*, Brown, found in Eastern Oregon and Idaho.

Habitat. Mountains of San Diego County, California. Only one specimen found.

Helicodiscus fimbriatus, var. Salmonensis.

This variety varies from the Eastern or typical forms in the absence of the revolving lines; otherwise the shells are alike.

Habitat. Banks of Salmon River, Idaho, Old Mission, Idaho, and Oakland, California.

Helix Kelletti, var. albida.

This is a beautiful clear white translucent variety, with no markings or stains of any kind. It is quite thin and frail, and a trifle smaller than the average size of *Kelletti*.

Habitat. Santa Catalina Island, California. Two specimens only found by me.

Helix Kelletti, var. castanea.

Among the numerous patterns of coloring assumed by *H. Kelletti*, none are more conspicuous than this well marked variety. The body whorl is of a deep shiny chestnut-color above the periphery, and becomes lighter as it follows the whorls of the spire to the apex. The band at the periphery is quite variable in the different speeimens; it is generally light, and well defined above, but below it is irregular and spreads over the base of the shell more or less.

Habitat. Santa Catalina Island, California. This variety is not rare.

Patula strigosa, var. Buttonii.

Shell umbilicated, elevated, or moderately depressed, nearly white, sometimes stained with light chocolate; whorls five, convex, with numerous oblique striæ; suture impressed, aperture circular; peristome thickened, not reflected, darker than the body of the shell; extremities nearly approached and joined by a callus; with or without a basal tooth; tooth when present very variable, generally consisting of a single tubercle; in some specimens it is nearly or quite square, as high as long; in other specimens it is long and bifid.

Diameter of the largest specimen, $\frac{7}{8}$ inch; height, $\frac{1}{2}$ inch. Diameter of the smallest specimen, $\frac{1}{2}$ inch; height, $\frac{3}{8}$ inch.

Habitat. Box Elder Co., Utah.

I dedicate this interesting form of *strigosa* to my friend, Mr. O. Button, of Oakland, California.

Selenites Duranti, var. Catalinensis.

Shell widely umbilicate, depressed, white, transparent when fresh ; whorls 4, flattened above and below, with fine oblique striæ; spire planulate ; aperture transversely rounded ; peristome simple, acute ; extremities approached and joined by a very thin callus in fully matured specimens.

Greatest diameter, $\frac{1}{4}$ inch ; height, $\frac{1}{16}$ inch.

Habitat. Santa Catalina Island, California.

My little shell differs from the typical *Duranti* in its greater size, smoother surface, broader umbilicus in specimens of the same size, but principally in its transparent shining surface. It is larger than the largest *Duranti* that I have seen, but not so large as the costate variety of that species described by Mr. Mazyck as distinct under the name of *S. cœlata,* which I have in my possession. My specimen of that species is larger than his measurements.

I can add the following to his locality : Los Angeles and San Diego, California, Point Abunda, and banks of San Tomas River, Lower California; thus giving it a range of about two hundred miles up and down the coast. I have collected the typical *S. Duranti* at the following places : Etna Springs, Napa Co., Healdsburg, Sonoma Co., Bolinas and San Rafael, Marin Co., Oakland, Alameda Co., Santa Cruz, Monterey, Santa Barbara Island, Santa Catalina Island, and San Clemente Island, a range of over one hundred miles north and south. It is confined to the Coast Range as far as we know at present.

EXPLANATION OF THE PLATES.

PLATE I

Fig. 1. Central tooth of lingual membrane of *Mesodon major*, the specimen labelled A (see p. 190).

Fig. 2. Central tooth, two adjoining lateral teeth, and two marginal teeth of lingual membrane of *Mesodon major*, the specimen labelled B (see p. 190).

Fig. 3. Same: an outer lateral tooth bearing a side cusp and cutting point (see p. 190).

Fig. 4. *Mesodon Andrewsi*: the genitalia.

ov.	oviduct.
g. b.	genital bladder.
d. g. b.	duct of same.
v. d.	vas deferens.
r.	retractor muscle of penis sac.
p. s.	penis sac.
or.	common orifice.
p.	prostate gland.

Fig. 5. Penis sac of another specimen of same.

Fig. 7. Lingual dentition of same, from specimen labelled E. Two central teeth, with an adjoining lateral tooth.

Fig. 8. Same: marginal teeth.

Fig. 9. Same: extreme marginal teeth.

Fig. 10. Same: first lateral tooth of specimen labelled F (see p. 191).

Fig. 11. Same: marginal tooth (see p. 191).

Fig. 12. Same: specimen labelled M (see p. 191), an outer lateral tooth.

Fig. 13. The fourth lateral tooth of *Limax Hemphilli* (see p. 205).

Fig. 14. *Succinea chrysis*, Westerlund, copied from the "Vega Expedition," Plate III. Fig. 10.

Fig. 15. *Succinea annexa*, Westerlund, copied from the same, Fig. 11.

PLATE II.

Lingual dentition of: —

Fig. 1. *Limax maximus*. A central tooth with two adjacent laterals; an outer lateral; two marginals, the left hand one the last.

Fig. 2. *Prophysaon* (see p. 216). A central tooth with its adjacent lateral tooth; an outer lateral tooth; an extreme marginal tooth.

Fig. 3. *Limax Hemphilli.* A central tooth with two adjacent laterals; an outer lateral tooth; two outer marginal teeth.

Fig. 4. *Limax Hewstoni.* A central tooth with adjacent lateral on either side; incorrectly numbered on the plate; two extreme marginals.

Fig. 5. *Microphysa dioscoricola* (see p. 196).

PLATE III.

Fig. 1. *Prophysaon Andersoni*, J. G. C., received from Dr. Cooper.

Fig. 2. *Pupilla Floridana*, Dall, from original figure.

Fig. 3. Genitalia of *Hemphillia*, from Old Mission, Cœur d'Alene, Idaho (see p. 217): —

t.	testicle.
ep.	epididymis.
ov.	ovary.
ovid.	oviduct.
pr.	prostate.
g. b.	genital bladder.
d. g. b.	duct of same.
v. d.	vas deferens.
r.	retractor muscle of penis.
p. s.	penis sac.
or.	common orifice.

Fig. 4. *Helix exigua*, from an original drawing by Dr. Stimpson.

Fig. 5. *Zonites lasmodon*, Phillips, enlarged. Drawn by Miss Helen E. Lawson.

Fig. 6. Central portion of jaw of *Microphysa dioscoricola*, greatly enlarged.

Fig. 7. *Bulimus Floridanus* (see p. 201). Drawn from original specimen in Mr. Cumings's collection, by G. B. Sowerby.

Fig. 8. Lingual dentition of *Polygyra hippocrepis*.
a. central and two lateral teeth.
b. marginal teeth.

Fig. 9. *Bulimus Hemphilli.*

Fig. 10. Dentition of *Onchidium Floridanum.*

PLATE IV.

Fig. D was drawn by W. G. Binney, the other figures by Arthur F. Gray: all from life.

Fig. A. *Hemphillia glandulosa*, twice the natural size.

Fig. B. The same; animal in motion, natural size; the slit on the mantle partially open.

Fig. C. The same; partially contracted and at rest.

Fig. D. The same; the very young animal.

Fig. E. The same; dorsal view of posterior portion of the animal, twice the natural size; pore closed

Fig. F. The same; lateral view, pore closed.

Fig. G. The same; dorsal view, pore open.
  a. mucus beads exuding.
  b. slit widely opened, the walls or lips rolled out.
  c. mucus accumulations.
Fig. H. The same; lateral view, pore open.
Fig. I. The same as last.
Fig. J. The same; the internal shell plate.

PLATE V.

Figs. F, H, drawn by W. G. Binney; A, C, D, by Arthur F. Gray; B, E, G, I, by T. D. A. Cockerell, of West Cliff, Custer Co., Colorado: all from life.

Fig. A. *Ariolimax niger*, fully extended.
Fig. B. *Ariolimax Hemphilli*, var. *maculatus*, Cockerell; animal contracted in alcohol.
Fig. C. *Ariolimax niger;* the caudal mucus pore, twice the natural size, dorsal view, the pore open.
  a. mucus exuding.
  b. b. ridges each side of slit or channel.
  c. mucus channel or pore.
  d. little channels conducting mucus from back of animal into channel *c*.
Fig. D. The same; posterior view.
Fig. E. *Ariolimax Californicus*, in motion, natural size.
Fig. F. *Ariolimax Andersoni*, restored from an alcoholic specimen.
Fig. G. *Ariolimax Hemphilli*, in motion, with end of tail and pore.
Fig. H. Portion of genitalia of E.
  p. s. the penis sac.
  f. the flagellum.
  r. the retractor muscle.
  v. d. the vas deferens.
Fig. I. *Ariolimax niger*, partially extended.

PLATE VI.

Figures B, C, D, E, H, were drawn by A. H. Baldwin, the last from life, the others from specimens preserved in spirits; Figures F, G, by W. G. Binney, from life; A, from life, by Arthur F. Gray.

Fig. A. *Ariolimax Columbianus*, var. *maculatus*, Cockerell, natural size; from a specimen collected by Mr. Hemphill.
Fig. B, C. *Onchidium Floridanum*, three times natural size; from type.
Fig. D, E. *Onchidella Carpenteri*, twice natural size.
Fig. F. *Tebennophorus Wetherbyi;* from type.
Fig. G. Portion of genitalia of A.
  p. s. the penis sac.
  r. the retractor of same.
  v. d. the vas deferens.
Fig. H. *Tebennophorus Hemphilli*; from the type.

PLATE VII.

All the figures drawn by T. D. A. Cockerell, excepting I, which was drawn by Miss Annie Roberts.

Fig. A. *Prophysaon fasciatum.*
Fig. B. “ *Pacificum.*
Fig. C. “ *Andersoni.*
Fig D. “ *Hemphilli.*
Fig. E. “ *pacificum,* jaw
Fig. F. “ *humile,* jaw.
Fig. G “ “ the animal contracted in spirits, and the surface.
Fig. H. “ *Pacificum;* the same views as last.
Fig. I. “ *cæruleum.*
Fig. J. “ “
Fig K. “ *flavum.*
Fig. L. “ *humile.*
Fig. M. “ “

PLATE VIII.

Figure C was drawn by F. W. Earl, from life; A, from life, by W. G. Binney; B, D, G, I, from life, by T. D A. Cockerell; E, F, H, were restored by Mr Cockerell from specimen in spirits

Fig. A. *Phenacarion foliolatus,* natural size; the tail eaten off.
Fig. B. Internal shell of A.
Fig. C. The same, var. *Hemphilli,* natural size.
Fig. D. *Limax Hewstoni;* in motion and at rest.
Fig. E. “ *Hemphilli;* same views as last, and surface
Fig. F. “ *hyperboreus;* same views as last.
Fig. G. “ *montanus;* same views.
Fig H. “ *occidentalis;* same views.
Fig. I. “ *Hewstoni;* a larger individual.

PLATE IX.

Figures A, B, C, D, G, H, were drawn by W. G. Binney; E, F, by T. D. A. Cockerell; I, J, by Arthur F. Gray.

Fig. A. Jaw of *Hemphillia glandulosa.*
Fig. B. Jaw of *Phenacarion foliolatus.*
Fig. C. Lingual membrane of same; one central tooth, with its adjacent lateral and three extreme marginals.
Fig. D. Genitalia of same; one half of natural size.
ov. ovary.
ovid. oviduct.
t. testicle.
g. b. genital bladder.
p. s. penis sac.
v. d. vas deferens.

Fig. E, F. *Veronicella olivacea*, from one of original lot from Folvon.
Fig. G. Lingual membrane of *Glandina decussata*.
Fig. H. Genitalia of *Phenacarion foliolatus*, var. *Hemphilli;* same references as in D; one half of natural size.
Fig. I. *Prophysaon Andersoni;* surface magnified sixteen times.
  a. a. a. reticulations of the body.
  b. b. foliolated spaces between reticulations.
  c. lower edge of the body.
  d. locomotive disk.
Fig. J. The same, magnified eight diameters; upper surface; same references as the last.

PLATE X.

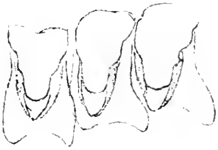
Drawn by A. H. Baldwin, Smithsonian Institution.

Fig. A. *Aglaja fidelis;* the large, elevated black variety.
Fig B. Sculpturing of same.
Fig. C. The same; small, black, elevated form.
Fig. D. Sculpturing of last.
Fig. E The same; small, depressed form.
Fig. F *Aglaja infumata;* sculpturing.
Fig. G. *Arionta Mormonum;* sculpturing of the form figured on Plate XI. Figs. G, H.

PLATE XI.

Drawn by A. H. Baldwin.

Fig. A. *Arionta arrosa.*
Fig. B. Variety of last, approaching *A. exarata.*
Fig. C. Sculpturing of last.
Fig. D. *Arionta exarata;* type.
Fig. E. Sculpturing of last.
Fig. F. *Arionta Mormonum.*
Fig. G, H. Variety of last, connecting with *Hillebrandi.*



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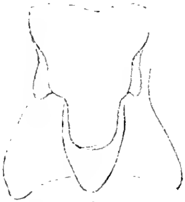

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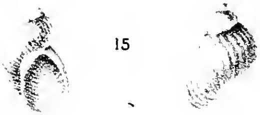

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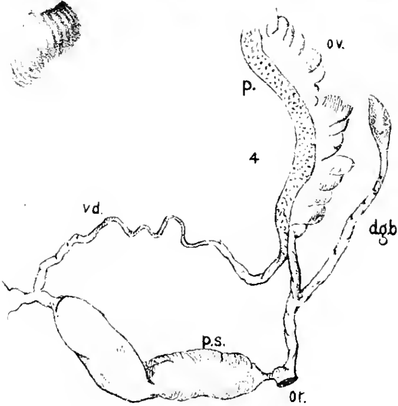

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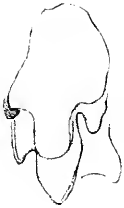

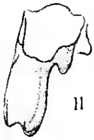

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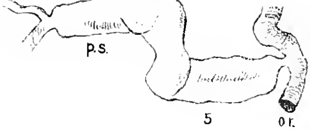

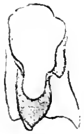

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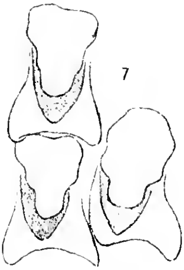

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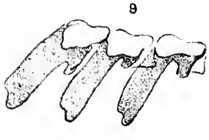

W.G B.del.

Buffords

Binney; 3d Suppl. to Terr Moll. Plate. II

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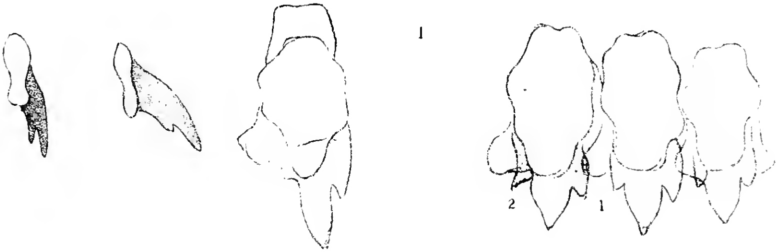

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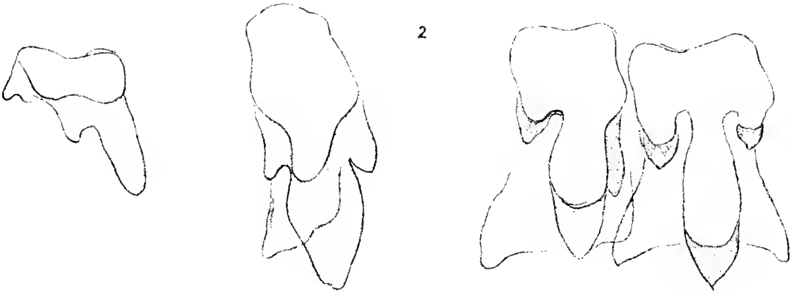

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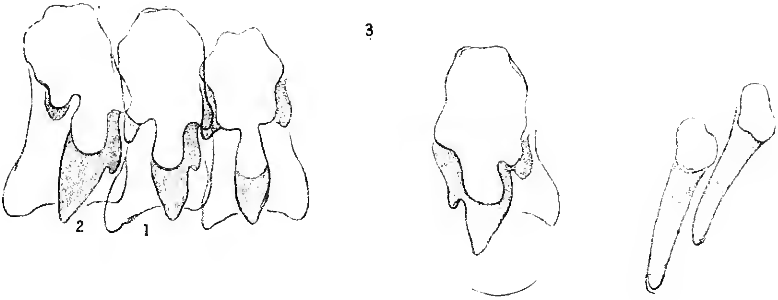

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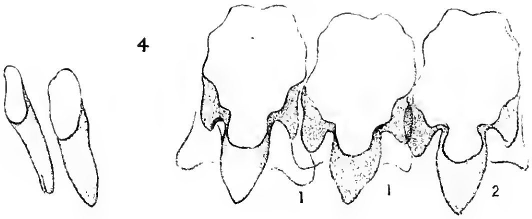

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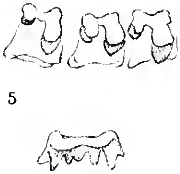
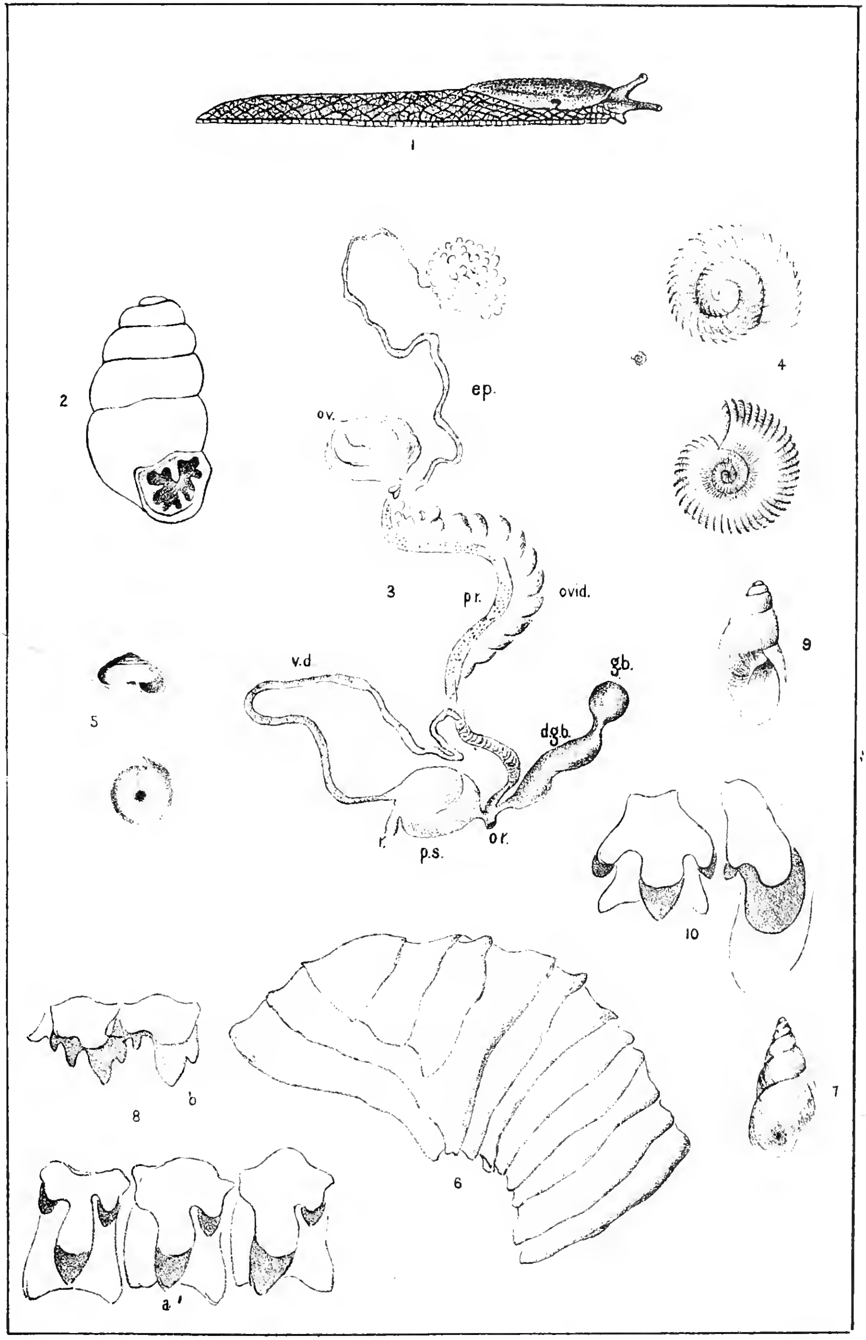


W.G.B. del.

Buffords

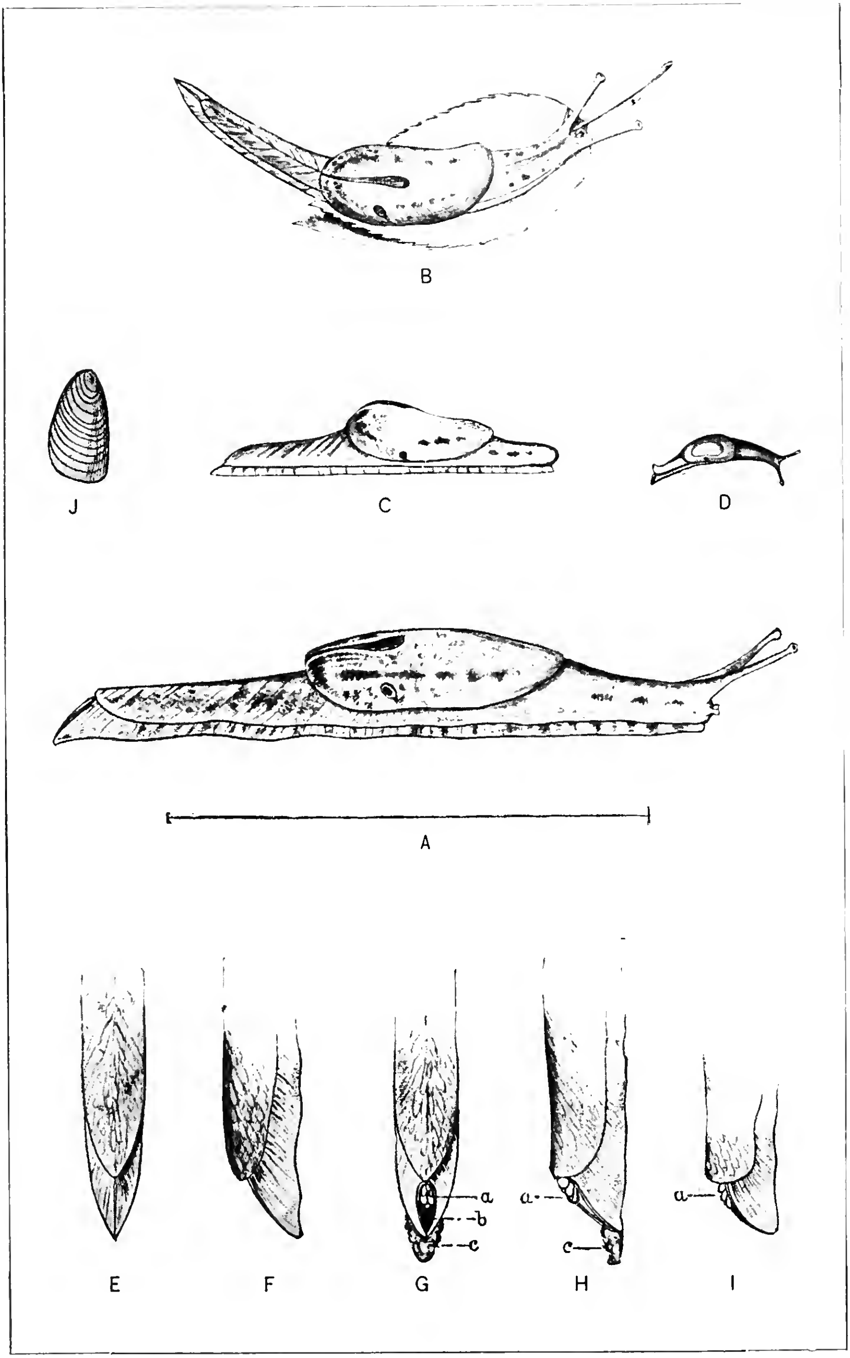
Binney; 3d Suppl to Terr Moll. Plate. III

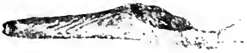


W.G.B.del.

Buffords

Binney; Suppl. to Terr. Moll. Plate IV.





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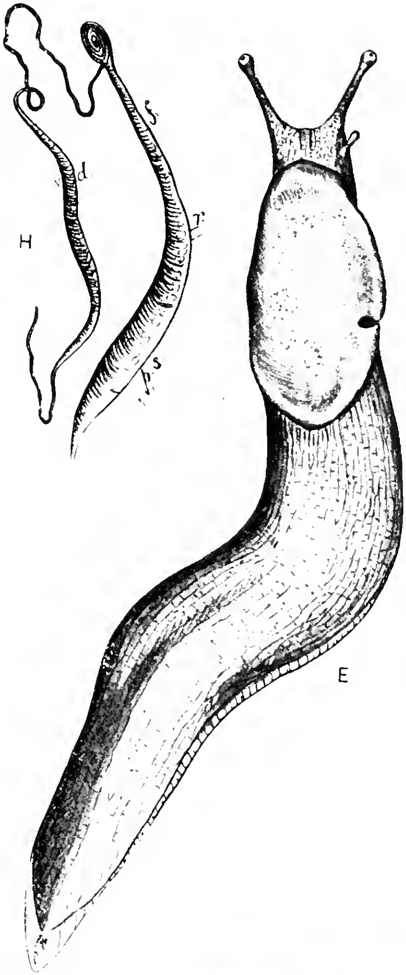

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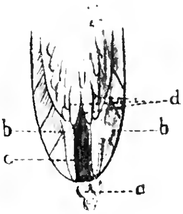

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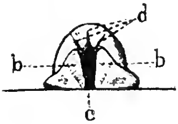

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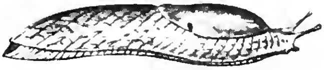

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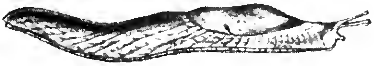

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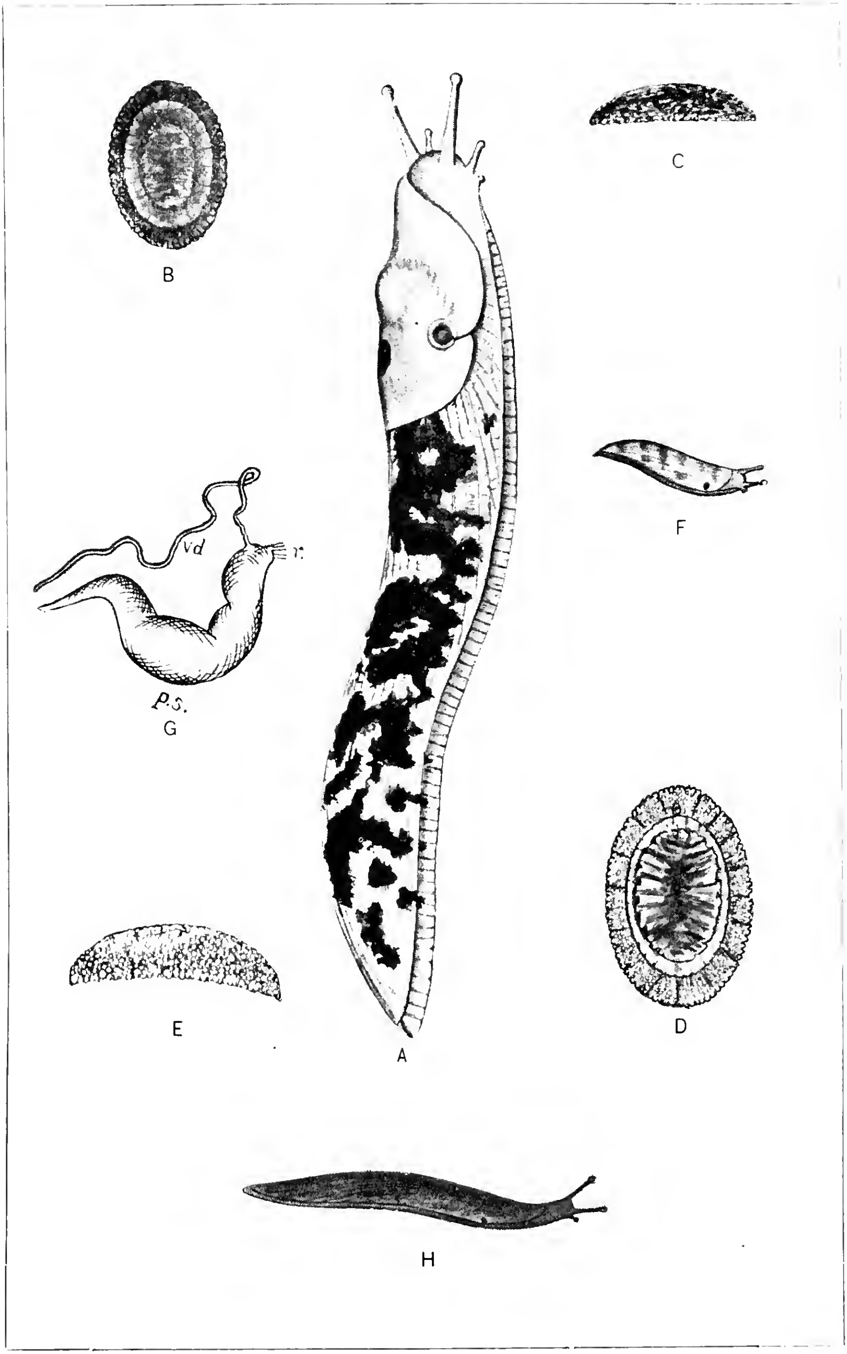
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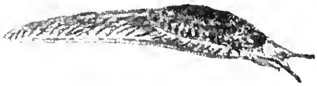

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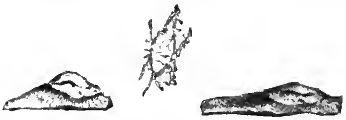
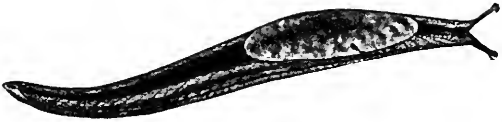


H

Binney; Suppl. to Terr. Moll. Plate VIII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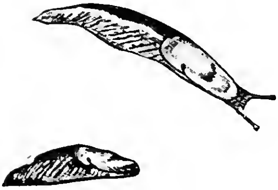
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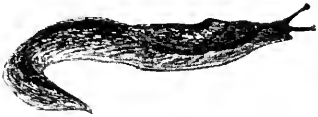
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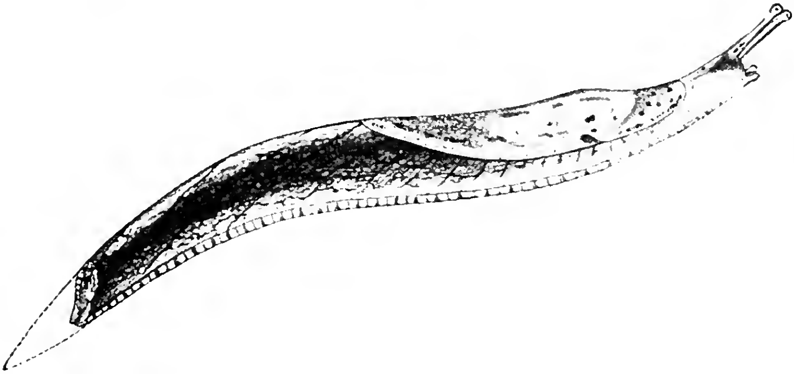
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Binney; Suppl. to Terr. Moll. Plate IX.

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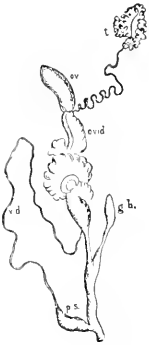

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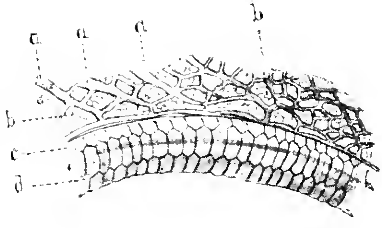

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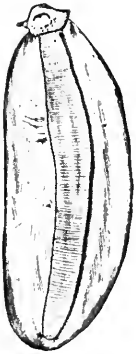

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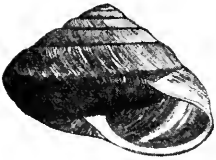

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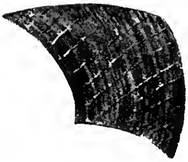

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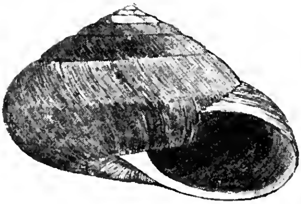

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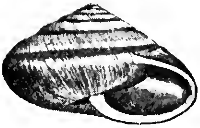

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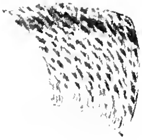

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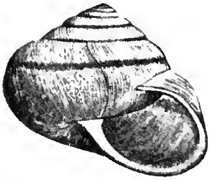

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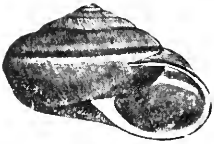

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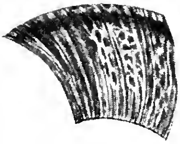

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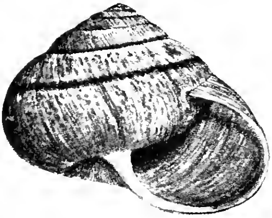

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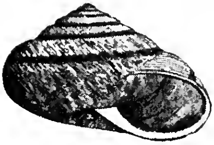

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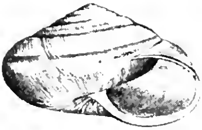

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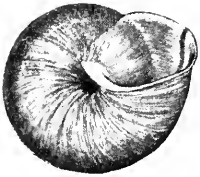

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