

INVERTEBRATE
ZOOLOGY
—
DREW

Fifth Edition
Revised

MARINE BIOLOGICAL LABORATORY.

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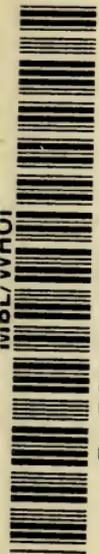
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A LABORATORY MANUAL OF INVERTEBRATE ZOOLOGY

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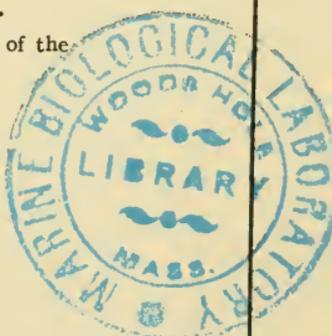
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FIFTH EDITION, RESET

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PHILADELPHIA

REVISERS OF THE FIFTH EDITION OF DREW'S
INVERTEBRATE ZOOLOGY

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PREFACE TO THE FIFTH EDITION

THE present revision of this Manual has been made by the undersigned for the purpose of modifying and enlarging the fourth edition. Some important changes in classification have been made and the instructions for the study of certain commonly used animals have been made more complete. Such errors as had not been eliminated in earlier editions have, it is hoped, been corrected. The glossary has been thoroughly rewritten and enlarged.

J. A. DAWSON,
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NEW YORK CITY, N. Y.
March, 1936

PREFACE

THIS manual has for its basis a set of laboratory directions prepared by the instructors in the Zoölogy Course given at the Marine Biological Laboratory at Woods Hole, Massachusetts, for the use of students in that course. These manifolded outlines were first used in 1901. Associated with me in the preparation of the first notes were Dr. Robert W. Hall, Dr. James H. McGregor, Mr. Robert A. Budington, and Dr. Caswell Grave. For several years the notes were modified and additions were made before there was any thought of publication. During this period other instructors became members of the staff and added to the directions. These instructors were Dr. Winterton C. Curtis, Dr. D. H. Tennent, Dr. Otto C. Glaser, Dr. Grant Smith, Dr. John H. McClellen, and Dr. Lorande L. Woodruff.

Since publication, the instructors in this course have offered many suggestions and criticisms that have aided greatly in revisions. I am especially indebted to Dr. Lorande L. Woodruff, who has given much attention to the revision of the Protozoa, and to Dr. Winterton C. Curtis, Dr. Caswell Grave, and Dr. W. C. Allee, who have successively been in charge of this course.

Probably few instructors will find it desirable for their students to follow closely all that is given in this manual, but it has seemed better to arrange the matter in a logical order, and in some of the forms to call attention to only the important points of anatomy or adaptation, than to try to make the directions for each form complete in themselves.

Since the first appearance of the manual in book form there have been many suggestions that directions for other forms be included, or that more complete directions be given for some of the forms treated. These suggestions have been followed in many cases. There is, however, danger that students will

learn only to follow directions, while they should be encouraged to devise their own methods of getting at the facts. For the comparative study of related forms complete directions are not needed and should not be given. The method sometimes used, evidently the favorite method of Agassiz, of giving a student an animal without directions and letting him work out his own salvation, is the true research method, and to this all who continue with Zoölogy must come in time. It is, of course, laborious and time consuming and not adapted to course work, but there is danger that its great value will be overlooked. It is so much easier for both instructor and student to follow directions.

The type method of laboratory study has for many years been the prevailing method, but care needs to be exercised to keep students from making everything conform to type, and in leading them to see the wonderful adaptations that fit the different animals for their particular lives. The manual is not intended to lead students to a knowledge of comparative anatomy alone, but to an appreciation of adaptation as well.

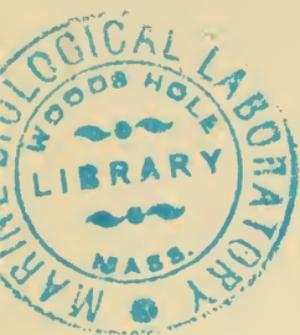
There has been no attempt to make the literature list at all complete, but it seems desirable to refer students to some of the available papers, for by consulting them in connection with their laboratory work they become acquainted with methods of work and develop the spirit of research that is the beginning of real understanding.

Certain books that have not been mentioned under the special heads, as they apply to practically all groups, should be used freely for reference. Among these may be mentioned Parker and Haswell, *Text-book of Zoölogy*, Macmillan; Lankester, *A Treatise on Zoölogy*, Black; Harmer and Shipley, *The Cambridge Natural History*, Macmillan; Lang, *Lehrbuch der Vergleichenden Anatomie*, Fischer; or the English translation, Macmillan; Korschelt und Heider, *Lehrbuch der Vergleichenden Entwicklungsgeschichte*, Fischer; or the English translation, Macmillan; Delage et Hérouard, *Traité de Zoölogie Concrete*, Schmidt; Pratt, *A Manual of Common Invertebrate Animals*, McClurg & Co.; MacBride, *Text-book of Embryology*, Vol. I, Macmillan; Verrill and Smith, *Invertebrate Animals of Vineyard Sound*, Bul. U. S. F. C., 1871; and Sumner, Osborn,

Cole and Davis, A Biological Survey of the Waters of Woods Hole and Vicinity, Bul. U. S. Bur. Fish., 30, 1911.

It has been my part to put the original directions into book form and to see that desirable changes were made in them, but much credit belongs to the men who have been associated with me in the instruction work at the Marine Biological Laboratory.

THE AUTHOR.



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INVERTEBRATE ZOOLOGY

PROTOZOA

Unicellular Animals

Subphylum 1. Zoomastigophora (Animal Flagellates).

Animal flagellates with no chromatophores or chlorophyl. Locomotor organs of adult phases consist of one or more motile structures called flagella. (The animal flagellates are similar in other general respects to the plant flagellates—Phytomastigophora of many older zoological classifications—now almost universally classed as members of the Algae.)

CLASS 1. Protomastigota.

Order 1. Protomonadida.

Minute forms. One flagellum, or a principal flagellum and 1 or 2 accessory flagella. (Mastigamoeba, Multicilia, Trypanosoma, Peranema, Monas, Cercomonas, Bodo.)

CLASS 2. Metamastigota.

Order 1. Hypermastigida.

With numerous flagella and complicated internal structure. All but one genus (Lophomonas) parasitic in termites. (Lophomonas, Trichonympha, Joenia.)

Order 2. Polymastigida.

Minute forms with highly developed kinetic elements. 3 to 8 flagella. Characteristic parasites of digestive tract. (Giardia, Trichomonas, Pyrsonympha, Streblomastix.)

Subphylum 2. Sarcodina.

No permanent locomotor organs in "adult" phase of the life history; the cells moving and ingesting food by temporary pseudopodia. "Young" phases may be amoeboid or flagellate. (Minchin, pp. 178 and 234-237.)

CLASS 1. Actinopod.

Chiefly spherical floating forms with slender unbranched radiating pseudopodia supported by an internal axial filament.

Subclass 1. Heliozoa.

Fresh-water forms without a "central capsule" separating ectoplasm and endoplasm. (Actinosphaerium, Actinophrys, Clathru-lina.)

Subclass 2. Radiolaria.

Marine forms with a central capsule. (Thal-assicola.)

CLASS 2. Rhizopoda.

Forms with branched, rootlike pseudopodia. Locomotion chiefly by creeping.

Subclass 1. Proteomyxa.

Forms with raylike pseudopodia frequently branching and with no axial filaments. (Nu-clearia.)

Subclass 2. Mycetozoa.

Semiterrestrial forms with myxopodia and plasmodium formation. (Stemonitis.)

Subclass 3. Foraminifera.

Chiefly marine forms with reticulate pseudo-podia and complex tests. (Lecythium, Glo-bigerina.)

Subclass 4. Amoebaea.

Simple amoeboid forms, typically with lobose pseudopodia; with or without a simple test. (Amoeba, Arcella, Diffugia.)

Subphylum 3. Infusoria.

With motile organs in the form of cilia during all or part of the life cycle. Nucleus generally dimorphic (macronucleus and micronucleus). Reproduction is by simple transverse division or by budding.

CLASS 1. Ciliata.

With cilia throughout the life history.

Subclass 1. Holotricha.

Cilia are of approximately equal length and equally distributed over body. Trichocysts frequently present. No adoral zone of mem-branelles. (Opalina, Prorodon, Lacrymaria, Coleps, Lionotus, Nassula, Frontonia, Col-pidium, Didinium, Paramecium.)

Subclass 2. Spirotricha.

With adoral zone of membranelles right-wound toward the mouth. Peristome not drawn out like a funnel. Includes as chief orders Heterotrichida, Oligotrichida and Hypotrichida. (Spirostomum, Stentor, Oxytricha, Stylonychia, Euplotes. Halteria.)

Subclass 3. Peritricha.

With adoral zone of membranelles left-wound toward the mouth. Cilia usually limited to those in the adoral zone. (Vorticella, Zoothamnium, Carchesium, Epistylis.)

Subclass 4. Chonotricha.

Adoral zone right-wound toward the mouth. Peristome drawn out like a funnel. (Spirochona.)

CLASS 2. Suctoria (Acinetaria, Tentaculifera).

Usually possessing cilia only during embryonic stages. Tentacles adapted for piercing and sucking are present in the adults. (Podophrya, Ephelota, Acineta.)

Subphylum 4. Sporozoa.

Without cilia or flagella in the "adult" period of the life cycle. Reproduction is by spore formation (multiple fission). All are endoparasites.

CLASS 1. Telosporidia.

Sporulation phase of the life cycle is distinct and follows the trophic phase.

Subclass 1. Gregarinina.

Typically lumen-dwelling parasites of invertebrates. Reproduction by sporogony alone or by sporogony and schizogony.

Order 1. Eugregarinida.

Comprises most of the gregarines. Sporozoites, usually 8 in number, formed only after sexual phenomena. (Monocystis, Gregarina.)

Order 2. Schizogregarinida.

Parasites of the digestive tract of arthropods, annelids and tunicates. With an asexual cycle. (Schizocystis.)

Subclass 2. Coccidiomorpha.

Found in all groups of animals. Typically intracellular in all stages of life history. Life cycle varies greatly in complexity.

Order 1. Coccidiida.

Suborder 1. Eimeriina.

With few exceptions, epithelial-cell parasites with sporoblasts in a capsule. (Eimeria.)

Suborder 2. Hemosporidia.

Typically blood parasites of vertebrates. In many forms the entire sexual period of the life cycle takes place in an intermediate host, as the mosquito. (Plasmodium.)

Suborder 3. Babesiina.

Blood parasites of vertebrates. They lack melanin pigment. (Babesia.)

Order 2. Adeleida.

With no flagellated gametes. Sexual process similar to pseudoconjugation in gregarines. (Adelea.)

CLASS 2. Cnidosporidia.

(This and the following class were formerly classed under Neosporidia.) Sporulation takes place during the "trophic" phase of life cycle. Sporozoites are amoebulae.

Order 1. Myxosporidia.

Typically parasites of fishes. Free or tissue-inhabiting. Spore capsule with 2 valves. Usually 2 polar capsules. (Myxidium.)

Order 2. Actinomyxida.

Parasites of annelids. Spores with 3 polar capsules. (Sphaeractinomyxon.)

Order 3. Microsporidia.

Minute organisms—rarely more than 1 polar capsule, sometimes none. (Nosema.)

CLASS 3. Acnidosporidia.

Includes the group formerly known as the Sarcosporidia. The initial stage of the life cycle occurs in the muscle cells of vertebrates. Spores with a single polar capsule. (Sarcocystis.)

PROTOPHYTA

ALGAE. In this group are now included flagellates formerly classed under the Protozoa as Phytomastigophora. Volvox (p. 33) is now classed under the Class Isokontae (formerly under Order Phytomonadida); Euglena under Class Eugleninae (formerly Order Euglenida); Ceratium and Noctiluca under Class Dinophyceae (formerly Order Dinoflagellida).

Blochmann: Die Mikroskopische Tierwelt des Süßwassers. Abt. 1. Protozoa, 1895.

Bütschli: Protozoa. Bronn's Thierreich, 1889.

Calkins: Biology of the Protozoa, 1933.

—: Marine Protozoa of Woods Hole. Bull. U. S. Fish. Com., 1901.

Cleveland: Effects of Oxygenation and Starvation on Intestinal Flagellates of Termopsis. Biol. Bull., 48, 1925.

Conn: Fresh Water Protozoa of Connecticut. Bull. State Nat. Hist. Surv., 1905.

Doflein and Reichenow: Lehrbuch der Protozoenkunde, 1929.

Edmondson: Protozoa of Iowa. Davenport Acad. Sci., 1906.

Hartmann and Kiskalt: Praktikum der Bakteriologie und Protozoologie, 1910.

Jennings: Behavior of the Lower Organisms, 1906.

—: Life and Death, Heredity, and Evolution in Unicellular Organisms, 1920.

Kent: Manual of the Infusoria, 1881.

Kudo: Handbook of Protozoology, 1931.

Lankester: Treatise on Zoölogy. 1. Protozoa.

Leidy: Fresh Water Rhizopods of North America, 1879.

Maupas: Studies on Infusoria, in Arch. Zool. Exp. et Gen., 1883, 1888, and 1889.

Minchin: Protozoa, 1912.

Prowazek: Einführung in die Physiologie der Einzelligen (Protozoen), 1910.

—: Taschenbuch der Mikroskopischen Technik der Protistenuntersuchung, 1909.

Stokes: Contribution Toward a History of the Fresh Water Infusoria of the United States. Jour. Trenton Nat. Hist. Soc., 1, 1888.

Ward and Whipple: Fresh-Water Biology, 1918.

Whipple: Microscopy of Drinking Water, 4th ed., 1927.

Woodruff: Observations on the Origin and Sequence of the Protozoan Fauna of Hay Infusions. Jour. Exp. Zool., 12, 1912.

SARCODINA

AMOEBA PROTEUS

Amoebae are usually just discernible under the low power of the microscope as irregular, semi-transparent, granular bodies. Find a specimen in the material provided, which is known to contain amoebae, and determine the following points:

1. With the high power observe the peculiar method of locomotion, the constant but slow change in the shape of the body by means of projections, *pseudopodia*, or "false feet."

Make sketches at intervals of one or two minutes to show the changes in the form of the body.

2. Observe the peripheral zone of hyaline protoplasm, the *ectoplasm*, and compare this with the inner protoplasm, the *endoplasm*. Observe in detail the formation of a pseudopodium. Does the endoplasm extend into the pseudopodium? Can you explain how the movement is caused?

3. Find a clear space which appears and disappears at intervals; this is the *contractile vacuole*. Determine the length of time between successive contractions. Are the intervals regular? When the vacuole contracts what becomes of the contents? What is its function?

4. Note the *nucleus*. In order to determine the actual shape of this structure observe it carefully as it is moved through the endoplasm. The nucleus of *Amoeba proteus* may appear at one time with a circular outline and in another view may seem much flattened or even somewhat biconcave.

5. Food materials in process of digestion are readily seen. Of what do they consist? They are contained in *food vacuoles*. By careful watching, it is often possible to observe the manner in which food is ingested and the manner in which the undigested matter is egested.

6. Observe the *crystals* within the endoplasm. What is their shape? Is more than one type present?

7. Determine whether the surface of the amoeba shows ectoplasmic ridges.

The large free-living amoebae are identified chiefly by the following characters: (a) type of pseudopodia and the appearance of these when the animal is crawling freely, (b) the shape of the nucleus, (c) presence or absence of ectoplasmic ridges, (d) character of the contained crystals.

Make a careful drawing of an Amoeba.

If time and material permit, study *Amoeba dubia*, *A. verrucosa*, *Arcella*, and *Diffugia*, and compare them with *A. proteus*. Do you understand how the shells of the last two genera are made, and of what service they are?

Drawings of these forms are desirable.

Calkins: Genera and Species of Amœba. Trans. Fifteenth International Congress on Hygiene, 1912. The Fertilization of *A. proteus*. Biol. Bull., 13, 1907.

Dawson, Kessler and Silberstein: Mitosis in *Amœba dubia*. Biol. Bull., 69, 1935.

Dellinger: Locomotion of Amœba and Allied Forms. Jour. Exp. Zool., 3, 1906.

Metcalf: Amœba Studies. Jour. Exp. Zool., 9, 1910.

Popoff: Ueber den Entwicklungscyclus von *A. minuta*. Arch. f. Protistenk., 22, 1911.

Schaeffer: Notes on the Specific and Other Characters of Amœba Proteus, etc. Arch. f. Protistenk., 37, 1916.

—: Taxonomy of the Amebas. Carnegie Inst., Washington, 1926.

THE FORAMINIFERA

With very few exceptions Foraminifera are marine and provided with shells. Specimens may be obtained from material scraped from wharf pilings. Examine them with a low power by reflected light.

1. Carefully examine various shells, compare them with each other and with figures. Notice the great variety in size and shape and determine how the chambers must have been added during growth.

2. Observe a single opening in a shell, and determine whether the general surface has any finer perforations. Be sure to understand the relation of the live animal to the shell.

Make drawings of several types of shells.

- Cushman: Foraminifera, Their Classification and Economic Use, 1928.
Farmer: Foraminifera, pp. 133-139, Lankester's Treatise.
Flint: Recent Foraminifera. Rep. U. S. Nat. Mus., 1897.
Calkins: Marine Protozoa of Woods Hole. Bull. U. S. F. C., 1901.

ACTINOSPHERIUM OR ACTINOPHRYS

Find, as usual, with the low power, and increase the magnification as occasion demands. On account of its large size it is better to study Actinosphaerium in a culture dish or depression slide using the low power only.

1. Note the many fine radiating *pseudopodia*. These are quite stiff compared with those of *Amoeba* and for a considerable time show little change, not being pushed out and retracted constantly as in *Amoeba*. Is the animal flat or spherical?

2. Both *ectoplasm* and *endoplasm* are so filled with vacuoles that they present a frothy appearance characteristic of most Heliozoa. The endoplasm of all Protozoa is alveolar in structure, but in Actinosphaerium the vacuoles are exceptionally large, though not as large as those in the ectoplasm. In Actinophrys the endoplasm is not so sharply separated from the ectoplasm.

3. The *nucleus* of Actinophrys is present in the center of the organism, but it is somewhat difficult to demonstrate in the live animal. In Actinosphaerium there are many nuclei. These can be seen well only in stained specimens.

4. At some point near the periphery, the *contractile vacuole* can usually be seen. When it is found notice its action, and immediately after it has contracted look among the pseudopodia of that region for indications of its extruded contents.

Draw a specimen, indicating all of the points observed.

5. When the contractile vacuole discharges, or when any foreign body touches the ends of the pseudopodia, notice the way in which this type of pseudopodium is moved. What does this indicate in regard to its structure? How far do the pseudopodia extend? They may be seen to contain

minute granules when studied with the high power and best light.

6. If possible, observe the process of catching food with the tips of the pseudopodia and the manner in which it is drawn toward the body. Note any motion on the surface of the body as the food is drawn closer, and also the manner in which the food is finally ingested. Are there any indications that the pseudopodia extend as still finer filaments beyond the point to which it is possible to trace them with the highest magnification at hand? If the capturing of food is observed, make a series of diagrams to illustrate the process. (Minchin, p. 50.)

If possible, observe a specimen undergoing *division*.
Draw.

It is desirable to examine *Clathrulina*, noting the stalk and skeleton. Look over figures.

R. Hertwig: Ueber die Kernteilung, Richtungskörperbildung und Befruchtung bei Actinosphaerium. Abt. d. Math. Phys. Kl. d. Ak. d. Wiss., München, 19, 1898.

INFUSORIA

PARAMECIUM

Place a drop of the culture on a slide, cover, and examine with the low power.

1. In an animal not closely confined note the shape and movements. Is it possible to distinguish an anterior and a posterior end? A forward and backward movement? Is one side of the animal kept constantly uppermost? Is there a dorsal and a ventral surface? Do the animals change their shape either permanently or temporarily? Individuals tend to collect about air bubbles and at the edge of the cover-glass. Why?

Indicate by a sketch all the points which can be determined with the low power.

2. Draw off all superfluous water by means of filter paper, add a trace of powdered carmine, and then find a specimen

which is narrowly confined and examine it with the high power.

The particles of carmine are taken into the body. Determine how and where. Note that the carmine collects in *food vacuoles*. What do you think is probably the nature of the fluid in the vacuoles? In watching them do you notice any definite movement of the protoplasm? Try to see the undigested material ejected.

3. Determine the arrangement of the *cilia*, and the nature of their motion. Is there a reversal of the direction of the stroke, etc.?¹

4. Observe the *contractile vacuoles*. How many are there? Is their position constant? What is their action? In compressed specimens the *contractile vacuoles* and their *reservoirs* are usually conspicuous. Note the order of appearance and disappearance of the vacuoles and reservoirs.

5. Focus carefully on the margin of the body and note a very thin outer *cuticle*. A thick layer, the *ectoplasm*, devoid of granules but containing radially arranged, minute, oval bodies, the *trichocysts*, is just internal to the cuticle. The inner mass of protoplasm, containing the contractile and food vacuoles, and small granules, is the *endoplasm*.

6. If possible distinguish the clear, centrally located *nucleus (macronucleus)*.

Make a sketch showing all of the above points.

7. Kill the animal by running a drop of methyl green under the coverglass. What happens to the cilia? It is possible to watch the process of staining in the macronucleus if specimens are observed very soon after the methyl green is added. In *Paramecium caudatum* the *miconucleus* may also be seen. If *P. aurelia* is being studied the miconuclei usually cannot be detected by this method.

¹ It is possible to decrease the rate of movement of the animals by placing them in a solution of quince-seed jelly or by teasing a small piece of lens-cleaning paper in the medium containing the paramecia. Specimens so treated remain alive for some time.

8. Place a drop of culture containing a number of animals on a slide and put a drop of Waterman's fountain pen fluid on a coverglass. Invert the coverglass and place it with its hanging drop of ink on the slide. What happens to the trichocysts?

Make a drawing showing the structures revealed by the treatment in these two cases.

9. Observe, if possible, animals dividing and conjugating.

10. Study demonstrations of permanently stained specimens for finer structure.

Calkins and Cull: Conjugation of *P. caudatum*. Arch. f. Protistenk., 10, 1907.

Jennings: Effect of Conjugation in Paramecium. Jour. Exp. Zool., 14, 1913.

Metalinkow: Contributions à l'étude de la digestion. Arch. d. Zool. Exp. et Gen., 9, 1912.

Wenrich: Eight Well Defined Species of Paramecium. Trans. Amer. Micr. Soc., 47, 1928.

Woodruff: Paramecium aurelia and Paramecium caudatum. Jour. Morph., 22, 1911.

Woodruff and Erdmann: A Normal, Periodic Reorganization Process (Endomixis) Without Cell Fusion in Paramecium. Jour. Exp. Zool., 17, 1914.

Woodruff: The Structure, Life History, and Intrageneric Relationships of Paramecium Calkinsi, sp. nov. Biol. Bull., vol. 41, 1921.

SPIROSTOMUM

1. Compare Spirostomum with Paramecium, noting the method of locomotion, the shape of the body, the ciliation, the *buccal groove* and *mouth*, and the large excretory reservoir, filling the posterior end of the body and in communication with the anterior end of the body by a *canal*.

2. Note the highly refractive, long, bandlike (*moniliform*) *macronucleus*. In a less common species of Spirostomum the macronucleus is similar to that of Paramecium.

3. Note the sudden contractions of the body. When these occur spiral lines appear on the surface. Can you distinguish these lines when the animal is extended? These are primitive structures (*myonemes*) functioning as muscles.

Make a drawing of the extended animal and a diagram showing the form when contracted. (See Doflein and Reichenow.)

VORTICELLA

Place a number of individuals on a slide and cover loosely to avoid crushing. As usual, study first with the low power and then with the high.

1. Notice that the body of *Vorticella* has the general shape of an inverted bell. The covering of the body is a very thin transparent layer, the *cuticle*, underneath which is the peripheral layer of *ectoplasm* enveloping the more fluid and granular *endoplasm*.

2. The *peristome* is the rounded rim about the base of the bell.

3. The elevated and inclined area included within the peristome, and ciliated around the edge, is the *disk*. It is somewhat convex.

4. The marked depression between the disk and the peristome is the *vestibule*. It is also lined with cilia. The vestibule defines the ventral surface of the animal.

5. The *gullet*, a slender canal, leads from the vestibule toward the center of the body.

6. The feces escape from the body by the side of the vestibule. The opening is temporary.

7. Within the endoplasm are situated the clear *contractile vacuole*, several *food vacuoles*, the long U-shaped *macronucleus*, and the small round *micronucleus*. The macronucleus may be made more distinct by treating with methyl green.

8. The *stalk* is composed of a sheath, which is continuous with the cuticle of the body, and, within the sheath, the contractile axis or *myoneme*, which is continuous with the body ectoplasm. Notice that this myoneme is situated within the sheath in a very loose spiral, and that the stalk quickly contracts into a close spiral when the animal is stimulated. Observe also the manner in which the peristome folds over

simultaneously with the contraction of the stalk. What purpose does the contraction of the stalk serve?

Vorticella is distinguished from its allied genera by its simple unbranched stalk and also by the spiral form assumed by the contracted stalk. In which order of the Ciliata does the ciliation of Vorticella place it? Compare with Zoothamnium.

Make a drawing of an expanded individual and a sketch to show the condition when contracted. (Minchin, p. 434.)

9. Study, by means of finely powdered carmine, the vortex currents set up by the cilia. Note how the particles are collected in the gullet, and at intervals are forced in rounded masses into the endoplasm to form *food vacuoles*. Is there a definite circulation in the endoplasm?

10. Endeavor to find several stages of reproduction by division.

Large fresh-water species of Vorticella are preferable for study, but marine species may be substituted when necessary. If time and material permit, study *Lichnophora*, a marine peritrichous form parasitic on *Crepidula*. (See Calkins' Protozoa, p. 203.)

Schröder: Beiträge zur Kenntnis von *V. monilata*. Arch. f. Protistenk., 7, 1906.

OXYTRICHA

Infusoria belonging to the genus *Oxytricha*, or the genera *Stylonychia*, *Pleurotricha*, *Euplotes*, etc., may be used for the following study. These forms belong to the order Hypotrichida. Hypotrichous forms are among the most highly organized of the class Infusoria, as well as of the entire phylum of Protozoa, and present a complexity of structure and function which probably is not exceeded within the limits of a single cell elsewhere in the animal series.

1. In an animal which is becoming quiet, note the mode of locomotion, the shape of the body, the *buccal groove*, the *contractile vacuole*, etc., as in other forms studied. Compare

the *ciliation* with that of other forms. Refer to Calkin's *Biology of the Protozoa*, pp. 152-162, and understand the relation of *cirri*, *membranelles*, etc., to cilia.

Draw, showing the structure in detail.

2. Run some methyl green under the coverglass. What is the shape of the *macronucleus*? The shape varies considerably in the different genera. Is it possible to distinguish the *micronuclei*?

3. Prepare a fresh slide and observe in detail the characteristic movements and manner of creeping over various objects. As the animal turns sidewise, note the marked dorso-ventral compression of the body.

Represent this diagrammatically beside the previous drawing.

It is desirable to examine permanently stained preparations for division stages, finer details of the nuclei, etc.

Maier: Ueber den feineren Bau der Wimperapparate der Infusorien. *Arch. f. Protistenk.*, 2, 1903.

Wallengren: Zur Kenntnis des Neubildungs und Resorptionsprocess bei den Teilung der Hypotrichen Infusorien. *Zool. Jahrb.*, 15, 1901.

EPHELOTA

Mount a small piece of hydroid (*e. g.*, *Bougainvillia*, *Campanularia*, *Sertularia*, or *Obelia*) under a supported coverglass and with a low power observe the suctorians attached by delicate stalks. Select a field where the animals are abundant and study under a high power.

1. Note the general shape of the cell and the distribution of the *tentacles*. Are all of the tentacles of one kind? Observe the movements of the tentacles and their use. Is there any morphological relation between tentacles and cilia? (See Minchin's *Protozoa*, p. 458.)

Draw.

2. Study the method of exogenous budding. What is the relation of this type to simple division? Is the number of buds in process of formation the same on all specimens? If

a budding specimen is found keep it well supplied with water and watch for the escape of the buds as free-swimming ciliated embryos.

3. Fix, stain, and mount in balsam a piece of hydroid with many Ephelota attached. Under the high power note the character of the macronucleus and its relation to the buds. Are micronuclei visible?

4. Examine carefully the relation of the stalk to the cell body. Compare with that of Vorticella.

If the material is available study Podophrya and allied forms, with particular reference to the method of budding.

Collin: Étude monographique sur les Acinétiens. Arch. Zool. Exp. et Gen., 1911 and 1912.

Root: Reproduction and Reactions to Food in the Suctorian, Podophrya Collini, n. sp. Arch. f. Protistenk., 35, 1914.

SPOROZOA

GREGARINA

Remove the head and posterior end of a larval or adult meal beetle and pull out the digestive tract with a pair of forceps. Place the digestive tract on a slide, split it open lengthwise with a sharp scalpel, and then spread it out, with the inner wall exposed, and cover. The operation should be performed rapidly to prevent the material from drying. If the beetle is infected, numerous gregarines will be visible under the microscope. Study with low and high powers.

1. Does the animal move? A great number of refractive granules are present in the protoplasm. They are regarded as reserve nourishment. They can be removed with acid.

2. Note that the body is covered with a *membrane*, and is divided into a dense superficial layer, the *ectoplasm*, and a central, more fluid mass, the *endoplasm*.

3. The endoplasm is separated into two parts by a portion of the ectoplasm. The anterior part is termed the *protomerite*, and the posterior part the *deutomerite*. In which is the *nucleus* situated?

4. Is it possible to distinguish a layer of *myonemes* just external to the endoplasm?

5. Is there another section of the body just anterior to the protomerite? If so, this is the *epimerite*.

6. Note that occasionally two (or more) individuals are united. These aggregations are termed *syzygies*.

Before reproduction Gregarina throws off the epimerite, leaves it in the cell host, and falls into the lumen of the digestive tract. It then encysts, and the protomerite and the deutomerite form one spore-producing individual. The attached stage in the life history of Gregarina is termed the *cephalont*, and the detached stage, the *sporont*. (Minchin, Fig. 7.)

Make a drawing.

Examine digestive tract of *Phascolosoma gouldi* for *Schizocystis sipunculi*, an acephaline gregarine.

Berndt: Beitrag zur Kenntniss der im Darne der Larve von *Tenebrio molitor* lebenden Gregarinen. Arch. f. Protistenk., 1, 1902.

Minchin: Sporozoa, pp. 177-179, Lankester's Treatise.

PROTOPHYTA

EUGLENA

Understand its habitat and with what forms it is usually associated.

1. Observe the free-swimming movements of the organism, and the *euglenoid* changes in the form of the body.

Make drawings showing the changes in the shape of a single individual.

2. Distinguish anterior and posterior ends. Is there any dorso-ventral differentiation? Note the motile organ, the *flagellum*. Where is it attached? What relation does it bear to the gullet? How is it directed during locomotion of the organism? Does it serve any other purpose besides locomotion? (Minchin, p. 52.)

3. The green color of *Euglena* is due to chlorophyl, and

this enables it to live in clear water, being nourished like other green plants. (Minchin, p. 14.)

4. Note the absence of color near the anterior and posterior ends of the organism. Near the anterior end also notice the red pigment spot, or *stigma*. What is its probable function?

5. Stain a specimen with iodine and look for the *nucleus*. It is somewhat obscured by the chlorophyl.

6. Observe specimens in the resting stage.

Make a drawing showing all of the points observed.

Look through the stock cultures for other forms of plant flagellates, such as *Trachelomonas*, *Phacus*, etc.

It is desirable to make drawings of the different forms.

Klebs: Ueber die Organisation einiger Flagellatengruppen und ihre Beziehungen zu Algen und Infusorien. Unt. Bot. Inst. Tübingen, 1, 1883.

—: Flagellatenstudien. Zeit. f. wiss. Zool., 55, 1893.

Walton: Review of the Order Euglenoidina. Ohio State Univ., 1915.

Baker: Studies in the Life History of Euglena. Biol. Bull., 51, 1926.

VOLVOX

Volvox globator is better for study than *V. aureus*. It may be distinguished from the latter by the larger size of the colony, the greater number of cells that compose it (about 15,000), the angular shape of the individual cells, and the stout connecting processes of protoplasm, into which *chromatophores* may enter.

Observe the movements of colonies in a watch glass of water, with the naked eye and with a low power of the microscope.

1. Do the colonies tend to collect toward a particular side of the dish? What reason is there for the reaction? Does an individual *Volvox* colony move with reference to one axis?

2. Place a number of colonies on a slide with enough water to allow them to be covered without crushing them. Study first with the low and then with the high power and

determine the species. Understand the relation of the individual cells to the colony. (See Doflein and Reichenow.)

Draw a figure showing several cells and their protoplasmic connections.

3. Compare in detail an individual cell with *Euglena*.

4. Observe, if possible, certain cells, called *parthenogonidia*, which are specialized for asexual reproduction. These divide and form the daughter colonies, which become detached and swim in the interior of the parent colony. They are finally liberated by the rupture of the wall of the parent colony.

Make a figure of a parent colony that incloses several daughter colonies of different sizes.

5. *Volvox globator* is monoecious. Look for *macrogametes* and bundles of *microgametes*.

Figure them.

6. Be sure to recognize the significance of the fact that the cells of *Volvox* are differentiated into somatic and germ cells, and to understand the resulting physiological division of labor.

7. Consider the reasons for and against regarding *Volvox* and allied organisms as plants rather than animals.

Meyer: Ueber den Bau von *V. aureus* and *V. globator*. Bot. Cent., 63, 1895.

CERATIUM

1. Examine this form with a high power, and in a favorable specimen notice the sculptured outer surface of the *cellulose test*. The living animals are green or brown owing to the presence of *chromatophores* in the protoplasm.

2. Note the furrow encircling the body. Does it extend completely around it? Is there a short furrow on one side at right angles to the first, or a depression of considerable size? Understand the position of the *flagella*.

Draw the animal, showing the points observed.

Look for examples of the earlier stages of division, and

of later stages, which appear as chains of fully formed individuals attached together.

Kofoid: Exuviation, Autotomy, and Regeneration in Ceratium. Univ. Calif., Pub. 4, 1908.

—: The Free Living Unarmored Dinoflagellates. Mem. Univ. Calif. Pub., vol. 5, 1921.

NOCTILUCA

If living specimens are not to be had for study, material preserved in alcohol, after suitable fixation, can be used. Specimens are best examined in a cell-slide under a cover-glass.

1. Observe the nearly globular shape, and on one side a groove from which arises a large *flagellum* or "*tentacle*." Is there a deep groove near it? At the bottom of this groove it is possible to see the mouth in a living specimen. Another smaller flagellum is visible in living specimens inserted at the bottom of the mouth, but in preserving the organism it is usually destroyed.

2. Note the appearance of the preserved protoplasm. The *endoplasm* appears parenchymatous. At one point a more compact mass is seen, from which strands appear to radiate. This has been found to contain the *nucleus*.

Noctiluca is luminescent, and frequently causes very brilliant displays.

Make a drawing.

Calkins: Nuclear Division in Noctiluca. Jour. Morph., 15, 1899.

Kofoid: Craspedotella. Bull. Mus., Harvard, 46, 1905.

PORIFERA

Cells not differentiated to form definite organs. Water admitted through surface pores and ejected through an osculum or through oscula.

CLASS 1. Calcarea.

With a skeleton composed of calcareous spicules.

Subclass 1. Homocoela.

With the gastric layer continuous so the collar cells line the whole gastric cavity. (Leucosolenia.)

Subclass 2. Heterocoela.

Gastric layer discontinuous. Collar cells restricted to the flagellated chambers. (Sycon.)

CLASS 2. Hexactinellida.

With a skeleton composed of silicious six-rayed spicules.

Order 1. Lyssacina.

Spicules separate or becoming united. (Euplectella.)

Order 2. Dictyonina.

Spicules united from the first into a firm framework. (Eurete.)

CLASS 3. Demospongiae.

Great diversity of structure. Dominant forms of today.

Subclass 1. Tetraxonida.

Typically with four-rayed spicules. (Corticella.)

Subclass 2. Monaxonida.

Simple, usually unbranched spicules. Spongin frequently present. (Cliona, Suberites, Chalina, Spongilla.)

Subclass 3. Keratosa.

Skeleton of spongin fibers. No true spicules. (Euspongia, Aplysina.)

Subclass 4. Myxospongida.

Without skeleton. (Oscarella.)

- Galtsoff: The Amoeboid Movement of Dissociated Sponge Cells. Biol. Bull., vol. 45, 1923; Regeneration of Microciona (2 papers) Jour. Exp. Zool., 42, 1925.
- Lankester: A Treatise on Zoölogy, Porifera, and Cœlenterata, Pt. 2, 1900.
- Moore: A Practical Method of Sponge Culture. Bull. U. S. Bur. Fish., 28, 1908.
- : The Commercial Sponges and the Sponge Fisheries. Bull. U. S. Bur. Fish., 1908.
- Parker: The Reactions of Sponges with a Consideration of the Origin of the Nervous System. Jour. Exp. Zool., 8, 1910.
- : The Elementary Nervous System, 1919.
- Wilson, H. V.: On Some Phenomena of Coalescence and Regeneration in Sponges. Jour. Exp. Zool., 5, 1907.
- : Development of Sponges from Dissociated Tissue Cells. Bull. U. S. Bur. Fish., 30, 1910.
- and Penney: The Regeneration of Microciona from Dissociated Cells. Jour. Exp. Zool., 56, 1930.

SYCON (*Grantia*)

This form is quite common along the New England coast, where it occurs attached to rocks, seaweeds, and submerged woodwork from just below the lowest tide mark to a number of fathoms in depth. You should visit an old wharf where specimens may be found, and study their relation to the forms with which they are associated. Specimens will be found to vary considerably in size. The largest sometimes reach an inch in length.

1. Examine a dry specimen and notice its general shape, manner of attachment, and *osculum*. The *osculum* is surrounded by a funnel of rather long spicules. Distributed over the general surface, more or less hidden by the numerous spicules, are many small *pores*. Their presence may be demonstrated more satisfactorily later.

2. Look for indications of *budding*. If your specimen does not show this, examine others.

Make an enlarged drawing of a sponge.

With a razor or sharp scalpel cut a dry specimen into halves, with a stroke from base to *osculum*, and notice:

3. The central cavity or *cloaca*.

4. Many *apophyses*, the inner openings of tubes that are

embedded in the walls of the sponge, will be seen opening into the cloaca. Are the apopyles arranged in any order?

5. With the low power of your microscope (with the light turned off) examine the cut wall and find that it is traversed by parallel tubes. Determine that these tubes are of two kinds.

(a) Regular, nearly cylindrical tubes that open into the cloaca through the apopyles and that bear tufts of spicules on their closed ends, at the surface of the body. These are the *radial canals*. It is frequently hard to see their openings into the cloaca, as the apopyles are narrow, so the section only occasionally passes through them.

(b) Smaller and less regular tubes that open on the outer surface between the clusters of spicules, and do not open into the cloaca. These are the *incurrent canals*. In life there are small pores, *prosopyles*, that open from the incurrent canals into the radial canals. These openings are very minute and are apparently capable of being closed. They are never visible in dried material.

6. Examine thin, transverse sections of a dry sponge and determine the positions of radial and incurrent canals.

Make a drawing that will show the arrangement of the canals.

7. Examine the spicules and determine their positions as regards canals. Boil a portion of a sponge in caustic potash until only the spicules remain and examine the spicules. See if more than one kind occurs.

Draw specimens of the spicules.

LIVING AND SECTIONED MATERIAL

1. Place a living sponge in a watch glass of sea water, add a little powdered carmine, and examine it with the low power of your microscope for currents of water. See if particles are moving in a definite direction near the general surface and near the osculum.

2. With a sharp razor cut tangential sections of the wall,

as thin as possible, mount in sea water under a cover, and examine with a low power. This will show both incurrent and radial canals in cross-section. How can you distinguish one from the other? In a favorable place look for moving *flagella*. Are *flagella* in all of the canals? In favorable situations it can be seen that the cells that have *flagella* possess *collars* also. (Collars may be withdrawn by cells so they protrude but slightly.) You see now what causes the current of water. Do you understand how a sponge feeds? The *choanocytes* of the sponge resemble choanoflagellate protozoons.

Make a drawing showing the arrangement of choanocytes.

Examine transverse sections of a specimen that has been decalcified and stained.

1. The cloacal chamber is lined by a *pavement of epithelium*.

2. The radial canals are lined by more conspicuous cells, the *gastric epithelium*, or *choanocytes*.

3. The incurrent canals and the outer surface of the sponge are covered with flattened cells, the *dermal epithelium*.

4. In a part of the section where a considerable area of choanocytes appears in surface view, look for the prosopyles, through which the water passes from the incurrent to the radial canals. (They may not be found.)

5. Make out any structures you can in the area lying between the dermal and gastric layers. What cells are found here?

Make a drawing of several adjacent canals to show the above points and indicate the course of the water by arrows.

6. In the stained sections, look for single *ova* and for spheres containing many spermatozoa, the *sperm spheres*. Look also for *segmenting eggs*, which are frequently to be found. The ova are evidently formed by growth of undifferentiated cells that lie between the definite cell layers and are fertilized while still lying where they have developed, just within the choanocyte layer. Remaining in place, they undergo cleavage and develop as far as the amphiblastula

stage (see figures in the textbooks). They then break through the choanocyte layer into the radial canals and pass out with the current of water. Living specimens are frequently found with such embryos issuing from the oscula in the outgoing current of water. The sperm spheres, when fully developed, also break through the choanocyte layer and, separating into their component spermatozoa, pass out with the outgoing water.

Ova and sperm are formed by the same individual, and the animal is therefore hermaphroditic, but the products ripen at different periods and are seldom both present in an individual at the same time.

If the time allows, draw ova, sperm spheres, segmenting eggs, and embryos.

It is desirable to examine specimens of *Leucosolenia*, a still simpler sponge, and of some of the more complicated forms, like commercial sponges, *Spongilla*, *Cliona*, and *Chalina*. Why is more than a single osculum desirable in such forms? Understand the relation of the internal structure of the complicated forms to the more simple forms. What reason is there for the complication?

The individual cells of sponges (*Microciona*) may be separated by squeezing through fine silk bolting cloth. Such cells will come together in a dish of sea water to form aggregates that will develop into new sponges. (See Wilson and Galtsoff, *loc. cit.*)

COELENTERATA

With a single continuous coelenteron or gastro-vascular cavity. With tentacles and nettle cells. With two cellular layers and a mesoglea.

CLASS 1. Hydrozoa.

Coelenteron simple, without septa. Gonads usually ectodermal. Fully formed medusae have a velum. (Craspedote medusae.)

Order 1. Leptolinae.

With a fixed zoophyte stage.

Suborder 1. Anthomedusae. (Gymnoblastea.)

Without hydrothecae or gonothecae. The medusa bears gonads on the manubrium. (Bougainvillia, Eudendrium, Clava, Hydra, Hydractinia, Pennaria, Tubularia.)

Suborder 2. Leptomedusae. (Calyptriblastea.)

With hydrothecae and gonothecae. The medusa bears gonads on the radial canal. (Campanularia, Gonionemus, Obelia, Sertularia, Tima.)

Order 2. Trachylinae.

Without fixed zoophyte stage.

Suborder 1. Trachymedusae.

Tentacles from the margin of the umbrella. Gonads on the radial canals. (Petasus.)

Suborder 2. Narcomedusae.

Tentacles from the exumbrella. Gonads on the manubrium. (Aeginopsis.)

Order 3. Hydrocorallina.

Massive calcareous exoskeleton. (Millepora.)

Order 4. Siphonophora.

Pelagic. Colonial. Colony usually shows extreme polymorphism of its zooids. (Physalia.)

CLASS 2. Scyphozoa.

Body wall of polyp thrown into four ridges (Taenioles) which project into the coelenteron. Medusa generally without velum and with gastric tentacles (acraspedote medusae). Medusoid form predominating.

- Order 1. *Stauromedusae*.
Conical or vase-shaped umbrella. No tentaculocysts. (*Lucernaria*, *Tessera*.)
- Order 2. *Peromedusae*.
Conical umbrella with transverse constriction. Four interradial tentaculocysts. (*Pericolpa*.)
- Order 3. *Cubomedusae*.
Four-sided umbrella. With perradial tentaculocysts. Velum present. (*Charybdea*.)
- Order 4. *Discomedusae*.
Saucer-shaped umbrella. Perradial and interradial tentaculocysts. (*Aurelia*, *Cyanea*.)

CLASS 3. *Actinozoa*.

With a stomodaeum, and with mesenteries extending into the coelenteron. Fixed forms.

- Subclass 1. *Zoantharia*.
Mesenteries and tentacles usually very numerous.
- Order 1. *Actiniaria*.
Usually single. No skeleton. (*Metridium*, *Sargatia*.)
- Order 2. *Madreporaria*.
Usually form colonies and always have calcareous exoskeleton. (*Astrangia*, *Orbicella*, *Meandrina*.)
- Order 3. *Antipatharia*.
Treelike. Mesenteries and tentacles comparatively few. Chitinoid skeleton. (*Cirripathes*.)
- Subclass 2. *Alcyonaria*.
Mesenteries and tentacles eight in number. Tentacles branched.
- Order 1. *Alcyonacea*.
Skeleton in the form of small, irregular bodies, frequently calcareous spicules. (*Alcyonium*, *Tubipora*.)
- Order 2. *Gorgonacea*.
Treelike, with calcareous or horny exoskeleton. No siphonoglyphes. (*Gorgonia*.)
- Order 3. *Pennatulacea*.
Colony with one end usually embedded in the sea bottom. (*Pennatula*, *Renilla*.)

Hargitt, C. W.: The Anthozoa of the Woods Hole Region. Bull. Bur. Fish., xxxii, 1912, Doc. No. 788.

—: The Medusæ of the Woods Hole Region. Bull. Bur. Fish., xxiv, 1904.

Mayer: Medusæ of the World. Carnegie Inst., Washington, 1910.

Nutting: The Hydroids of the Woods Hole Region. Bull. U. S. Fish. Com., 19, 1899.

HYDROZOA

HYDRA (Fresh-water Polyp)

Hydra, the common fresh-water coelenterate, is frequently found in quiet pools or sluggish streams that contain lily pads, decaying leaves, and other vegetable matter. The animals may frequently be found by examining the surfaces of submerged leaves, but it is usually better to allow such material to stand in glass jars for a day or two, as the animals then tend to collect on the lighter sides of the vessels. They are easily kept in balanced aquaria.

Examine specimens in an aquarium and find what you can about their mode of life. Do they form colonies?

Place a specimen in a watch glass of water and examine it with a lens.

1. What is its shape and color? Is it attached? If so, by what part of the body? Notice the circlet of *tentacles*. How many are there? Compare notes with others and see if all have the same number. How are they placed?

2. Does the Hydra move its body or tentacles? Is it sensitive?

3. Examine with a low power of the microscope and review the above points. You may also be able to see the *mouth* around which the tentacles are arranged. What is the shape of the mouth when open?

Make two drawings, one showing the animal expanded and the other contracted.

Place your specimen on a slide under a coverglass that is supported by the edge of another coverglass and examine with a high power. *Be careful not to crush it.* Notice:

4. The outer layer, *ectoderm*. What is its color? Is it

continuous over the whole outer surface? Does it vary in thickness? Are the cells of which it is composed apparently all alike?

5. The inner layer, *endoderm*. What is its color? If color is present, is it evenly diffused or is it collected in special bodies? Are the cells of which the endoderm is composed apparently all alike? Do they differ in appearance from those of the ectoderm other than in color? If the specimen is not deeply colored, look for flagella moving in the internal cavity.

6. Examine the ectoderm of the tentacles carefully and notice that each of the large, rounded, clear bodies, the *nematocysts*, shows a rather indefinite streak (the stinging thread) running from its outer end, back into the interior. See if you can find the trigger (*cnidocil*) on any of these cells.

Draw a portion of a tentacle showing the distribution of the nematocysts.

7. Place your specimen under the low power of the microscope, carefully run in a drop of safranin, and see if any of the nematocysts are discharged when the safranin touches them. Examine with a high power and notice the appearance of the thread. Notice the change in the shape of the nematocysts that have discharged. See if you can find two kinds.

Make an enlarged drawing of an exploded nematocyst.

8. Examine prepared transverse sections of Hydra. Notice that the body is composed of two layers of cells, between which is an almost structureless thin layer. Do the cells of the two layers differ in size, shape, and structure? Do you find more than one kind of cell in each or either of these layers? Where are they? What are they?

Make a careful drawing of the section showing the arrangement as you see it.

Examine longitudinal sections, for differences in the character of the ectoderm and endoderm in different parts of the body.

9. *Reproduction.* Examine living specimens in a watch glass of water for bud formation and for sexual organs. *Spermaries* are just beneath the tentacles; *ovaries*, lower down; *buds* may be found at different levels. What cells are involved in the formation of each of these?

Eggs are not formed at all seasons of the year and vary greatly in appearance according to their stage of development.

Make drawings of the stages of reproduction that you find.

Tannreuther: The Development of Hydra. Biol. Bull., 14, 1908.

Whitney: Artificial Removal of the Green Bodies from Hydra viridis. Biol. Bull., 14, 1908.

OBELIA

These small, colonial animals are common on submerged or floating wood, stones, and seaweeds, where the water is rather free from sediments. With the aid of a glass-bottomed pail they, in company with many other forms, may usually be seen about old wharfs.

Note the appearance of large colonies of this form that are growing on stones or on pieces of board or kelp.

1. Notice the treelike form of any single stem. Do the branches have a definite size and arrangement?

2. At the extremities of the branches are the single individuals, *hydranths* or *zooids*. Each is similar to a single Hydra in certain ways, but is inclosed in a vase-like formation, the *hydrotheca*.

3. The latter is a continuation of a tough, membranous sheath, the *perisarc*, which covers each part of the whole colony.

Do you notice any modifications of the perisarc below the hydrotheca? Do the modifications serve any purpose?

4. Trace the stem to the creeping, stolon-like portion of the colony, the *hydrorhiza*.

Make a drawing of a colony.

5. The fleshy continuation of the zooid down into the stalk is termed the *coenosarc*. Is it in close contact with the *perisarc*?

6. In an expanded hydranth, note the *mouth*, the arrangement of the *tentacles*, and the number of tentacles. How is the individual supported in the hydrotheca? Trace the *coelenteric cavity* through branches and hydranths and determine whether it is continuous.

7. Can you determine what keeps the fluid in the cavity in motion?

8. Examine a hydranth with a high power and look for the cell layers characteristic of coelenterates. Determine how its tentacles differ from the tentacles of Hydra, and explode nematocysts as in Hydra.

Make a drawing of a hydranth.

9. Look for certain extremities which show neither tentacles nor any opening in the outer covering. Such a condition signifies either an immature hydranth or a reproductive individual. If the latter, it is considerably swollen and is termed a *gonosome*. The central core of a gonosome, the *blastostyle*, should be examined for *gonophores*, frequently called *medusa buds*. This may require a high power. Determine how the gonophores are arranged around the blastostyle. Are all in equal stages of development? What relation has the end of the blastostyle to the outer covering, the *gonangium*?

Make a drawing of a gonosome.

10. The free medusae are small, transparent, and easily overlooked. During the breeding season they may usually be found in abundance in dishes in which colonies have been kept over night. Notice their movements and their positions while at rest on the bottom. The number of tentacles and the position of the sense organs is definite for the species. Two species that differ in the number of tentacles are common at Woods Hole. The inverted bell with the manubrium sticking out from the convex surface of the resting specimen

is characteristic for this form. Notice the quick reversal when the animal swims. The radial canals are easily seen, but the gonads are not developed at the time of liberation. The velum is very small.

Gonionemus is a more favorable medusa to study. This form is valuable for comparison.

CAMPANULARIA

In structure and habits this form is so much like *Obelia* that it is not easy to distinguish the two genera without studying the gonosomes. Several species are found at Woods Hole, two of which (*Campanularia flexuosa* and *C. calceolifera*) are usually abundant during the summer:

The gonosome of one species superficially looks like the gonosome of *Obelia*, while the other has a notch on one side near its extremity. In structure they are similar.

The *blastostyle* runs throughout the length of the gonangium and gives rise to buds that develop into imperfect gonophores. The structure of these gonophores is difficult to make out in fresh material. While they are comparable to medusae, they never become detached, and organs usually present are largely aborted.

The distinct manubrium of the male gonophore becomes charged with sperm which, as they develop, press the ectoderm of the manubrium against the ectoderm of the subumbrella. Ultimately the ectoderm of the manubrium ruptures and the sperm escapes through the subumbrellar cavity.

A female gonophore ripens usually one, sometimes two, eggs. The mature egg, which lies inside the ectoderm of the manubrium, before segmentation is flattened and molded between the mass of the manubrium and the subumbrellar wall. The growth of the egg presses the manubrium to one side. Such an egg appears as a brownish granular mass with a distinct, clear nucleus. The ectoderm of the manubrium ultimately ruptures and liberates the eggs into the subum-

brellar cavity. Cleavage stages are frequently found, and *planulae*, the larval stage that is finally set free, may be found. In old gonosomes, where most of the gonophores have matured their sexual products and the outer end of the blastostyle has broken down, especially large planulae may frequently be found. These may be liberated with needles and studied with a high power for cilia and the arrangement of cells. Older planulae will show a streak that indicates the formation of a cavity inside.

Planulae of this kind placed in a watch glass of sea water and covered to prevent evaporation will soon attach and develop into hydranths. When attached the sea water should be changed twice a day. Without feeding, development is not continued far.

Make drawings of gonosomes and of a planula.

SERTULARIA

In habits and relation of parts there is nothing fundamentally different from the other forms studied. The gonosomes present another modification.

1. The male gonosome has the blastostyle pressed to one side and carries a single gonophore with prominent manubrium and a mass of sperm. The sperm are actually between the ectoderm and endoderm of the manubrium.

2. The female gonosome has the blastostyle pressed to one side and from it originates, one at a time, vestigial gonophores that in turn push toward the distal end of the gonangium and discharge their eggs into a specially constructed brood pouch, the *acrocyst*. By opening acrocysts with needles stages in development up to planulae may be obtained.

Make a drawing showing a female gonosome with an acrocyst.

GONIONEMUS

This form, belonging to the suborder Leptomedusae, has a much reduced polyp generation. It is found in considerable

numbers throughout the summer in the border of eel grass in the Eel Pond at Woods Hole, where it may be obtained with a dip-net. It is more satisfactory to study than the medusa of *Obelia*, as it is much larger and its movements and organs are more easily observed. In plan of structure the two are quite similar.

Put a living specimen in a jar containing sea water, or in a finger bowl, with a black tile beneath, and notice:

1. Its method of locomotion. To the contraction of what part of the bell is movement due? How large is the jet of water that is delivered from the bell? Why is the jet made narrow? Does the jet necessarily leave at the center or may it be thrown from one side? Should it be thrown from one side, what would be the result?

2. Its position in the water when quiet. Why is this position more desirable than the opposite? With a needle point prove that various parts of the body are sensitive.

With either fresh or preserved material, notice:

1. Its flattened dome shape. The convex face is called the *exumbrella* (aboral), while the concave portion is termed the *subumbrella* (oral).

2. The *velum* is the perforated diaphragm that partly closes in the subumbrella. All medusae possessing this structure are classed as *Craspedota*. Do you understand its use?

3. In the center of the subumbrella is seen the large pendent *manubrium*, at the extremity of which is a wide-lipped *mouth*. What is the shape of the mouth when open? How does it compare in this respect with the mouth of *Hydra*? If the medusa is alive, feed it with small bits of clam meat.

4. From the capacious sac at the base of the cavity of the manubrium, the *stomach*, the four *radial canals* lead to the periphery of the disk, where they open into the very delicate *circular canal*. The four radii marked out by these canals are called the *perradii*. Do you understand the use of these canals?

5. The *gonads* hang from beneath the radial canals into the subumbrellar space. They are lobulated in structure, and more or less prominent according to maturity and the breeding season. The eggs or spermatozoa, as the case may be, are dehisced from these into the water directly.

During the breeding season specimens placed in the dark in the latter part of the afternoon and left for two or three hours will shed eggs and sperm. The fertilized egg undergoes cleavage, a planula is formed that finally attaches at one end and develops into the hydra stage. Eggs are normally laid about 8 P. M.

6. The *tentacles*. Is their arrangement a radially symmetrical one? How are the *nematocysts* arranged on them? Look for *adhesive organs* on them. Of what use are such organs?

Turn your specimen with the velum side toward you and study the edge of the medusa with a low-power objective for the *sense organs*. These are of two kinds:

(a) The larger, round bodies at the bases of the tentacles communicate with the circular canal (which may possibly be seen along the edge of the bell). They are filled with a layer of strongly pigmented endoderm cells and are probably *light-percipient organs*.

(b) Other small sessile and transparent outgrowths, situated between the bases of the tentacles, are the so-called *statocysts* (*lithocysts*), which are probably static organs.

All of the tentacles are abundantly supplied with tactile, sensory cells. There is a well-established circumvelar *nerve ring* (not easily determined in living material) derived from the ectoderm, also scattering nerve cells beneath the ectoderm in connection with the muscular tissue. Exumbrellar and subumbrellar layers of muscle fibers are also present.

Make a drawing from the side, slightly tipped, to show the velum, and another as seen from the oral surface.

Murbach: The Static Function in *Gonionemus*. Am. Jour. Physiol., 10, 1903.

Perkins: The Development of *Gonionema murbachii*. Proc. Acad. Nat. Sci., Phila., 1902.

—: *Gonionemus*, Science, 1926, p. 93.

Yerkes: A Study of the Reaction of the Medusa *Gonionema murbachii* to Photic Stimuli. Am. Jour. Physiol., 9, 1903.

TUBULARIA (Parypha)

This form is frequently abundant on the piles of old wharfs and on rocks, where the colored colonies form conspicuous masses just below low-water mark.

Examine the general form of a colony and note, either with a hand lens or with the naked eye, the stem, or *hydrocaulus*, as it arises from the branching, matted *hydrorhizal portion* of the colony. The parts of the colony will be seen to differ from the Leptomedusan (Campanularian) form studied, especially in branching, rigidity, hydrothecae, and gonosomes.

Make a drawing to show the formation of the colony.

1. How does a hydranth differ from the hydranth of *Obelia* in the matter of *tentacles*? Is a *hydrotheca* present?

2. The *mouth* is terminal and is situated at the end of a *proboscis*.

3. The short but rather large body of the hydranth passes back to the *perisarc* as the fleshy axis, *coenosarc*.

4. Notice the *gonosomes* between the rows of tentacles. What is their origin and arrangement? This is a form in which the medusae are not set free, but remain vestigial. They show neither radiating nor circular canals. The gonads ripen on the partially developed manubrium of the medusa. The sexes are separate.

Make a drawing of a hydranth.

5. The male gonophores when nearly mature are rounded or elongated with the space apparently between the manubrium and subumbrellar surface filled with sperm. In fact, the sperm are enclosed between the ectoderm and endoderm of the manubrium, but the ectoderm is pressed over against

the ectoderm of the subumbrella so that this space is practically obliterated. These sperm become active when liberated in sea water.

6. The female gonophore when mature is more elongated, shows indications of tentacles at the free extremity, and there is an actual subumbrellar space. The eggs are formed in the ectoderm of the manubrium and are shed into the subumbrellar cavity. An egg develops into a larva called an *actinula*. With needles open a female gonophore and examine the developmental stages. These are: (a) somewhat irregular disk-shaped embryos with a variable number of projections (the forming tentacles) around the margin. (b) Older stages with the tentacles more developed and with disk- or lens-shaped bodies in which the coelenteric cavity can be easily seen. (c) Actinula stage. Essentially a small polyp. Notice the number of tentacles, the position of the mouth, and the method of locomotion.

Actinulae kept in a covered watch glass of sea water will attach and form the basis of new colonies.

Make drawings of gonosomes, gonangia, and developmental stages.

7. The arrangement of the attached medusae is best seen in sections.

Sections show the same body layers as Hydra, and the derivation of the medusa as an outpocketing of the wall of the hydranth is evident.

Hargitt: The Early Development of *Pennaria tiarella*. Arch. f. Entwicklungsmech., 18, 1904.

Pearse: Reactions of *Tubularia crocea*. Am. Nat., 40, 1906.

Torrey: Biological Studies on *Corymorpha*. I. Jour. Exp. Zool., 1, 1904; II. Univ. Calif. Pub. Zool., 3, 1907.

BOUGAINVILLIA

This form is not always obtainable during the summer months. It occurs in fair abundance at Woods Hole earlier in the season, attached to piles and floating timbers.

1. Examine the colony for arrangement of branches, and determine the relation of perisarc and coenosarc.

2. How do the hydranths differ from those of *Obelia*? Is the number of tentacles constant? Is the hydranth as contractile as it is in *Obelia*?

3. Look for *gonosomes*. The gonophores are borne singly or in clusters on the main stem and branches. By examining a number of buds the general method of medusa formation can be determined. If possible, find: (a) a young bud slightly swollen showing the thin perisarc with the cellular layers inside and a somewhat enlarged coelenteron. (b) A bud showing a thickening of the ectoderm at the distal end, in which a cavity appears, the subumbrellar cavity. (c) A bud showing the formation of the manubrium as a projection into this cavity. The manubrium involves both layers, as the subumbrellar cavity is wholly ectodermal. The ectodermal distal covering of the subumbrellar cavity will later perforate and form the velum. (d) A bud showing the perforated velum and the tentacles. The tentacles are at first directed through the opening of the velum into the subumbrellar cavity.

4. Find *medusae* that have become detached. Notice the arrangement and number of tentacles, the eye spots at the bases of the tentacles, the radial and circular canals, and the mouth appendages. Gonads are not developed at the time of liberation. Study the swimming movements.

Make drawings to illustrate development and adult structure of medusae.

HYDRACTINIA

This form is particularly abundant at Woods Hole on the shells of gastropods inhabited by hermit crabs, but at certain seasons is abundant on rocks or pebbles and sometimes on piles.

1. Examine a shell covered with a colony, and notice the distribution and size of the individuals.

2. Notice the hard secretion that sticks up as prominent points and ridges between the individuals.

3. Break a shell and place the fragments incrustated side up in a watch glass of sea water and examine with a low power. Three kinds of individuals will be apparent: (a) large individuals with long tentacles. These are the feeding hydranths. They differ somewhat in appearance in the male and female colony. The male individual has a large *proboscis*, while the female individual has only a slightly arched disk with the mouth in the center. (b) Reproductive individuals with knoblike tentacles, a proboscis that is usually retracted, a mouth, and with gonophores along their sides. In female gonophores the manubrium and a number of eggs may be seen. These gonophores never become detached and never show further medusoid structure. (c) Elongated individuals, especially near the outskirts of the colony, that have rounded tentacles, proboscis, and mouth like those of the reproductive individuals. These sometimes branch and have a habit of bending the head toward the base or even twisting the body into a spiral. They are not distinguishable from the reproductive individual except by shape and the fact that they have no gonophores.

4. Notice that the individuals are connected at the bases by a fleshy layer which is responsible for the deposit already mentioned.

Make a drawing of each kind of individual.

HYDROCORALLINA

To this group belong forms that have heavy calcareous exoskeletons. While material is generally not at hand to study the polyps, it is desirable to study and sketch the characteristic forms of colonies such as *Millepora* and *Stylaster*, and to note the difference in the distribution of pores. Later you will see how decidedly these differ from the ordinary stony corals.

SIPHONOPHORA

Examine living or preserved specimens of *Physalia*, and sketch the type with reference to showing, if possible, the

following structures: (a) *pneumatophore*, (b) *dactylozooids*, (c) *gastrozooids*, (d) *gonodendrons*, (e) *tentacles*. It will be well to refer to a textbook to find the positions and functions of each of these.

Bigelow: The Siphonophoræ. Mem. Mus. Comp. Zool., Harvard, 38, 1911.

SCYPHOZOA

AURELIA

This form is one of the common jellyfishes, and is found floating freely in the water. It is frequently washed up on shore. To be appreciated these medusae should be seen as they occur at the surface of the sea, before they have been handled or injured. Frequently vast numbers may be seen together, all gently pulsating and thus keeping near the surface. The movement is very different from that of most hydrozoan medusae, being very deliberate and graceful.

If living material is offered, study the method of locomotion and compare it with the locomotion of *Gonionemus*. Like the latter, the discoid animal *exumbrellar* (aboral) and *subumbrellar* (oral) surfaces, but the edges of the disk are indented, fringed with very numerous short tentacles, and a velum is wanting. What difference does the velum make in locomotion?

The exumbrellar surface presents little of interest. In the live specimens, however, you should prove that the animal is sensitive over this area as elsewhere.

Preserved and hardened material is better than living for the study of the rest of the anatomy of this form. With a specimen in water in a finger bowl, with a black tile for the background, find the following from the subumbrellar surface:

1. The shape of the animal. Is the margin perfectly circular or regularly indented? Are all of the marginal portions similar?
2. Four large, fringed *oral arms* or *lips* hang from the

corners of the nearly square *mouth*, which is located in the center. Notice how each arm is similar to a long, narrow leaf, with the sides folded, especially along their margins. Examine the arms for *nematocysts*. Do you understand how the animal gets its food? If the arm edges appear to be covered with dark specks and granules, examine to see if *embryos* are entangled there.

3. The mouth is found to lead by a short *gullet* into a rather spacious *stomach*, which is produced in the region between adjacent corners of the mouth to form a *gastric pouch*. In each of the pouches are a number of *gastric filaments*. Determine the shape of the stomach.

4. The remaining parts of the digestive (and also in this case circulatory) system include the numerous *radial canals* and the single *circular canal*.

(a) Directly beneath each oral arm a *perradial canal* is given off, which, at a short distance from the stomach, gives off a branch on either side. One portion of the perradial canal continues straight to the margin and joins the circular canal, without further subdivision, but the two side branches in turn subdivide several times.

(b) From the peripheral wall of each gastric pouch three canals pass toward the margin; the middle one (*interradial canal*) branches somewhat after the manner of the perradial canals, but the other two (*adradial canals*) continue to the circular canal without further branching.¹

5. The position of the gastric pouches is made clearly manifest by the *gonads*, which lie on the floor of the pouches, as frill-like structures, horseshoe-shaped, with their open sides toward the mouth. The *ova* or *spermatozoa* are shed into the stomach and pass out of the mouth. Embryos in various stages of development may frequently be found adhering to the oral arms. The sexes are separate. On the sub-

¹ In most cases the foregoing canals are very evident, but if they are not, they may be injected with water in which powdered carmine is mixed, by inserting a large-mouthed pipette into the stomach.

umbrellar surface, opposite each gonad, is a little pocket, the *subgenital pit*, which opens freely to the outside. Whatever purpose this may serve, it does not function to conduct the genital products to the outside.

6. Parallel with the inner or concave border of each gonad is a row of delicate *gastric filaments*. These are supplied with nematocysts, and they may aid in killing live food taken into the stomach. These structures are not present in the Hydromedusa.

7. At the marginal extremity of each perradial and inter-radial canal there is an incision, on the edge of the animal, in which there are sensory organs. In each incision find:

(a) A *tentaculocyst* in the form of a short, clublike structure containing a prolongation of the radial canal. At its outer extremity are calcareous concretions or *lithites*, and a pigment spot or *ocellus*. Each tentaculocyst is covered aborally by a hoodlike projection, and on the sides by marginal lappets.

(b) Two depressions, one above and the other below the tentaculocyst. These have been assigned olfactory functions, and are called the *olfactory pits*. Evidence of function is lacking.

Make a drawing showing the profile of the entire animal, and show the structure of at least one quadrant, as seen from the oral surface.

8. If time permits, study developmental stages.

The eggs are shed through the mouth and frequently become entangled in the oral arms, where they may develop into planulae. Most of the eggs are set free in the water, where they develop.

The *planula* after swimming for some time attaches by one end, acquires a coelenteron, mouth, and tentacles. Longitudinal ridges called *taeniolae* or *taenioles* are formed in the coelenteron, *septal funnels* are formed between the tentacles and mouth, and from the septal funnels ectoderm cells are budded off that form the four longitudinal *septal muscles*. This larva is called a *scyphistoma*.

The scyphistoma grows, acquires more tentacles, may bud to form other scyphistomae, and usually acquires *stolons*, which grow out from the body wall just above the base. From the stolons new scyphistomae arise. *Subgenital pits* make their appearance in the position formally occupied by the septal funnels, and an *ostium* appears in each taeniole near the oral surface. In this way a *ring sinus* is formed. *Gastric filaments* are formed on the edges of the taenioles.

From the oral side of the first eight tentacles, *sense organs* bud out. Eight lobes make their appearance opposite these sense organs, each lobe divides into two *lappets*, between which the sense organ lies. While these changes are taking place constrictions running around the body appear and deepen so the body is divided into a series of plates, each of which has eight lobes, eight sense organs, and sixteen marginal lappets. The disk at the free extremity is the oldest and most differentiated.

This stage is frequently called the *strobila*, but there is no definite dividing line between scyphistoma and strobila. The number of disks formed by a strobila seems to be dependent upon conditions, probably largely food supply.

Before the disks are ready to be detached as *ephyrae* the tentacles disappear. Ephyrae are detached one at a time from the free end as they mature.

Up to this point students will be able to determine only part of the points mentioned unless an abundance of material and sections are provided. The remaining points are easily determined.

Examine a free *ephyra*. If it is alive, watch it swim. Find the mouth, stomach, marginal lobes, marginal lappets, and sense organs. Use these as landmarks to determine the relation of parts to the adult. Are there any outgrowths from the stomach? Do the sense organs have any relation to branches from the stomach? Can you find gastric filaments?

From the shape of the mouth determine which of the lobes are perradial and which interradianal. What part of the adult is represented by the notches between the lobes?

Study a somewhat older ephyra and find the starting of the adradial canals and the beginning of the formation of *adradial cushions*. Examine a series of older stages and find how the adradial cushions expand, how the canals branch, and how the circular canal is formed.

Make drawings of the stages.

By way of comparison, examine demonstrations of *Cyanea*, *Dactylometra*, *Lucernaria*, or other forms belonging to this group.

Hargitt: Variations among Scyphomedusæ. Jour. Exp. Zool., 11, 1905.

Hargitt, C. W. and G. T.: Studies in the Development of Scyphomedusæ. Jour. Morph., 21, 1910.

Mayer: Rhythmical Pulsation in Scyphomedusæ. Carnegie Inst. of Washington, 1906.

ACTINOZOA

METRIDIUM (Sea-anemone)

Specimens are quite common on piles, as well as on rocky bottoms, and may be easily observed by means of a glass-bottomed pail. Most of the observations can be made much better on specimens in aquaria, but it is desirable to see their natural surroundings.

Specimens for laboratory study should be placed in aquaria, and left undisturbed until they are fully expanded. In experimenting be very careful not to overstimulate.

1. Notice the shape and attachment of expanded, living specimens in an aquarium, or in a deep finger bowl. The free end, called the *disk* or *peristome*, is fringed with *tentacles*, and the elongated *mouth* is located in the middle of this area. At one or both angles of the mouth the lips are thickened into what is called a *siphonoglyph*.

Make a drawing of the animal.

2. Drop a few grains of sand on the tentacles. Observe and record what happens. Repeat, placing the sand on the oral lips, the siphonoglyph, and the oral disk successively. Try the same using sawdust soaked in clam juice. Repeat, using clam meat.

What conclusions can you make: first, as to the ability to distinguish food; second, as to methods of obtaining food; and third, in regard to ciliary action?

3. Stimulate the animal with a needle at various points and try to determine where it is most sensitive. Observe its manner of contraction. When fully contracted, if the irritation is continued, threadlike structures, *acontia*, are thrust out through minute pores, *cinclides* (sing. *cinclis*), in the body wall.

Make a drawing of the contracted animal.

Internal Anatomy.—Using preserved material, place the edge of a razor across the peristomial area, at right angles to the mouth-slit, and divide the animal from disk to base into halves:

1. Note the extent of the *actinopharynx* and *siphonoglyphs*; they lead into the *coelenteric chamber*. Find the extent of this chamber, and the method of its subdivision by delicate partitions, the *mesenteries*, or *septa*. Are all of the mesenteries alike?

2. Forming the free edges of the mesenteries, below the *actinopharynx*, are the convoluted *mesenteric filaments*, which are secretory organs that are probably equivalent to the gastric filaments of the Scyphozoa.

3. Quite near the bases of the mesenteries are the attachments of the *acontia*. What relation have they to the mesenteric filaments? Mount living *acontia* under a cover slip in sea water and notice the central *muscle strand*, *nematocysts*, and *cilia*.

4. Also located on the mesenteries, and arranged parallel to the filaments, but back from the edge a bit, are the *reproductive organs* or *gonads*. Are they found on all of the mesenteries? The ova or spermatozoa are shed into the coelenteric chamber and pass out through the mouth.

Cut one of the halves of your specimen transversely in the region of the actinopharynx, and study the arrangements of the mesenteries, their attachments, etc.

5. How many pairs of *primary mesenteries*, *i. e.*, those attached both to the outer body wall and to the actinopharynx, are there? The *directive septa* are those at the angles of the actinopharynx. The portion of the coelenteric cavity between any two pairs of mesenteries is termed an *inter-radial chamber*. The space between the two mesenteries of each pair is called an *intradial chamber*.

6. Carefully determine the disposition of the *longitudinal retractor muscles* on the mesenteries. Do they occupy similar positions on all of the mesenteries?

7. Examine the upper parts of the mesenteries for openings, *septal stomata*, that put the chambers in communication.

8. Are the tentacles solid or hollow?

Make a drawing of a longitudinal section and another of a cross section. Put into these all of the points of the anatomy you have seen.

If time and opportunity permit, it is very desirable that this form should be compared with specimens of the order Madreporaria, and later with the Alcyonaria. Such a form as *Astrangia* may easily be obtained either alive or properly preserved, and will serve to show the relation of the hard parts of the coral to the polyp. You should understand the relation of the septa and the mesenteries, and of the polyps to each other. If specimens are at hand, compare such forms as *Orbicella*, *Favia*, and *Meandrina*, or any forms that show gradations from separate calices to fused groups, and understand the positions of mouths, the arrangement of the coelenteric chambers, and the way in which the colony has come to its present form. You should also examine large branching colonies and determine why branches are formed and how they arise.

Examine the structure of an alcyonarian colony and see how the polyps are placed. The structure of the expanded polyps is nicely shown by *Renilla*. The spicules of such forms as *Gorgonia* may be obtained by boiling a portion of

a colony in caustic potash. What purpose do such spicules serve?

Parker: The Reactions of *Metridium* to Food and Other Substances. Bull. Mus. Comp. Zool., Harvard, 29, 1896.

—: The Mesenteries and Siphonoglyphes in *Metridium marginatum*. Bull. Mus. Comp. Zool., Harvard, 30, 1897.

—: Longitudinal Fission in *Metridium marginatum*. Bull. Mus. Comp. Zool., Harvard, 35, 1899.

—: The Reversal of the Effective Stroke of the Labial Cilia of Sea-Anemones by Organic Substances. Am. Jour. Physiol., 14, 1905.

—: The Origin and Significance of the Primitive Nervous System. Bull. Mus. Comp. Zool., Harvard, 50, 1911.

—: The Elementary Nervous System. Lippincott, 1918.

CTENOPHORA

Single. Pelagic. Eight rows of meridional swimming plates. No nettle cells, but with adhesive cells. With aboral sense organ. This phylum consists of one class which comprises the following orders:

Order 1. Cydippida.

Nearly circular. Two tentacles, each of which may be retracted into a sheath. (Pleurobrachia, Mnemiopsis.)

Order 2. Lobata.

Compressed in the vertical plane. Two large oral lobes. No tentacle sheaths. (Deiopea.)

Order 3. Cestida.

Ribbon-shaped. Two tentacles with sheaths, and numerous other tentacles. (Cestus.)

Order 4. Beroida.

Laterally compressed. Without tentacles. (Beroe.)

PLEUROBRACHIA¹

This form belongs to the group of animals popularly called "comb jellies," and occurs along the coast in irregular abundance during the summer months. Specimens are very luminescent when disturbed, so, when they are abundant, the display caused by them while rowing at night is sometimes brilliant. They may frequently be seen during the daytime and can often be satisfactorily observed in the shade of a wharf when the water is calm.

Unmutilated, living material can be studied to best advantage, but preserved material may be had that is quite satisfactory for anatomic study.

¹ Although the following section is written especially for *Pleurobrachia*, little difficulty will be found in applying it to the related genus *Mnemiopsis* which is usually very abundant in the vicinity of Woods Hole during the late summer.

1. In general appearance a specimen resembles a hydrozoan medusa, with its *aboral* surface elongated until, as a whole, it approaches the shape of a fowl's egg.

2. The broader or *oral* end bears two small lip-like lobes, between which is the slit-like *mouth*. We may consider the elongation of the mouth to be in the anteroposterior plane. Bilateral symmetry is thus evident.

3. At the aboral pole is the "*sensory body*."

4. Leading away from this and extending as meridional lines toward the oral pole are eight *ctenophoral rows* of *swimming plates*. Examine the plates with a hand lens and determine their structure and function. Determine the positions of the rows with respect to the anteroposterior plane.

5. By the sides of the stomodaeum are a pair of yellowish or orange *tentacles* that may be retracted wholly into the *tentacle sheath* or extended through pores near the aboral pole. When extended the tentacles are seen to be branched. They are very sensitive and contractile.

Digestive System.—With a pipette inject a solution of carmin into the mouth opening.

1. You can then more plainly see the long ribbon-like *stomodaeum* which extends two thirds of the distance to the sensory body, where it joins the *infundibulum*.

2. From the stomodaeum are given off the *canals*, which in a successful injection will be seen to be as follows:

(a) The *axial funnel tube* extending to the sensory body.

(b) Two *paragastric canals*, one on each side, passing down along the stomodaeum.

(c) Two *tentacular canals*, one on each side, passing to the tentacular structures.

(d) Two *perradial canals*, one on each side, each of which bifurcates to form the *interradial canals* (four in all), each of which again bifurcates to form the *adradial canals* (eight in all), which are continued orally and aborally just beneath the swimming plates as the *meridional canals*. These canals end blindly without intercommunication.

Reproductive System.—The ctenophore is hermaphroditic and ova and spermatozoa are proliferated from the walls of the meridional vessels.

A portion of a ctenophoral row should be cut off, and examined under a microscope, to ascertain the arrangement and relation of plates and cilia.

Make a drawing of a side view.

Make a diagram that will show the appearance of a meridional cross-section.

Abbott: The Morphology of the Cœloplana. Zool. Jahrb., 24, 1907.

Agassiz, A.: Embryology of the Ctenophoræ. Am. Acad. Arts and Sci., 10, 1874.

Mayer: Ctenophores of the Atlantic Coast of North America. Carnegie Inst. of Washington, 1912.

Parker: The Movements of the Swimming-plates in Ctenophores, with Reference to the Theories of Ciliary Metachronism. Jour. Exp. Zool., 2, 1905.

PLATYHELMINTHES

Body elongated, flattened and unsegmented. Anus generally absent.

CLASS 1. Turbellaria.

Outer surface ciliated. Free living.

Order 1. Polycladida.

Intestine complexly branched. No separate vitellaria. (Planocera, Leptoplana, Stylochus.)

Order 2. Tricladida.

Intestine with anterior median, and two posterior lateral limbs. Vitellaria numerous. (Planaria, Bdelloura, Syncoelidium.)

Order 3. Rhabdocoelida.

Simple, saclike intestine. Body usually elongated. (Polychoerus, Microstomum.)

CLASS 2. Trematoda.

Parasitic. Generally with sucking disks. Well-developed digestive system.

Order 1. Monogenetica.

Ectoparasitic. Direct development. Three or more suckers. (Polystomum.)

Order 2. Digenetica.

Endoparasitic. Complicated development. Never more than two suckers. (Distomum.)

CLASS 3. Cestoda.

Endoparasitic. Without digestive cavity. Usually having a scolex, bearing clinging organs (suckers or hooks).

Order 1. Monozoa.

Body not divided into proglottids. (Caryophyllaeus.)

Order 2. Polyzoa.

Body consisting of scolex and proglottids. (Taenia, Crossobothrium.)

CLASS 4. Nemertinea.

Elongated, ciliated, with eversible proboscis not directly connected with the alimentary canal. Intestine usually with lateral diverticula. Anus present. (Tetrastemma, Cerebratulus.)

TURBELLARIA

PLANARIA MACULATA

This form is very common in fresh-water ponds throughout the United States. It is found during the day on the lower or shaded surfaces of stones and other submerged objects, a fact which suggests that it is nocturnal in its habits. Most fresh-water planarians have very opaque bodies and their internal organization cannot be studied in the fresh specimens.

1. Notice the general shape of the body.
2. The methods of locomotion. Look for cilia.
3. The *pharynx* and *mouth* near the middle of the ventral surface.
4. The *eye spots* on the anterior dorsal surface.
5. Try feeding specimens by crushing a live pond-snail and putting the fragments in the dish with them. If any of the worms are at rest, set them in motion by lifting one end of each with a bit of wood, a camel's-hair brush, or some blunt instrument. Observe the animals at intervals of a few minutes and see if any of them begin to feed. If so, by turn-in them over quickly with a camel's-hair brush, try to see how the pharynx is used. If not successful, try turning a specimen ventral side up, and placing a small bit of snail meat on its body in the region of the pharynx.

6. Look among the specimens in the dishes on the preparation table for animals that show marks of *normal fission*.

7. Clean a heavy watchglass thoroughly and pour it about two thirds full of clean pond water from the jar on the preparation table. Transfer all of the specimens to this dish, lifting each carefully with a camel's hair brush. With a scalpel mutilate them in various ways; cut one transversely, another longitudinally, another into several pieces of various shapes. Make memoranda, if necessary, of the shapes of the various pieces. Carefully cover the dish and set it away. Examine the pieces with a hand lens every twenty-four hours

for the next week or ten days. Change the water in the dish at least twice a week. Do not use water from the tap.

Curtis: The Life History, the Normal Fission, and the Reproductive Organs of *Planaria maculata*. Proc. Bost. Soc. Nat. Hist., 30, 1902.

Morgan: Experimental Studies of the Regeneration of *Planaria maculata*. Arch. f. Entwicklungsmech., 7, 1898.

Parker and Burnett: The Reactions of Planarians With and Without Eyes to Light. Am. Jour. Physiol., 4, 1900.

BDELLOURA OR SYNCOELIDIUM

Most triclads are free-living, but a few live on the external surfaces of other animals. The above-mentioned forms are found upon the proximal joints of the walking legs and in the gill books of *Limulus*. Owing to the absence of pigment, they are very favorable for the study of internal structure, and may be used to demonstrate the structures not observed in *Planaria maculata*.

1. Observe the movements of the living worms in a watchglass of sea water; then place a specimen on a slide, dorsal side uppermost, and cover with a slip.

If any of the points of structure mentioned for *Planaria* have not been observed, try to find them on this form.

2. Notice that the *gut* with its three main branches (triclad type) and many secondary diverticula is easily recognizable. The mouth can sometimes be made out as a small circular opening leading ventrally from the posterior end of the pharyngeal sheath.

Compress the specimen as much as possible by drawing off the water with filter paper and look for:

3. The *cerebral ganglia*, a bilobed structure beneath the eye spots, that appears as a slightly lighter area.

4. From the cerebral ganglia two longitudinal *nerve cords* pass backward, and several smaller nerves pass off in front. Examine the specimen by reflected light, looking particularly at the nervous system and pharynx. What relation have the nerve cords behind?

5. With the high power and good light, look for the *water-*

vascular tubules. These tubules are more easily seen in specimens that have been under the coverslip some time. The region anterior to the cerebral ganglia is a favorable place. They form a clear, branching tracery, a little lighter than the surrounding tissue. The flicker of the *flame cells* can usually be seen, but they may be more easily seen in *Crossobothrium*. Examine chart and textbook figures of the water-vascular system.

Make a good-sized drawing of a worm, showing the above points.

Reproductive Organs.—Turbellarian worms are hermaphroditic. In this form the various organs are so crowded together that it will be best to follow each system separately. Compress a specimen under the slip and find the male organs as follows:

(a) The *testes* are the numerous rounded masses between the lateral branches of the gut. They are connected by means of fine tubes which cannot be seen in fresh specimens.

(b) The *vasa deferentia*, two large tubes, one on either side of the pharynx, which unite posteriorly near the base of the *penis*.

(c) The *genital atrium*, within which the penis lies withdrawn, is situated behind the pharynx. The penis and atrium may be considered as a replica, in miniature, of the pharynx and its sheath.

If the above structures cannot be satisfactorily seen, try preserved, stained, and mounted specimens.¹

Draw the male reproductive system. Refer to charts and textbooks for anything that is obscure.

¹Specimens may be readily killed by compressing under a slip, being careful to draw the excess of fluid out on one side so that the animal cannot contract, and running in killing fluid. (Sublimate acetic is good.) As soon as they become opaque white, put on enough killing fluid to float the slip off and transfer the specimens to a dish of the fixative for five minutes, then 50 per cent alcohol a few minutes, 70 per cent several hours, stain with borax carmine or Delafield's hematoxylin; dehydrate, clear and mount in balsam. (See directions in the appendix for making permanent preparations.)

The female organs are as follows:

(a) Opening into the genital atrium are the two large sacs, the so-called *uteri*, which lie near the margins, just posterior to the end of the pharynx. Each has a separate opening on the ventral surface of the body, but has no direct connection with any other part of the reproductive system. These may not be homologous with the single uterus found in most triclad. (See Wheeler.)

(b) Place a worm ventral side up and look carefully between the second and third or the third and fourth anterior gut diverticula on either side of the main anterior ramus for the two *ovaries*.

(c) The *oviducts* pass backward from the ovaries, parallel to the vasa deferentia, and unite posterior to the penis. The common duct thus formed enters the posterior part of the genital atrium. The oviduct is difficult to demonstrate and it may be necessary to try both fresh and stained material.

(d) Along the margins of the animal, between the diverticula of the gut, are rounded bodies, the *vitellaria*. These discharge their products into the oviducts. What is their function?

Draw the female reproductive system.

Study stained and mounted specimens for any points which have not been found, and particularly examine the nervous system. Look for the marginal nerve running along the edge of the body, and for numerous transverse commissural nerves. How many of these are there? How regular is their arrangement?¹

Wheeler: *Syncœlidium pellucidum*, a new Marine Triclad. Jour. Morph., 9, 1894.

TREMATODA

Trematodes are flat worms which lead a wholly parasitic life, but which have retained, to a greater or less degree, those

¹A Polyclad, Planocera, can often be obtained from the mantle chamber of *Busycon*. If *Busycon* is allowed to remain out of water for some hours the Planocera usually crawl out. The form is fairly satisfactory for study.

organs that characterize free-living animals. Some Trematodes are parasitic upon the outside (or ectoderm) of other animals, and are hence called ectoparasites.

PNEUMONECES

This form is found as a parasite in the lungs of frogs. In some localities a large proportion of the frogs are infested and several specimens are frequently found in one frog. The host of the asexual generation of this species is not known, but in a closely allied species the asexual generation lives in the pond snail. The living worm is cylindrical and pointed at both anterior and posterior ends. With a low-power objective note:

1. The *anterior sucker*, surrounding the mouth.
2. The *ventral sucker*, near the middle.
3. Whether eyes are present or not.
4. The *alimentary canal*.

(a) The *mouth*.

(b) The muscular *pharynx*.

(c) The *intestine* which, soon after leaving the pharynx, divides into two equal branches, passing one on the left and one on the right side, to near the end of the body. These intestinal branches do not send out lateral branches as they do in *Bdelloura*.

The Water-vascular System.—A small opening will be found at the posterior end of the body from which a duct passes forward in a median position to a point a little posterior to the median sucker. Here it divides and sends a branch on either side of the worm to near the anterior end.

The Nervous System.—This is difficult to see, but in a mounted specimen a small, deeply stained mass, the *cerebral ganglia*, may be visible on either side of the pharynx. Three pairs of longitudinal nerves pass back to near the posterior end of the body.

Make a drawing showing the above structures indicating all you have been able to observe.

The Reproductive Organs.—*Male*: Two large bodies, the *testes*, very definite in outline, occupy the posterior end of the animal. A duct from each, the *vas deferens*, passes forward, and the two unite just posterior to the point where the intestine branches. By means of a median, common duct, they open to the exterior through the *male genital opening*. This is situated on the ventral surface, just below the point where the intestine branches.

Female: Some of the ducts are difficult to see, and in many cases they cannot be followed, but some of the organs can be found in most of the specimens.

The *ovary* is a lobed organ lying a little to one side of the middle of the animal, and just anterior to the testes. Lying against it is the saclike *ootype*, into which the ovary opens. From the posterior end of the ootype the long, coiled, ductlike uterus passes backward to near the posterior end of the animal, turns and passes forward, and finally opens at a point on the ventral surface near the male opening. The uterus of an adult usually contains embryos and fills the body, so as to obscure the other parts.

The *vitellaria* consist of numerous small, rounded masses that lie near the margins of the animal. The products of these organs are emptied into the ootype through a short common duct, just ventral to the ootype. Do you know what they are for? *Lawrer's canal* is a short duct which leads from the ootype to the exterior. Its function is doubtful.

Cort: North American Frog Lung Flukes. Trans. Am. Micr. Soc., 34, 1915.

Goto: Studies on the Ectoparasitic Trematodes of Japan. Jour. Col. Sci. Imp. Univ. Tokyo, 8, 1894.

Linton: The Process of Egg Making in the Trematode. Biol. Bull., 14, 1908.

Leuckart: Die Blasenwürmer und ihre Entwicklung. 1856.

—: Die Parasiten des Menschen.

Schauinsland: Beitrag zur Kenntnis der Embryonalentwicklung der Trematoden. Jen. Zeit. f. Naturwiss. Neue Folge, 9, 1883.

Thomas: Development of the Liver Fluke. Quart. Jour. Mic. Sci., 23, 1883.

CRYPTOCTYLE

One of the most favorable digenetic trematodes for study of the life history is *Cryptocotyle lingua*. Adults of this species are found in the intestine of fish-eating birds and mammals. Larval stages may be found in the common marine snail, *Littorina litorea*.

Observe the living and preserved adult *Cryptocotyle* and make a drawing to show the difference in morphology between this species and *Pneumoneces*.

The Rediae.—Remove *Littorina litorea* from its shell. If the liver is grayish, shrunken and irregular in appearance it will be found to contain numerous rediae and cercariae. Transfer to a slide and study. Note the following:

(a) Rediae with characteristic *mouth* and *pharynx*.

(b) The numerous *cercariae* in various stages of development.

The Cercaria.—Obtain, from the assistant, material containing mature cercariae of *Cryptocotyle* which have emerged from an infected *Littorina* kept over-night in a finger bowl. To this add a drop of 1:1000 neutral red solution and cover. As the cercariae become quiet look for the following: mouth, pharynx, "penetration glands," tail fin, flame cells, excretory vesicle, and germinal cells.

Encystment of cercariae.—To a watch glass filled with sea water, add first a piece of cunner fin and then several mature cercariae.

Observe and describe the activities of the cercariae during encystment.

Metacercariae.—Study and draw metacercariae of *Cryptocotyle* as they appear when encysted in a cunner fin.

Stunkard: The Life History of *Cryptocotyle lingua* (Creplin), with Notes on the Physiology of the Metacercariae. Jour. Morph. and Physiol., 50, 1930.

CESTODA

The Cestoda, or tapeworms, are endoparasites which possess very few of those organs that are characteristic of free-

living animals. They have no alimentary canal, probably no organs of special sense, and, except in the head, the nervous system is feebly developed. On the other hand, the organs needed for the reproduction of the species are enormously developed, so that in the more mature portions of the animal, the ovaries, testes, and accessory organs occupy nearly the whole space. Can you explain this condition?

CROSSOBOTHRIMUM LACINIATUM

This tapeworm passes its adult life in the intestine (spiral valve) of the sand shark. Cestode larvae which may be the young of this species are abundant in the cystic duct of the squeteague. How the developing eggs and embryos are conveyed from the shark to the squeteague is not known. The transfer of the larvae from the squeteague to the alimentary canal of the shark can be easily understood.

Adult Stage.—1. Notice specimens that are attached to the wall of the intestine of the shark.

2. Observe movements of specimens in a dish of sea water. Do the *suckers* have independent movements?

3. With a low power of the compound microscope, or with a hand lens, note that the worm is made up of a head portion, the *scolex*, and of numerous "segments," the *proglottids*. What is the relative size of the proglottids in the different regions of any specimen? Where are new proglottids produced? (See Curtis.) Are the proglottids attached to one another with equal firmness in all parts of the body? Note their peculiar shape, and how they are connected together. In the above examination, if living material is used it will often be desirable to stretch portions of the animal very gently with your forceps.

4. Note the number and arrangement of the disk-like suckers. How are they borne on the scolex? Do you find each sucker to be entirely simple?

Draw the adult worm.

5. Cut from the head end of a living specimen a piece

consisting of a scolex and not more than one or two proglottids. Place this on a slide, cover, being careful not to compress too much at first, and examine the scolex carefully again to make sure you understand its structure.

6. Look for transparent tubes coiling about in the scolex and its suckers. Compress the specimen by drawing off as much water as possible with filter paper, and look again for the transparent tubes. These are portions of the *water vascular system*. Recall the description of this system given in the lecture or in textbooks. The finer branches which lead from the main trunks are difficult to identify with certainty, but by using the high power of your microscope, and focusing just below the surface in the more transparent portions of the scolex, the *flame cells* may easily be seen. The "flame" appears like a short, thick whip lost in continual vibration. Find such flames and watch them carefully. If not found at once, let the preparation stand and examine in about half an hour. In the older preparation they are frequently easier to find.

7. In both scolex and proglottids of fresh specimens many clear, transparent, threadlike *muscle fibers* may be seen. There will also be found an abundance of clear, rounded granules of lime.

8. Watch the movements of the large, detached proglottids. Pull proglottids from the posterior end of the specimen to see how easily they may be detached. Very many tapeworms have these "motile proglottids," which in some cases remain alive for so long after being detached as to seem almost like independent animals. Ripe proglottids, taken from the intestinal fluid of the host and placed in sea water, begin within a few minutes to extrude eggs. Extrusion is accompanied by peculiar and extensive muscular contractions.

Mount stained specimens of proglottids in balsam and study the reproductive organs.¹

¹Specimens may be killed in the manner described for *Bdelloura*. Enough pressure should be used to flatten the proglottids decidedly.

1. On one side of the proglottid the lateral *genital aperture* will be seen. The *penis* is a long, slender organ, found protruding, or lying in its sheath near the lateral aperture. The *vas deferens*, a long, convoluted tube, extends from the penis to the *testes*, which form many rounded, deeply stained structures that lie about the oval outline of the *uterus*. On leaving the penis the vas deferens extends toward the pointed end of the proglottid, along the side of the uterus, until it reaches a point anterior to it, where it may sometimes be seen sending branches to the testes, but is frequently lost. Throughout its length it is greatly convoluted and is generally filled with spermatozoa.

2. At the base of the penis, in the lateral genital aperture, is the external opening of the female organs. From this point a small tube, the *vagina*, leads to a point below the saclike uterus, which is sometimes very large and sometimes collapsed and small. The vagina ends in a small pouch, the *ootype*, from which a short canal (sometimes visible, but more often obscured by the vagina, which lies above or below it) leads to the uterus.

3. The *ovary* consists of a large many-fingered mass in a median position, near the posterior end of the proglottid. It surrounds, more or less completely, the inner end of the vagina and ootype.

4. The *vitellaria* occupy the posterior corners of the proglottid, and may extend anteriorly along its margins, by the sides of the testes, nearly to its anterior extremity. The ducts from the *vitellaria* unite and join the ootype.

5. The *shell gland* is a small median mass that is situated between the lobes of the ovary around the ootype.

Understand the relation of the ducts of the *shell glands*, *vitellaria*, and *vagina* to the ootype and uterus, how and where the eggs are fertilized, and how they are finally lodged in the uterus. Why should hermaphroditism occur in this form?

Draw a figure of the proglottid showing all of the parts you have seen.

Larval Stage.—Examine and draw a specimen of the larva found in the cystic duct of the squeteague. The scolex with its suckers at the anterior end, and the opening of the water-vascular system at the posterior end, are readily seen. Compress slightly if the trunks of the water vascular system are not easily seen. They can always be seen in preserved and stained specimens that have been killed under pressure. If you have trouble in seeing them, examine such a specimen. Do you find proglottids? Understand the relation of this larva to a true cysticeroid.

Curtis: *Crossobothrium laciniatum* and Developmental Stimuli in the Cestoda. Biol. Bull., 5, 1903.

—: The Formation of Proglottids in *Crossobothrium laciniatum*. Biol. Bull., 11, 1906.

Linton: A Cestode Parasite in the Flesh of the Butterfish. Bull. U. S. Bur. Fish., 26, 1906.

Tennent: A Study of the Life-history of *Bucephalus haimeanus*: A Parasite of the Oyster. Quart. Jour. Mic. Sci., 49, 1906.

NEMERTINEA¹

Several representatives of this group are rather easily obtained. Some of these, as some species of *Cerebratulus* and *Meckelia*, are large, but they are generally unsatisfactory for anatomic study, as they are opaque and filled with a connective-tissue parenchyma that binds the organs together. Furthermore, they are especially likely to cut themselves into small pieces by contraction of muscles in the body wall.

TETRASTEMMA

This small animal lives among the forms that are generally found attached to piles. Specimens can usually be found by placing scrapings from piles in a glass jar with a little sea water and allowing them to stand from a half hour to three hours. The animals may then be found, with the aid of a lens, on the sides of the dish, usually near the surface.

With a pipette transfer a specimen to a slide, cover it,

¹This group is, by many, considered as a separate phylum.

and examine with low and high powers of the microscope. Notice:

1. The shape of the body, the four *eye spots*, and the sensory *ciliated grooves*.

2. The straight *alimentary canal*. The *diverticula* of the intestine and the terminal *anus*.

3. The enormous *proboscis*, consisting of a large anterior eversible portion, and a smaller posterior portion that is not eversible. *Stylets* are present in the eversible portion, near its inner end. Can you determine how the proboscis is protruded and retracted? Does the proboscis have any connection with the digestive system?

4. Beneath the posterior eye spots are the *cerebral ganglia*, from which lateral nerve cords extend posteriorly.

5. If the specimen happens to contain eggs, they will lie between the diverticula of the intestine. They are comparatively large.

Coe: Development of the Pilidium of Certain Nemerteans. Trans. Conn. Acad., 10, 1899.

—: On the Anatomy of a Species of Nemertean (*Cerebratulus lacteus*). Trans. Conn. Acad., 10, 1899.

—: Series of papers on Regeneration in Nemertinea. Jour. Exp. Zool., 54, 57, 61, 67 (1929-34); Biol. Bull., 66, 1934.

Verrill: The Marine Nemerteans of New England and Adjacent Waters. Trans. Conn. Acad. Sci., 8, 1892.

Wilson, C. B.: Habits and Early Development of *Cerebratulus lacteus*. Quart. Jour. Mic. Sci., 43, 1900.

NEMATHELMINTHES

Body elongated, cylindrical, and not segmented. They have a very general distribution and a great diversity of forms. Many are parasitic. Anus usually present. Coelom not filled with parenchyma. The classes may not be genetically related.

CLASS 1. Nematoda.

Many are internal parasites, but others are found in fresh and salt water and in damp earth. Body pointed at both ends. Mouth terminal, anus ventral. (*Ascaris*, *Trichinella*, *Gordius*.)

CLASS 2. Acanthocephala.

Formidable intestinal parasites. Proboscis bearing hooks. No alimentary canal. *Macracanthorhynchus* (*Echinorhynchus*).

CLASS 3. Chaetognatha.

Marine, and all but one species pelagic. With caudal and lateral fins and bristle-like jaws. (*Sagitta*.)

ASCARIS

Animals belonging to this genus are common in the intestine of the horse and pig, and are not uncommon in man. Examine specimens and see if they have any organs that would aid them in clinging to the intestinal wall. How can they retain their positions?

1. Can you determine which is anterior and which is posterior? Is there any indication of segmentation? Can the ventral side be distinguished from the dorsal?

2. Find the *mouth* and see that it is bounded by three *lips*. Notice how these are placed and find the *papillae* on the ventral ones. Find the *anus* and note its position. This serves also as a reproductive aperture for the male. In the female the reproductive aperture is situated about one third

back from the anterior end. It can be seen only in favorable specimens.

3. Open a well preserved or fresh specimen along the dorsal line and notice the definite *cavity*, and the straight *alimentary canal*. If the specimen is a female, find the Y-shaped *genital organs*, the free, ovarian ends of which are slender and somewhat tangled. The position of the external genital opening has already been noted. In the male there is a single, tangled, threadlike *testis*, which joins the enlarged *seminal vesicle* that extends to the cloaca. The *nervous system* consists of a circumesophageal ring, six longitudinal nerves, the dorsal and ventral of which are larger than the others, and anterior nerves. It is not easily seen.

A drawing is desirable.

Montgomery: The Adult Organization of *Paragordius varius*. Zool. Jahrb., 18, 1903.

TRICHINELLA

Encysted specimens may occasionally be found by examining thin pieces of pig muscle obtained from the meat market. Pigs fattened in small pens and fed on table waste, or in slaughter yards and fed on the offal of butchered animals, are much more likely to be infected than others. Scavenger rats and cats are frequently infected.

1. Flatten a piece of muscle containing trichinellae between two slides in a little glycerin and notice the relation of the animal to the muscle fibers. Notice the *cyst* that surrounds it and see if you can determine whether this was formed by the host or the parasite. There are frequently fat cells at the ends of the cyst. Just after the parasites are encysted, the cysts are surrounded by capillaries that may be injected by injecting the vessels of the host. These may be found only at a definite stage after encystment. Why are they formed? Do they indicate how the cysts were formed? If the trichinellae are abundant see if you can find more than one in a cyst.

2. Notice the shape that is assumed by the parasite. Is the coiling always the same? If your material is fresh, mount some of the muscle between slides without glycerin, warm the slide, and see if the encysted animals will move.

3. Are the anterior and posterior ends alike? Is there any indication of a *mouth*? The large cells that form the *intestine* can frequently be seen. It should be borne in mind that the encysted specimen is not fully adult and that the animal grows after reaching the alimentary canal of the next host.

Make a drawing of an encysted animal.

Glazier: Report on Trichinæ and Trichinosis. U. S. Treas. Dept. Doc. No. 84, Marine Hospital, 1881.

METONCHOLAIMUS¹

This species, *Metoncholaimus pristiurus*, is a free-living nematode found in the mud in shallow salt water. It belongs to a large marine group, the Oncholaiminae (type genus, Oncholaimus, "tooth in the throat").

1. Make a preliminary examination of several specimens in a Syracuse dish using a binocular dissecting microscope. Observe the characteristic coiling and uncoiling movements. Distinguish the blunt *anterior end* from the more pointed *posterior end*. In some specimens large beadlike structures may be seen near the middle of the body. These are *eggs* and serve to identify the females.

Select a female and place in a drop of fresh water for one to two minutes until it is quiet and then mount immediately in clear sea water. Flatten the animal slightly by removing water from under the cover glass.

2. **The Digestive System.**—Note that the posterior end tapers rapidly and is slightly curved. The anterior end tapers gradually. Along the sides of both are numerous *sensory setae*. The *mouth* is at the truncated extremity of the anterior end. Behind the mouth is the short *pharynx* with

¹The laboratory directions given above have been adapted from directions originally furnished by the late Dr. N. A. Cobb.

sharply pointed teeth. The *esophagus* running posteriorly from the pharynx is a thick-walled tube. A *sphincter valve* at its posterior end marks the beginning of the *intestine*, a yellowish-brown tube running nearly the entire length of the body. Focussing on the anterior part of the intestine will show that its wall is made up of *columnar epithelium*. The inner ends of most of the epithelial cells are filled with granules giving the intestinal wall its color. The *anus* lies about half way along the tapering *tail* and the *rectum* runs forward from it at an angle.

3. The Nervous System.—At the anterior end of the worm the *nerve ring* may be seen encircling the esophagus about midway of its length. Running backward from the nerve ring are strands of nerve fibers which connect with *ganglion cells* between the esophagus and the body wall. In a specimen previously stained with methylene blue the distribution of the ganglion cells with relation to the sensory setae may be noted. The longitudinal nerve cords, characteristic of many nematodes, are not well developed in *Metoncholaimus*.

4. The Female Reproductive System.—A short distance anterior to the large thick-shelled eggs, lying in the uterus, may be seen a row of cuboidal cells, each approximately equal to the diameter of the body. These are *oocytes* and progressively more advanced stages may be seen toward the anterior end, where mature oocytes as large as the eggs may sometimes be observed. At the posterior end of the ovary a cluster of small rounded *oogonia* may be seen by careful focussing. The funnel-like opening of the oviduct may be observed at the anterior end of the ovary. The oviduct itself is difficult to find. It runs backward parallel to the ovary and opens into the anterior end of the uterus. A varying distance posterior to the last egg in the uterus may be seen a low *papilla* with an opening, the *vulva*, the external orifice of the female genital system.

5. The Demanian System.—This system is found only in the female. Its function seems to be accessory to the female

reproductive system. It consists of the following structures—the *moniliform glands*, two large, clear, cross-striated tubes, lying a short distance anterior to the rectum. These glands open posteriorly by separate pores. Anteriorly these tubes unite near a very obvious structure, the *rosette*. From the rosette a tube runs to the intestine, and another tube, starting as a wide ampulla, soon narrows rapidly to form a thin tube, which joins the uterus in the vicinity of the vulva.

Make a large drawing of a female Metoncholaimus to show as many of the above features as you have been able to make out. If possible make also diagrammatic cross sections of the body (1) in the region of the esophagus and nerve ring (2) in the midovarian region and (3) through the moniliform glands.

The Male Metoncholaimus.—In the manner already described mount several specimens as large as the female but showing no eggs. These are probably males. Compare the posterior end with that of the female. Do you find any trace of the demanian system? The opening of the male genital system is just anterior to the anus. Two slender rodlike *spicula* may be seen running forward from it for some distance. Anterior to the spicula is the *ejaculatory duct*, which is connected with the *vas deferens*. The latter is usually difficult to see. Careful focussing in the midregion of the body will enable one to see the clear, colorless cells which constitute the elongated *testes*, one anterior and one posterior. In each testis there is a progression of stages *from before backward*. A study of the length of this organ will show *spermatogonia* and all stages in spermatocyte growth. Occasionally mitotic figures are observable.

Make a drawing of the male Metoncholaimus.

TROCHELMINTHES

Minute animals whose adult structure seems to be related to that of the trochophore larva. Mouth usually surrounded by a cirlet of cilia. Three classes (Rotifera, Dinophilea, and Gastrotricha) are referred to this phylum, but they may not be genetically related.

ROTIFERA

Mostly fresh-water forms, but a few are marine. All are of microscopic size. The pharynx is provided with a masticatory apparatus, and the anterior end bears a trochal disk. Most rotifers are free, but a few are permanently attached, and some, as *Melicerta*, live in tubes of their own formation.

BRACHIONUS (A Rotifer)

These animals are frequently quite abundant in ponds and aquaria. They are not very active, and spend most of their time near the bottom among the plants and débris. Owing to their minute size, they must be studied with a high power of the microscope.

1. The body is divided into a *trunk*, which is inclosed in a transparent cuticular *lorica*, and a movable *tail* or *foot*. The tail is tipped with two processes which form forceps, by means of which it attaches itself to plants. Can you see how these are used? Why does the animal need to attach itself?

2. Projecting anteriorly from the lorica is the retractile *trochal disk*. Notice the cilia on the margin of this disk. Is the disk used in locomotion? Does the animal always move when the cilia are active? What other use has the disk? Is the animal entirely dependent upon the cilia of the disk for locomotion?

3. The *mouth* is at the ventral border of the trochal disk and leads by a short buccal cavity to the *mastax*, which is a

muscular apparatus provided with three chitinous *trophi* (a median *incus* and two *mallei*). It is used in grinding the food. The grinding movements are easily seen. A very short *gullet* leads from the mastax to the large *stomach*. The *intestine* is short and thick and opens into a *cloaca*. The *anus* is near the base of the tail, on the dorsal surface.

4. The reproductive and excretory systems are not easily seen. An *ovary* and a large *vitellarium* are present. The *oviduct* opens into the cloaca. Two long *nephridial tubes* open into a *contractile vesicle* that in turn opens into the cloaca.

5. There is a single *ganglion* in the anterior dorsal region, immediately beneath two red *eye spots*. Anterior to the eye spots is a *dorsal feeler*, which is a tactile organ.

There are many common rotifers that have no lorica and some of them have the trochal disk two-lobed.

Jennings: Rotatoria of the United States with Especial Reference to those of the Great Lakes. Bull. U. S. Fish Com., 19, 1899.

Whitney: The Desiccation of Rotifers. Am. Nat., 42, 1908.

MOLLUSCOIDA

Lophophore present. Mouth and anus closely approximated. Coelom usually present.

CLASS 1. Bryozoa or Polyzoa.

Usually colonial. Zooids of small size and protected by a firm cuticle.

Subclass 1. Entoprocta.

Colonial or solitary. Anus and mouth both inside lophophore. Epistome present. Tentacles not retractile. Stalk contractile. (Loxosoma, Pedicellina.)

Subclass 2. Ectoprocta.

Colonial. Anus outside lophophore. Mouth inside it. Tentacles retractile. Stalk not retractile.

Order 1. Gymnolaemata.

Recent, marine. Lophophore circular. Epistome absent. (Crisia, Bugula, Flustrella, Membranipora, Lepralia, Schizoporella.)

Order 2. Phylactolaemata.

Fresh water. Lophophore horseshoe-shaped. Epistome present. (Plumatella, Pectinatella.)

CLASS 2. Brachiopoda.

Marine. Solitary. Bivalve shell. Usually attached by a peduncle.

Order 1. Inarticulata.

Valves not united by a hinge. (Lingula.)

Order 2. Articulata.

Valves hinged. Usually with a shelly loop to support the lophophore. (Terebratulina.)

BRYOZOA

BUGULA (Sp.)

The colonies are very common in shallow water along shore, attached to rocks and piles. They may be examined with the aid of a glass-bottomed pail in the positions they occupy on the sides of the piles of almost any old wharf.

What must be the source of their food? What part of the colony is likely to be best nourished? Collect specimens by scraping the piles and see what forms are associated with them.

1. Examine a colony in a dish of water and see how it branches. Does it present any regularity?

Make a drawing of a colony.

2. Remove one of the flat branches, place it in a watch glass of water, and examine it with a low power. What more can be observed regarding the branches? How are the *cups* arranged? Are the cups on the two sides of a twig placed in definite relations to each other? Where are the empty cups found? Explain. Can you find connections between the cups of the two sides?

Make a drawing showing the arrangement of the cups.

3. Allow a living branch to remain undisturbed for a few moments and with a microscope see how the thin outer margins of the cups are unfolded as the zooids protrude.

4. Mount a specimen on a slide, cover, and compare the *tentacles* of an expanded zooid with those of the hydroids that you studied. How do they differ? How must the animal feed?

5. How are the tentacles arranged around the distal end of the body? How many tentacles are there? Look for the *mouth*.

6. Can you see the parts of the alimentary canal? Is there food in the *stomach*? How does the zooid pull itself back into its cup?

7. Look for *avicularia* and observe their movements and structure. Where is the jaw hinged? Where are the muscles that open it? Where are the muscles that close it? Of these muscles, which are largest? Why? See if "sense hairs" can be found between the jaws. What is their probable use?

Draw an avicularium.

8. *Ooecia* with embryos will be found in some specimens. Where are they placed?

9. Put powdered carmine in the water with a living branch and see if the zooids will eat it.

10. Put a small living branch in a drop of sea water under a supported cover glass and see if any of the zooids will expand. If any do expand they may be examined, with a high power, to good advantage.

Study specimens that have been killed while expanded. Stain with iodine, wash in water, mount in glycerin, study with a high power. Find the *retractor muscles*, the *funiculus*, *germ cells*, and, if possible, the shape of the *alimentary canal*. As the alimentary canal bears a definite relation to the position of the zooid on the branch, its shape can be readily determined only when the branch happens to be twisted so the zooid is to be seen in side view.

Make drawing showing the structure.

If time permits study *Flustrella*, *Membranipora*, *Lepralia*, or *Schizoporella*, as type incrusting forms to determine methods of branching, colony formation, how the apertures are closed, and specific characters.

Bissonnette: A Method of Securing Marine Invertebrates. Science, 71, 1930.

Grave: Natural History of *Bugula flabellata*. Jour. Morph., 49, 1930.

PLUMATELLA¹

If the zooids of this fresh-water form will expand in a watch glass of fresh water, notice the shape of the *lophophore* and the position of the *epistome*. In such a specimen the *ganglion* may be seen as a rounded mass just beneath the lophophore, between the mouth and the anus. Study the *statoblasts* with a microscope.

Allman: Monograph of the Fresh-water Polyzoa. Ray Soc., 1856.

Calvet: Contribution à l'Histoire Naturelle des Bryozoaires Ectoproctes Marins. Trav. Inst. Zool. Montpellier, N. S., Mem. No. 8, 1900.

¹Slices of the large gelatinous form, *Pectinatella*, placed in watch glasses of fresh water, make very satisfactory objects for study, as the zooids will soon expand, and they are then in the best possible position for study.

Nitsche: Beiträge zur Kenntnis der Bryozoen. Ueber die Anatomie und Entwicklungsgeschichte von *Flustra membranacea*. Zeit. f. wiss. Zool., 21, 1871.

O'Donoghue, Charles H., and Elsie O'Donoghue: Second List of Bryozoa from Vancouver Island Region. Contrib. to Canadian Biol. and Fisheries, N. S. III, pp. 47-132. (See Bibliography list, 1926.)

Osburn, T. C.: Bryozoa of Woods Hole. Bull. Bur. Fish., xxx, 1910, Doc. No. 760, 1912.

BRACHIOPODA

TEREBRATULINA

Examine specimens on the demonstration table and notice:

1. *Shell*. The difference in the size and shape of the two *valves* and their position in relation to the body. How are the valves articulated? How are they opened?

2. *Peduncle*. Its position. What is its use?

3. *Muscles*. Those used in opening and closing the shell.

4. *Lophophore*. Consisting of two elongated arms with a double row of tentacles on each.

5. *Mouth*. Notice its relation to the grooves running between the rows of tentacles on each of the arms of the lophophore.

Brooks: Development of *Lingula*. Ches. Zool. Lab. Sci. Results, 1878.

Conklin: The Embryology of a Brachiopod, *Terebratulina septentrionalis*. Proc. Am. Phil. Soc., 41, 1902.

Hancock: On the Organization of Brachiopoda. Trans. Roy. Soc., London, 148, 1858.

Morse: Observations on Living Brachiopoda. Mem. Bost. Soc. Nat. Hist., 5, 1902.

ANNELIDA

Body elongated, generally divided into somites. Coelom usually extensive. Appendages when present form parapodia.

CLASS 1. Archi-annelida.

Without setae or parapodia. Nervous system not separate from the epidermis. (Polygordius.)

CLASS 2. Chaetopoda.

With numerous, distinct somites that are provided with setae.

Subclass 1. Archi-chaetopoda.

Setae retractile. Nervous system not separate from the epidermis. (Saccocirrus.)

Subclass 2. Polychaeta.

With numerous setae per segment. With a great variety of structure. (Amphitrite, Arenicola, Autolytus, Chaetopterus, Clymenella, Diopatra, Hydroides, Nereis, Pectinaria, Polynoe, Sabella, Spirorbis.)

Subclass 3. Myzostomida.

Disk-shaped. Without external segmentation. Parasites on Echinodermata. (Myzostoma.)

Subclass 4. Oligochaeta.

Without parapodia. Setae few and simple. (Tubifex, Lumbricus.)

CLASS 3. Gephyrea.

No segmentation. With or without setae. With introvert or proboscis.

Order 1. Inermia.

With introvert. Anus dorsal. No setae. (Phascolosoma.)

Order 2. Armata.

With proboscis. Anus posterior. Setae few. (Echiurus.)

CLASS 4. Hirudinea.

Somites constant in number, with more external annuli than there are somites. With sucking mouth and posterior sucker.

Order 1. Rhynchobdellida.

Anterior end of body forming a proboscis or introvert. No jaws. (Glossiphonia, Macrob-della, Clepsine.)

Order 2. Gnathobdellida.

No proboscis or introvert. Mouth usually with three teeth. (Hirudo.)

Hatschek: Studien über Entwicklungsgeschichte der Anneliden. Arb. Zool. Inst. Wien, 1, 1878.

Norman: Dürfen wir aus den Reactionen niederer Thiere auf des Vorhandensein von Schmerzempfindungen schliessen? Arch. ges. Physiol., 67, 1897.

CHAETOPODA

NEREIS VIRENS (Clam-worm)

These animals may be found inhabiting mud flats from which the water flows at low tide. Occasionally they may be seen with their head ends protruding from their burrows, but generally specimens will have to be dug. Notice the conditions under which the animals live and the forms with which they are associated. It should also be understood that many of their worst enemies are present only when the water covers their burrows.

External Structure.—1. Examine a living worm in a dish of sea water, noting the motions of the body and of the *parapodia* or *swimming feet*.

2. Is the general surface clean or slimy? Compare with the earthworm in this respect and explain the basis for the difference.

3. Determine the direction of the peristaltic waves in the *dorsal blood vessel*.

4. Is the median *ventral nerve cord* visible through the body wall?

5. In an anesthetized or dead worm, count the *segments* or *metameres* and compare it with your neighbor's to ascertain whether the number is constant. What segments, if any, are devoid of parapodia? Explain.

6. In the head distinguish the *prostomium*, which bears the four *eyes* and a pair of short *terminal tentacles*. At each

side of the prostomium is a thick *palp*. Determine which parts of the worm are most sensitive by gently stimulating with a needle.

7. Also in the head find the *peristomium*, the segment which surrounds the *mouth* and bears four pairs of *peristomial cirri*. Stretch the mouth with forceps.

Make an enlarged drawing of the head.

8. Hold it down against the bottom of the dish or place in fresh water for a few minutes to induce it to protrude the *proboscis*, the protrusible anterior portion of the alimentary canal. This is lined with chitin and armed with numerous *denticles* and a pair of lateral *jaws*.

9. Find the small terminal *anus* and a pair of *caudal cirri* on the last segment.

10. With scissors cut off a parapodium close to the body and observe that it has a dorsal blade and a ventral blade (*notopodium* and *neuropodium*). Each of these contains a bundle of bristles or *setae*. What use can you ascribe to the *setae*? In each bundle is one very thick seta, the *aciculum*, which extends into the body and is attached to muscles. Of what use is the *aciculum*? Examine a few of the small *setae* microscopically. What is their structure? Why is it desirable to have so many of the small *setae*? Can you give any reason for *Nereis* having more *setae* than the earthworm?

Observe that each parapodium has a small dorsal and a small ventral cirrus, and that the main portion of both *notopodium* and *neuropodium* has the form of a flattened blade, somewhat divided into lobes. The largest lobe of the *notopodium* is very thin and vascular. What function can you ascribe to it?

Draw a parapodium.

11. Look for the *nephridiopores*, minute apertures which are segmentally placed on the ventral surface near the *neuropodial cirri*.

Internal Structure.—For dissection use a specimen that has been killed and fasten it down by a pin through the head

and one through the posterior part. With scissors cut through the body wall, longitudinally, near the mid-dorsal line.

A preserved specimen can easily be segmented transversely with a sharp razor at the somatic boundaries. These sections are valuable for comparison during dissection.

Find the *septa* which divide the *coelom*, or *body cavity*, into metameric chambers. Cut through the *septa* with scissors and pin the edges of the body wall apart, progressing toward the head.

Circulatory System.—The dorsal blood vessel lies along the dorsal surface of the alimentary canal and gives off branches in each segment, which ramify through the body wall and viscera and connect with the longitudinal, ventral blood vessel. The blood plasma contains hemoglobin in solution.

Digestive System.—The *buccal cavity* leads into a muscular *pharynx*. The eversible buccal region and the protrusible pharynx form the *proboscis*. Examine carefully the muscles of the pharynx, *protractors* and *retractors*, and ascertain their attachments. Posterior to the pharynx find a small dilation and a narrow *esophagus* with a *digestive gland* at each side. Where does the duct of the gland open? In the very long *stomach-intestine* note the constrictions and their relations to the dissepiments. Can you demonstrate dorsal or ventral mesenteries? Cut open the pharynx and the anterior end of the stomach-intestine and note the character of their walls.

Make a drawing of the digestive system.

Muscular System.—How many distinct bands of *longitudinal muscles* can be distinguished? Examine with a hand lens the *parapodial muscles* attached to the base of the acicula. Can you make out a layer of *circular muscles*? Of what layers does the body wall consist?

Excretory System.—The *nephridia* are not nearly so easily found or studied as they are in the earthworm. Near or just beneath the lateral edges of the ventral muscle bands

find the minute pear-shaped nephridia. Determine their distribution in the body. Each nephridium consists of a tortuous canal in a multinucleate mass of protoplasm. The external opening is the *nephridiopore* above mentioned. The inner end perforates the septum anterior to the body of the nephridium and opens into the coelomic cavity of the segment next in front, by a ciliated funnel, the *nephrostome*. With a hand lens try to find the nephrostome. Remove a nephridium by means of fine forceps and examine it with a microscope.

Reproductive System.—The sexes are separate, but no permanent gonads are present. At the breeding season the ova or spermatozoa are proliferated from the coelomic epithelium of a large number of segments and escape by rupture of the body wall.

Nervous System.¹—On lifting the alimentary canal you will see the ventral *ganglionated nerve cord*. Note the nerves passing off laterally from the ganglia. How many pairs of nerves per segment are there, and how are they placed? Are the ganglia metameric? Is there any indication that the nerve cord is double? At the anterior extremity of the cord note the *infra-esophageal ganglia* and, extending from them and encircling the anterior end of the alimentary canal, the *circumesophageal connectives* which unite above in the bilobed *brain* or *supra-esophageal ganglia*. *Sensory nerves* connect the brain with the eyes, tentacles, and palps.

Make a drawing of the nervous system.

Binard et Jeener: Morphologie du lobe préoral des polychaetes. Inst. Zool. Torley-Rousseau Recueil (Bruxelles), 2, 1928.

¹The nervous system can be most readily studied by tearing it out with needles in a specimen which has been macerated in 20 per cent nitric acid for twenty-four hours. Sensory cells and their neurites can be identified in the parapodia by placing them in a 1 per cent solution of ammonium picrate after having let vigorous worms crawl around for three or four hours in a small amount of 1 per cent solution of methylene-blue. Mounts of the parapodia should be made in a mixture of glycerin and ammonium picrate solution.

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- : Breeding Habits of the Heteronereis Form of *Heteronereis megalops* at Woods Hole. Biol. Bull., 27, 1914.
- : A Cytological Study of Effects of Ultra-violet Light on the Egg of *N. limbata*. Zeit. Zellforsch., 17, 1933.
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- Wilson, E. B.: The Cell-lineage of *Nereis*. A Contribution to the Cytology of the Annelid Body. Jour. Morph., 6, 1892.
- Woodworth: The Palolo Worm, *Eunice viridis*. Bull. Mus. Comp. Zool., Harvard, 51, 1907.

AUTOLYTUS CORNUTUS

This polychaete lives in cylindrical tubes of its own construction that it attaches to seaweeds and hydroids, and is especially interesting because of its method of reproduction, by budding.

Study live and preserved specimens with the naked eye and with the hand lens, in order to form a correct idea of its natural color, size, and movements, and then study stained specimens with the low power.

1. Observe two individuals attached end to end. The anterior one is a *nonsexual zooid* (or original "stock") and is giving rise to a new *sexual zooid* by *budding*. Counting the peristomium as one somite, on what somite does the bud begin and what does it represent?

2. Study the *head* of the anterior, nonsexual zooid. Find three *prostomial tentacles*. How are they arranged? Find the *eyes*. How many pairs are there? Do you find *palps*?

On the peristomium find the two *tentacles* and a *tentacular cirrus* on each side.

3. On the succeeding somites study the *parapodia*. Observe the large *dorsi cirri* and the knoblike *notopodium* with the short unjointed setae. There is no *neuropodium*.

4. Identify the *pharynx*, *gizzard*, and *intestine*.

5. Compare the sexual bud with the nonsexual individual. The adult male and female differ. The outer prostomial tentacles of the male are forked. Is this bud to be a male or a female? In an older sexual individual make out a so-called *thoracic* region in which the setae are short, and an *abdominal* region in which the setae are long. Look for evidences of germ cells in the body cavity, between the intestine and body wall. There is a ventral brood pouch on the adult female and the young partly develop in it. Find the anal cirri.

A drawing illustrating the method of reproduction should be made.

LEPIDONOTUS (POLYNOE) SQUAMATUS

The family Aphroditidae, to which this belongs, can be distinguished from all others by the presence of peculiar plates (*elytra*) on the dorsal surface. They lead sluggish lives under stones and are carnivorous. Note the size, color, and shape of the worm.

1. Examine the *elytra*. How are they arranged? What purpose do they serve? How many are there? With a hand lens observe the fringed condition of the outer edge and the small tubercles covering the surface. Note the color of the *elytra* and the notches in the inner edges of the posterior pair.

Remove with forceps all of the *elytra* on one side of the specimen and the first two or three on the other side. Note the stumps to which the *elytra* were joined. Do all segments have *elytra*? If not, do the scaleless segments have structures which correspond to *elytra*?

2. Examine the dorsal aspect of the head, and note the small prostomium, with two pairs of eyes, three slender ten-

tacles, and a pair of fleshy palps. Outside the palps are two pairs of cirri arising from the peristomium. The significance of these will be understood later.

3. Find the mouth, placed ventrally in the first or peristomial somite. The mouth leads into a buccal region, which is eversible and fringed at the end with papillae, each having a dark spot at the base. If the pharynx is retracted, expose the buccal cavity by a median ventral incision. In the anterior end of the pharynx are four black chitinous jaws. Do you infer that this species is carnivorous or herbivorous? The eversible buccal region and the protrusible pharynx form the proboscis.

4. The *anus* is dorsally placed, and can be found beneath the notches in the last pair of elytra.

5. Examine the lateral appendage or parapodium of the third or any subsequent somite. Note that it consists of a stout ventral or neuropodial division, and a less prominent dorsal or notopodial division, each supported internally by a chitinous rod or aciculum, and bearing externally a tuft of setae. If there is time, compare the form of the notopodial and neuropodial setae. The typical parapodial structure is completed by a soft neuropodial cirrus ventrally, and a notopodial cirrus dorsally. Did you find any evidence that the elytra are modified notopodial cirri?

6. Make a careful study of the appendages of the first or peristomial and the last or anal segment. Cut off close to the body, mount in glycerin, and examine with low power of the microscope. Determine the homology of the parts observed.

Draw the dorsal aspect of the head, to show the appendages and the proboscis, if exposed. Diagram the structure of the parapodium as seen in a transverse section of the body.

Unlike most other worms, many of the Aphroditidae have a fixed number of somites. Count the number in your specimen, including in the enumeration the peristomial and anal segments. How many pairs of elytra? The number and po-

sition of the elytra are also characteristic of various genera, and may be conveniently represented by an elytral formula consisting of the numbers of the somites on which elytra are borne, *e. g.*, 2, 4, 5, etc. Determine the elytral formula of your specimen. *Draw one of the elytra, noting its form, surface, and border markings, etc.* These points are of importance in defining species. Each of the last pair of elytra is notched on the medium side over the anus, which in this form opens dorsally instead of terminally.

DIOPATRA¹CUPREA

This worm belongs to the family Eunicidae. Specimens live on mud and sand flats, sometimes above low-tide mark, but usually where the burrows are covered by water. This form is especially interesting because of its feeding and tube-building habits, parapodial gills, and complex jaw apparatus. Study the preserved specimens for the structure and specimens in an aquarium for the habits.

Make a special, comparative study of the reactions of *Nereis* and *Diopatra*. What types of movements can you distinguish? What is the significance of each? Which worm is better adapted for a pelagic life? Locate any respiratory structures which may be present. What structural differences can you see which may be correlated with different habits? Place a glass tube in the dish with a worm and gently move the head of the worm so that the anterior end projects into the tube. Observe results. Study the activities of a worm after it has been in the tube a few minutes. Are these identical with the movements of the worm when free in the water? Supply a *Diopatra* in a glass tube with bits of sea weed or shells and observe the method of tube formation.

1. Notice the size of the body, also its gradual attenuation posteriorly. Account for this condition. Observe how degenerate the posterior parapodia are from the same cause.

2. The *prostomium*. Identify the *tentacles*. What is their number and arrangement? Find a pair of *eyes* dorsally

placed behind the tentacles, also a pair of *palps* in front of them. Note a second, larger pair of palps which serve as an upper lip.

3. The *peristomium*. What appendages does it carry? Note the lower lip formed from the ventral edge of the peristomium.

4. The position of the *jaw apparatus* can be identified as being in a pouch ventral to the buccal region. Find both by means of a probe. What kind of food are such jaws fitted for?

5. The *parapodia* vary greatly, depending upon their position on the body. Notice that the notopodia are vestigial, being represented only by the *dorsal cirri* and, toward the anterior end, *branchial cirri* or *gills*. Acicula can be seen projecting into the base of the dorsal cirrus. The *neuropodium* shows two kinds of setae: (a) stiff and unjointed, (b) crochets. It also bears an accessory cirrus and the ventral cirri, which are curiously modified in most cases as glands for use in tube building. Make out all these modifications and where they occur.

CHAETOPTERUS

This is one of the most aberrant of our Polychaeta. It lives on mud flats below low tide in a U-shaped, parchment-like tube both ends of which protrude above the mud. In the body three regions can be distinguished. Examine a tube and see the size of its outer openings. Specimens may be made to live in tubes of glass, bent to correspond to their tubes, and their normal movements may thus be studied in aquaria. What must be the source of the animal's food?

1. The *anterior region*. Identify ten modified *parapodia*, the fourth of which is supplied with a group of much stouter setae. Observe that the tunnel-like *mouth* is placed dorsally and surrounded ventrally and laterally with flaring peristomial lips. Find the pair of peristomial cirri. The region between these cirri represents the prostomium.

2. The *middle region* consists of five somites. The first, the eleventh segment, is marked by the great pair of wings which are used to bring food to the mouth. Their dorsal surfaces are grooved and supplied with cilia, as is the median dorsal line. Hence a current of water passes continually toward the mouth. The twelfth somite is marked by a dorsal and a ventral sucker, which are modified parapodia. Somites thirteen, fourteen, and fifteen carry notopodial folds or fans, for keeping up a stream of water through the tube. Their neuropodia are mere knobs.

3. The *posterior region* is less highly modified. Of how many segments does it consist? Notice their gradual diminution in size. Homologize the parts of their appendages.

4. The living Chaetopterus contains a green coloring matter and is very luminescent.

5. The eggs are orange yellow and the sperm milky white. Determine their location. The sexes are separate.

A drawing is desirable.

Lillie: Observations and Experiments Concerning the Elementary Phenomena of Embryonic Development in Chaetopterus. Jour. Exp. Zool., 3, 1906.

AMPHITRITE ORNATA

This belongs to the family Terebellidae and lives under stones, or in mud or sand, along shore in stout muddy tubes.

1. Find the *prostomium*, which forms an upper lip and bears a transverse group of long, retractile tentacles.

2. The *peristomium* forms the under lip, but bears no appendages.

3. Find three pairs of racemose *gills*. These are modifications of the dorsal cirri.

4. Notice again the feeble development of the *parapodia* and the absence of ventral cirri and neuropodial setae. Setae are not found posteriorly. Exactly which segments have setae?

5. Find the ventral *shield glands* which are concerned in building the tube. How many are there?

6. The live worm is of a bright pinkish color, due to its red blood. There is only one internal septum and its forms a so-called *diaphragm*. Anterior to the diaphragm the nephridia are large and excretory in function. Posterior to the diaphragm the nephridia serve as genital tubes.

A drawing is desirable.

CISTENIDES (PECTINARIA) GOULDI

This very aberrant worm belongs to the family Amphictenidae.

1. Study the beautiful tube of sand and the manner in which the grains are fitted together. It is said that the worms can carry the tubes about.

2. See how the *peristomium* and the large golden *setae* close the shell. The *setae* are said to belong to the second somite. Notice the ends of the tentacles protruding from the tube.

3. Find the *tentacles*, two pairs of *gills*, and the *parapodia*. Notice how the latter diminish in size posteriorly and how each typically consists of a ridge-like notopodium without *setae* and a reduced neuropodium with long golden *setae*. If the specimen is complete you can see a much degenerated portion (the *scapha*) at the posterior end, which serves to close the small end of the tube.

A drawing is desirable.

CLYMENELLA TORQUATA

This worm belongs to the family Maldanidae. It makes tubes of sand and generally lives in sheltered places on sandy or muddy shores.

1. Study the structure of the tube.

2. Observe the diameter and length of the worm, the small number of somites, their great length as compared with somites of *Nereis*, and the reduced *parapodia*. How many segments are there? Which are setigerous? Notice the characteristic *collar* on the fifth somite, and the funnel at the posterior end, with the *anus* within it. The mouth is

more or less ventral and is overhung by a narrow prostomium surrounded by a peristomial rim.

A drawing is desirable.

Sayles: External Features of Regeneration in *Clymenella torquata*.
 Jour. Exp. Zool., 62, 1932.

—: Regeneration in the Polychaete *Clymenella torquata*. Physiol.
 Zool., 7, 1934.

ARENICOLA² CRISTATA (Lug-worm)

This worm lives in sand flats in U-shaped burrows. During the breeding season the burrows of females may be located by the large, elongated, gelatinous egg masses.

External Structure.—1. Examine a living worm, noting its movements. Are they associated with locomotion? Compare with *Nereis* and *Amphitrite*. What is the effect of the tube habit on locomotion? Compare the general shapes of *Arenicola*, *Amphitrite* and *Nereis*. In what respects are the shapes of the first two similar? How do you account for this? Is the color of *Arenicola* a pigment or is it due to iridescence?

2. On the *head* locate the *prostomium*, a small, three-lobed structure embedded in the dorsal surface of the first segment or *peristomium*. Unless the specimen has been well narcotized, the prostomium will be withdrawn into the *nuchal groove*, which lies just posterior to it. Prostomial tentacles and palps are absent. Compare with *Amphitrite*. Eyes are present as minute, subepidermal structures not visible externally.

3. The body is divisible into three regions: an anterior one bearing parapodia but no gills; a middle one bearing both parapodia and gills; a posterior one bearing neither.

4. Examine a parapodium and find both *notopodium* and *neuropodium*. Explain the position of the gills so near the dorsal surface. Compare with *Amphitrite*. Examine setae from both divisions of the parapodium. In the posterior region of the body note the small *papillae* which occur on the fourth annulus and are probably vestigial parapodia.

5. Locate the anterior, terminal *mouth* and posterior, terminal *anus*.

6. *Nephridiopores*, situated just ventral to the neuropodia of the fifth to tenth setigerous segments are not easily visible under ordinary conditions.

7. Study the segmentation of the worm and note that the *annuli* and *segments* are not homologous. Where parapodia

RELATION OF SEGMENTS TO VARIOUS OTHER STRUCTURES

Segments.	Annuli.		Gills.	Nephridia.	Gonads.	Septa.
	Number per segment.	One on which setae found.				
1st	1	no setae	
2nd	2	no setae	
3rd	2	1st	← 1st
4th	3	2nd	← 2nd
5th	4	3rd	← 3rd
6th	5	4th	
7th	5	4th	1st	
8th	5	4th	2nd	1st	
9th	5	4th	1st	3rd	2nd	
10th	5	4th	2nd	4th	3rd	
11th	5	4th	3rd	5th	4th	
12th	5	4th	4th	6th	5th	
13th	5	4th	5th	
14th	5	4th	6th	
15th	5	4th	7th	
16th	5	4th	8th	
17th	5	4th	9th	
18th	5	4th	10th	
19th	5	4th	11th	
20th	5	no setae	
21st	5	no setae	
22nd	5	no setae	
etc.	5	no setae	

* Septa begin again in extreme posterior part of worm. There are dorsal and ventral mesenteries in the 3rd and 4th segments.

are present they offer a clue to the number of segments, since there is ordinarily one pair of parapodia per segment. Where parapodia are absent, internal structures (*e. g.*, septa) may help to define segments. See table on page 103.

Internal Structure.—If fresh material is used, anesthetize first in 8 per cent alcohol in sea water. Pin the specimen out in a dissecting pan, dorsal side up, sticking two pins through the sides of the first segment and two through the sides of the tail region. Cover the specimen with water. Open the worm by making an incision with a fine pair of scissors in the mid-dorsal region near the center of the gill-bearing portion. Before continuing with the cut, notice the coelomic fluid. Examine some of this under the microscope. Observe the *coelomic corpuscles* and possibly the *reproductive cells*. There are two kinds of coelomic corpuscles: *amoebocytes* and *fusiform cells*. These cells form clots when removed from the coelom. They are also phagocytic.

Next raise the point of incision carefully with forceps and extend it to within an eighth of an inch of the prostomium. Posteriorly continue the cut about an inch into the tail region. The tail region is difficult to open and, unless you are careful, you may pierce the intestine. The flaps of the body wall should now be pinned out right and left so that the worm is moderately well stretched both longitudinally and transversely. At the breeding season ova or spermatids are so abundant that they may obscure some of the organs. In this case the reproductive cells should be carefully washed away.

Note that the *septa* are confined to the anterior and posterior regions of the body. On the anteriormost septum there is a pair of *diaphragmatic pouches*, which extend posteriorly. It is possible that these may have some function in connection with the extension of the proboscis. What advantage is there in the absence of septa throughout the principal part of the worm?

Digestive System.—The protrusible *proboscis* region is

followed by an *esophagus* which extends through several segments. At the junction of *esophagus* and *stomach* note the two conical, yellowish *esophageal glands*. The stomach is covered with a vascular *gastric plexus* between the branches of which is the yellow *chlorogogue tissue*. When you have finished all dissection, observation and drawing, slit open the alimentary canal and locate the ventral *ciliated groove*.

Vascular System.—There is a closed blood system. The general course of the circulation is anteriorly in the dorsal and longitudinal intestinal vessels and posteriorly in the ventral vessel. The principal vessels are:

1. *Dorsal Blood Vessel*.—Arises near anus, extends along dorsal side of intestine and terminates in small vessels on *esophagus*. It communicate with the following:

(a) *Intestinal Vessels*.—One pair in each posterior segment, more numerous on anterior part of intestine.

(b) *Efferent Branchial Vessels*.—From last seven pairs of gills.

(c) *Gastric Plexus*.—Numerous vessels over wall of stomach.

(d) *Nephridial Vessels*.—To first three nephridia.

(e) *Esophageal Pouch Vessel*.

(f) *Septal Vessels*.—To second and third septa.

2. *Ventral Blood Vessel*.—Arises by fusion of small vessels at anterior end of body. It communicates with the following:

(a) *Septal Vessels*.—From the first three septa and the diaphragmatic pouches. Branches of second and third septal vessels connect with the neural vessels (see p. 106). There is also a connection between ventral and each neural at level of second setigerous sac.

(b) *Segmental Vessels*.—One pair per segment, associated with the setigerous sacs and with the nephridia and gills where the two latter are present.

(c) *Intestinal Vessels*.—To intestinal wall.

3. *Subintestinal Vessels*.—A pair running most of the

length of the stomach. These communicate with the gastric plexus and receive the *efferent branchial vessels* from the first four pairs of gills.

4. *Lateral Gastric Vessels*.—One on each side receives blood from the gastric plexus. They are clearly distinguishable only on the anterior half of the stomach. Each communicates anteriorly with a heart.

5. *Hearts*.—Paired structures, located laterally on each side of anterior end of stomach. Each consists of an *auricle* and a *ventricle*. Each auricle is really a swelling at the anterior end of a lateral gastric vessel. The auricle gives off anteriorly a *lateral esophageal vessel* as well as discharging into the ventricle. The ventricle sends blood into the ventral blood vessel.

6. *Neural Vessels*.—One runs along each side of ventral nerve cord. They arise in capillaries at the anterior end of the body. Each communicates with the ventral vessel in segments two to six, inclusive.

7. *Nephridial Longitudinal Vessels*.—Lie just ventral to nephridiopores, one on each side of the body. Each runs the length of the region where nephridia are found.

8. *Dorsal Longitudinal Vessels*.—One on each side running parallel to the nephridial longitudinal but dorsal to the level of the notopodial sacs.

9. In each setigerous segment there is a ring of blood vessels formed by the connectives between the neurals, nephridial longitudinals and dorsal longitudinals. These vessels can best be seen in young, transparent worms.

Muscular System.—Note the conspicuous bands of *longitudinal muscles*. External to these are the *circular muscles* which can be seen only if some of the longitudinal layer is teased away carefully. Inside these muscles are the *oblique muscles* which consist of many bands which pass from the midventral region to the dorsolateral part of the body wall on each side. Associated with each setigerous sac, there are six to ten *protractor muscles*, which are attached to the dorso-

lateral body wall, and a single *retractor muscle*, which has its origin on the midventral body wall.

Nephridia.—Location? Number? To see them clearly, cut the oblique muscles. With a hand lens, distinguish the *funnel* with its fringed *dorsal lip* and its simple *ventral lip*. The *excretory tubule* is divided into an *excretory portion* and a *bladder*. The latter communicates with the outside through the *nephridiopore*.

Gonads.—Each is a minute body attached to the posterior margin of the lip of the nephridial funnel. They occur on the last five pairs of nephridia. The reproductive cells are discharged to the outside through the nephridia.

Nervous System.—Push aside the digestive tract and follow the *ventral nerve cord* forward to the first septum. The *brain* and *circumesophageal connectives* can be found more easily when the proboscis has been completely retracted. Remove the pins at the anterior end, pull in the proboscis with a pair of forceps, and then pin the specimen down again. Usually an *otocyst* can be found on each side attached to the connective about one third of the way from brain to ventral nerve cord.

Ashworth: Arenicola (the Lug-worm). Liverpool Mar. Biol. Com. Mem., 11, 1904.

Gamble and Ashworth: The Anatomy and Classification of the Arenicolidae with Some Observations on their Post-larval Stages. Quart. Jour. Mic. Sci., 43, 1900.

Strunk: Excretions-Physiologie der Polychäten Arenicola marina und Styelaroides plumosus. Zool. Jahrb., Abt. Allgem. Zool. u. Physiol. Tiere, 47, 1930.

PARASABELLA MICROPHTHALMA

This worm belongs to the family Sabellidae. It builds leathery, muddy tubes on piles, among tunicates, algae, etc.

1. In addition to the general size, form, and color of the worm, observe the reduced condition of the *parapodia*, and the arrangement and general structure of the *branchiae* or *gills*. These structures are modifications of the palps and not of the parapodia, as in the other species which have been

studied. Observe the two irregular rows of small *ocelli* or *eye spots*. Account for the presence of eyes in their position. A pair of short *tentacles* can be seen by pushing the *branchiae* aside.

2. Find a *collar* which is used in smoothing the orifice of the tube. This is a peristomial structure and is so extensively developed in some species as to hide the prostomium entirely.

3. Identify eight setigerous somites anteriorly, in which the *capillary setae* are in the notopodium and the *uncini*, or hooked setae, are in the neuropodium. With the peristomium they form a "thorax" of nine somites. In the somites which follow (the "abdomen") observe that the *uncini* and the capillary setae stand in the reverse order. How do you interpret the above fact?

4. Find the ventral *shield glands*. A furrow (sulcus or fecal groove) divides them into pairs toward the posterior end of the worm.

A drawing is desirable.

HYDROIDES

This is a member of the family Serpulidae. Study living specimens and their heavy calcareous tubes. Notice the banded *branchiae* (modified palps) and the dorsally placed *operculum*, a modified gill filament. Look for "eyes" on the gill filaments.

When eggs and sperm are mature these animals will shed them immediately upon being removed from their tubes and placed in sea water. The larvae are typical trochophores.

A drawing is desirable.

Hatschek: Entwicklung der Trochophora von *Eupomatus uncinatus*, Philippi. (*Serpula uncinata*.) Arb. Zool. Ins., Wien, 6, 1886.

Okada: Remarks on the Reversible Asymmetry in the Opercula of the Polychaete Hydroides. Jour. Mar. Biol. Assoc. Unit. Kingd., 18, 1933.

Shearer: On the Development and Structure of the Trochophore of *Hydroides uncinatus* (*Eupomatus*). Quart. Jour. Mic. Sci., 56, 1911

SPIRORBIS

This animal is also a member of the family Serpulidae. Specimens are very abundant along the shore, attached to *Fucus*.

1. Study the tube and notice the way in which it "parallels" the form of a small snail shell.

2. Remove a live specimen from the *Fucus* on which it grows and crack the tube away with a needle. Study the animal in a watch glass with a low power. Identify the *gills*, the *operculum* (which serves as a "brood pouch"), the *setae*, and the *collar*. Are any "eyes" on the gills?

3. Study the egg strings which are lodged in the tube, and the young embryos which are to be found in the brood pouch.

A drawing is desirable.

LUMBRICUS (Earthworm)

Earthworms feed mostly at night. What reason is there for this habit? You should look for earthworms with a lantern some mild, calm summer evening when the ground is quite moist. See if they leave their burrows entirely. How much of the body is generally protruded? Can you determine what the worms are doing? Are they disturbed by walking near them? Are they ever disturbed by flashing the light suddenly upon them? Of what service to them is the ability to distinguish light? Look for castings near the burrows. During daylight look for castings and thus determine the relative abundance of worms in lawns, gardens, etc. (As the worms come to the surface only when it is moist, castings will be abundant only at such times.) Do the casting indicate anything about the feeding habits?

Place a living specimen upon moist filter paper and observe the direction and method of movement. How can it reverse its direction? Gently touch different parts of the body to see which are the most sensitive.

Observe the movement of the blood in the dorsal vessel. In what direction does it move? Does the vessel change in shape?

Place a preserved specimen in a dish with a little water.

1. Note the difference in shape of the two ends of the body. The *mouth* is at the anterior end, below the protruding lobe of the *prostomium*. The *anus* is a vertical slit at the end of the last somite.

2. How do the ventral and dorsal sides differ?

3. The right and left sides are symmetrical. Count the somites of the body, compare with others, and record the result.

4. On the anterior third of the body certain somites are swollen and form the *clitellum*. What somites are swollen? The clitellum is not present in young individuals. It is used in making egg cases and providing food for developing embryos. Understand how this is accomplished.

5. On the ventral side of the fifteenth somite are small swollen areas where the *vasa deferentia* open.

6. *Setae* project slightly from the surface of each somite. These light colored spines are easily felt with the fingers. See if you can determine the number and position of the rows by stroking gently. How are they used?

Draw a ventral view of the anterior end, including the clitellum, and another view of the posterior end.

Taking care not to cut deep, with fine scissors cut through the dorsal wall of the body of a preserved specimen, and extend the cut the whole length of the body. Carefully spread and pin the animal open. In doing this you must tear or cut the septa, but be careful not to tear or break the organs that perforate them.

Alimentary Canal.—This consists of a straight tube that runs the length of the body.

1. Immediately behind the mouth is a muscular, white organ, the *pharynx*. Through how many somites does this extend? It is connected with the body wall by numerous, radiating muscle fibers. What function do these fibers perform?

2. Behind the pharynx is the narrow and long *esophagus*. This runs posteriorly between lobed, light colored organs, the

seminal vesicles, which will be studied in connection with the reproductive organs. Press these aside and notice the small *calciferous glands*.

3. The esophagus leads to the *crop*, which lies just anterior to and in contact with the *gizzard*. In what somites are these organs placed? What is their shape? Do you understand the function of each?

4. Leaving the *gizzard* is the *stomach-intestine*, which runs through the remainder of the body, giving off lateral diverticula in each somite. Do you know its function?

Notice the relation of the septa to the alimentary canal.

Circulatory System.—1. Lying dorsal to the alimentary canal is the blood vessel that could be seen pulsating in the living specimen. In most cases this vessel is full of blood and appears brown.

2. Near the anterior end of the body large side branches, the *aortic arches*, are given off on either side and pass down around the esophagus. How many aortic arches do you find? In what somites are they placed?

3. Examine with a lens and see whether you find other vessels connected with the dorsal aorta. If you do, determine how they are placed. Do they appear like the aortic arches?

Make a drawing of the anterior end of the body, showing the points you have seen.

4. Gently press the *stomach-intestine* to one side and see if you find a blood vessel beneath it. Do the aortic arches join this? Other connections between blood vessels are too small to be studied in dissections, but you should understand from textbooks or lectures what they are, and the probable course of circulation.

Excretory System.—1. A pair of *nephridia* occurs in each somite, one nephridium on either side of the alimentary canal. (The first three or four somites are not provided with nephridia.) Each nephridium is a coiled tube, appearing to the unaided eye as a fluffy mass, that opens externally between the groups of setae, in the position already observed, and internally by a small opening near the *funnel*. The inner

opening is not in the somite in which the most of the tube lies, but in the somite anterior to it. That is, the nephridium that occupies the space in somite twenty opens externally on somite twenty, but internally perforates the septum directly anterior and opens into somite nineteen.

2. Remove a nephridium with your forceps and examine it with your microscope. Notice that it consists of a coiled tube of varying diameter. The funnel is not easy to find and is hard to remove. It may be found by removing the portion of the septum through which the nephridium passes and examining it with a microscope.

Draw the nephridia into your previous figure.

Cut the stomach-intestine behind the gizzard and pull it forward, *carefully separating the tissue from it as it is drawn forward*, so underlying organs will not be disturbed. In this way free the alimentary canal to the position of the pharynx.

You can now see the extent of the nephridia, and possibly see where they perforate the septa.

Reproductive System.—1. The *seminal vesicles* are large white bodies, united in the median line. They send three lobes on either side, that normally overlap the posterior part of the esophagus. In what somites do the lobes occur?

2. Carefully open the seminal vesicles near the median dorsal line and examine their contents microscopically.

3. With a pipette wash out the contents and notice the two pairs of *convoluted funnels*, the inner openings of the *vasa deferentia*. The *testes* are hard to find, as they are the same color as the coagulated mass that filled the seminal vesicles. They are attached to the septa just anterior to the funnels. The narrow tubes of the *vasa deferentia* may sometimes be seen leaving the seminal vesicles. They open externally on somite fifteen.

4. The *ovaries* are a pair of very small organs attached to the posterior surface of the septum that separates the twelfth from the thirteenth somite, near the midventral line. They may sometimes be found with a lens, but usually are

not visible otherwise. If possible, remove an ovary and examine it with a microscope to see its shape, and to find which portion has the most mature eggs. The *oviducts* open into the cavity of the thirteenth somite and externally through the ventral wall of the fourteenth somite, in line with the nephridia. They can seldom be seen in dissections.

5. Between the ninth and tenth and the tenth and eleventh somites, on the ventral side, are two pairs of white, rounded pouches, the *seminal receptacles*, that open externally but not internally. Understand their function. *Make a drawing of the reproductive system.*

Nervous System.—1. On the dorsal surface of the pharynx, near its anterior end, are the two *cerebral ganglia*. They lie on either side of the median line and are connected by a stout commissure. In what somite do they lie?

2. The remainder of the ganglia lie ventral to the alimentary canal. The first ventral ganglia are connected with the cerebral ganglia by connectives that pass around the sides of the pharynx. Adjacent ganglia of the ventral chain are united by connectives. The ganglia of each somite, and the cords that connect those of adjacent somites, are fused so that the original paired condition is not very apparent. How far does the ventral chain of ganglia extend? Where do nerves leave it?

Draw the nervous system into the figure that shows the reproductive system.

Notice the sacs that inclose the setae and indicate them in the above figure.

Examine prepared serial microscopic sections.¹

¹Small worms should be kept in a dish and fed on clean moistened filter paper, which they will eat readily, until the alimentary canal is free from grit, before they are preserved for sectioning. It is well to narcotize them by placing them in a small quantity of water and adding a little alcohol from time to time (never enough to make the worms squirm violently) until they cease to move. They may then be killed with sublimate acetic or other killing agent and treated in the usual manner.

1. The *cuticle* will probably be absent in most sections, in which case the outer covering will be the cellular *hypodermis* or *skin*. How many cells thick is this layer? Look for the gland cells that keep the living worm moist. Do you know how the cuticle is formed?

2. Beneath the hypodermis is the *circular muscle layer*, which is followed by the *longitudinal muscle layer*. The fibers of the latter are arranged in conspicuous bundles. Lining the body wall is the thin *peritoneal layer*. Do you understand the function of each of these layers? How is the body elongated?

3. Find the *setae* and determine where they are placed, how many are in each group, how many groups there are, how they pierce the body wall, and what muscles are attached to them. Why are setae not in every section?

4. The alimentary canal consists of a *lining epithelium*, followed by *connective tissue* and *muscle*, and, on its outer wall, *peritoneal cells*, which in the region of the stomach-intestine are large, very numerous, and are known as the *chlorogogue cells*.

5. Lying in the midventral line, beneath the alimentary canal and close to the body wall, is the ventral *nerve cord*. Examine its structure. See if any of the sections show nerves leaving it.

6. Dorsal to the alimentary canal is the *dorsal blood vessel*, on its ventral side is the *ventral blood vessel*, and ventral to the nerve cord the *subneural vessel*.

7. Find sections of the nephridia. Where are they placed? How do the sections appear? Why?

Other organs will appear in most of the sections. See if you can identify them.

Draw an enlarged cross section.

Darwin: The Formation of Vegetable Mold through the Action of Worms. Appleton and Co., 1888.

Harrington: The Calciferous Glands of the Earthworm, with Appendix on the Circulation. Jour. Morph., 15, 1899.

- Parker and Arkin: The Directive Influence of Light on the Earthworm, *Allolobophora foetida*. Am. Jour. Physiol., 4, 1901.
Sedgewick and Wilson: General Biology.
Wilson: The Embryology of the Earthworm. Jour. Morph., 3, 1889.

MACROBELLA (Leech)

If you have living specimens notice their methods of locomotion both in crawling around the dish and in swimming. A considerable volume of water is usually necessary to get the animals to swim.

Specimens may be killed with chloroform, narcotizing materials, or killing agents, such as weak chromic acid.

1. Observe the shape of the body. Which is the anterior end?

2. Do the dorsal and ventral surfaces differ in shape and color?

3. Note the *rings* which encircle the body. Determine their number. There is good evidence that these do not represent *somites*. The somites are fewer in number and each is composed of from one to five of these rings.

On the dorsal surface:

1. Near the anterior end is a series of ten small black spots arranged in the form of a horseshoe with the arched end forward. These are the *eyes*. They are arranged in pairs on the first, second, third, fifth, and eighth rings. These are believed to be on the first five somites. The first and second somites comprise a single ring each; the third includes the third and fourth rings; the fourth—the fifth, sixth, and seventh rings; the fifth—the eighth, ninth, and tenth rings.

2. Near the lateral edges notice the black pigment spots. The larger spots are situated mostly on a single ring, but may be extended on to others. Smaller pigment spots may occur on other rings along the same line. There is evidence that these larger spots mark the anterior rings of each somite wherever they occur. How many rings are commonly included in a somite?

3. On the midline between these pigment spots are white spots.

4. If the specimen is favorable you may find with a lens a series of *segmental sense organs* on the first ring of each somite. They are of unknown function, the eyes are supposed to be developed from certain of these organs, and they are landmarks in determining the morphological boundaries of somites.

5. On the median line in the groove that separates the most posterior ring from the sucker find the *anus*.

Make a drawing of the dorsal surface.

On the ventral surface:

1. The *mouth*, at the anterior end of the body, is bounded by the proboscis dorsally and anteriorly and by the fourth ring ventrally. Determine its shape. The mouth with the region around it forms the *anterior sucker*.

2. The *male reproductive aperture* is on the median ventral portion of the thirtieth ring. This is surrounded by a thickening.

3. The *female reproductive aperture* is on the median line at the posterior margin of the thirty-fifth ring. This is not marked by a distinct thickening.

4. Find four apertures with thickened margins arranged in the form of a square between the thirty-ninth and fortieth and the fortieth and forty-first rings. These are the apertures of the *mucous glands*.

5. Find the external apertures of the *nephridia* about half way between the median line and the margins, on the posterior edge of the last ring of a somite. The spots are light colored and elliptical. These are important landmarks in determining the boundaries of somites. Some of the anterior and posterior somites do not bear them.

6. Observe the size, shape, and structure of the *posterior sucker*.

Preserved specimens if very hard should be placed in water some time before dissection. Cut through the body wall along the mid-dorsal line, being careful not to cut underlying organs. Lift up the flap of integument and cut the connective tissue loose so it may be turned and pinned back under water. Work forward and backward from the middle of the back.

1. The digestive tract consists of a *buccal pouch*, *pharynx*, *stomach*, and *intestine*.

2. The *pharynx* is thick walled, elongated, and bound to the body wall by radiating muscle fibers. What is their function? The pharynx is muscular and is provided with bands of longitudinal and with circular muscles. What function is performed by these fibers?

3. The *stomach* joins the pharynx, is large, and has diverticula that nearly fills the body cavity. It occupies a considerable portion of the length of the body. How many pouches has it? Do the pouches bear any relation to the somites? The posterior end of the stomach narrows and projects into the intestine.

4. The intestine is enlarged a little at its anterior end and tapers posteriorly to a slight dilatation, sometimes called the colon. From this a short rectum runs to the *anus*.

Make a sketch of the digestive tract.

5. Open the digestive tract, wash it out, and examine with a lens. Especially study the pharynx and see how the sucking action is produced, and how the blood is forced into the stomach. The cavity of the mouth will be studied later.

Cut the digestive tract at the rectum and at about the middle of the pharynx and carefully dissect it loose.

Beneath it notice:

1. The *nerve cord*. Do the ganglia have any relation to somites? Find the lateral nerves leaving them. Trace the connectives between them. This will receive more attention later.

2. The *ventral blood vessel* and the lateral branches.

3. The *nephridia*, of which there are eighteen pairs.

4. The *male reproductive organs*. On the midventral line ventral to the nerve cord, and opposite the external opening already observed, is the globular muscular *penis*. Just anterior and laterally are a pair of white *seminal vesicles*. A short broad duct leads from each vesicle to the penis and a long narrow duct leads posteriorly to connect with the nine *testes* on the same side. The anterior pair of testes are four somites behind the penis and the eight others are in the succeeding somites. The testes are rounded white organs near the nerve cord.

5. The *female reproductive organs* which lie behind the penis. The *vagina* is a muscular sac on the median line opposite the opening already described. From the anterior dorsal side the *oviducts* run to the two small white *ovaries*, which are near the vagina, but a little anterior and lateral to it.

6. Four *mucous glands*, behind the vagina and opposite the openings on the ventral side.

Draw.

Nervous System.—The location of the ventral cord has already been noticed.

(a) How many ganglia?

(b) Are the ganglia of equal size? Do they all give off the same number of lateral nerves?

(c) Find the *supra-esophageal ganglia*, above the pharynx.

(d) Observe the *connectives* that join the supra-esophageal ganglia to the first pair of the ventral chain, the *infra-esophageal ganglia*.

(e) Find the nerves that run from the supra-esophageal ganglia to the eyes.

(f) There are three *stomogastric ganglia*, one on each side of the muscular lobes of the buccal pouch and one on

the median line. These are joined to the supra-esophageal ganglia.

Draw the nervous system.

Open the buccal cavity by cutting along one side and notice the three large buccal muscles, one dorsal and two lateral. These bear many minute denticles that may be seen, in the right position, with a compound microscope. It is by means of these that the leech makes its wound.

GEPHYREA

PHASCOLOSOMA

This form is commonly found buried in sand between tide marks. Specimens sometimes occur on the same flats with *Nereis*, but they are generally more abundant where the mud is of a slightly different, more sticky character.

1. Handle a living specimen and see how turgid it is. If you touch a specimen that has been allowed to expand in a dish of sea water you will find it is rather soft, but becomes turgid upon being touched. How is this accomplished?

2. Examine a living animal in a dish of sea water. The anterior portion of the body, the *introvert*, is drawn in, but may occasionally be extended, when it will be seen to bear at the anterior extremity a crescentic crown of *tentacles*, which partly surrounds the *mouth*.

3. Compare with a preserved specimen which has been killed with the *introvert* extended.

Make drawings showing the animal with the introvert protruded and with the introvert concealed.

4. The *anus* is located on a dorsal papilla, anterior to the middle of the body. Near the anus a pair of lateral papillae mark the position of the nephridiopores. The coiled intestine and brown *nephridial tubes* can probably be seen through the body wall. Note carefully the character of the integument. Is there any indication of spines, appendages, or eye spots?

For dissection use both fresh and preserved specimens.

With scissors open the worm from end to end near the mid-dorsal line, and pin the body wall out flat.

5. In opening the fresh worm, note the pinkish *coelomic fluid* which fills the *coelom*. Examine a drop under the microscope. What functions has this fluid to perform?

Alimentary Canal.—Trace the alimentary canal (stomach-intestine) from mouth to anus. Do any digestive glands open into it at any point? Note the mesenteric thread which runs through the axis of the intestine spiral. Where is it attached? Does it seem to be contractile in the fresh worm?

Muscular System.—Note the silvery white *longitudinal muscles* composing the inner layer of the body wall. Are they arranged in distinct bands or in a continuous sheet? Remove some of these muscles carefully to expose the layer of *circular muscles*. How many *retractor muscles of the introvert* are there? How is the mechanism of protrusion of the introvert to be explained?

Circulatory System.—This system is very difficult to observe. Dorsal and ventral blood sinuses are present, and communicate anteriorly by a circular sinus. A blood sinus, purplish red in living specimens, occurs, as an irregular tube, along the anterior portion of the esophagus and intestine.

Excretory System.—Find a pair of brown *nephridia*, an inch or more in length. Cut off a nephridium (from the fresh worm) as close as possible to the body wall, and examine it under a microscope. Near the cut (the attached) end find the coelomic opening or nephrostome. Is it ciliated?

Reproductive System.—The sexes are separate. Oogonia and spermatogonia are detached from the coelomic epithelium, at the points where the ventral retractor muscles are attached to the body wall. These cells become mature while floating in the coelomic fluid. They pass out through the nephridia, which function as *gonoducts*.

Nervous System.—Does the ventral nerve cord seem to be double? Is it ganglionated? Does it give off lateral nerve branches? Trace the *circumesophageal connectives* to the

supra-esophageal ganglion. The ganglion is small and situated behind the crown of tentacles, to which sensory nerves extend. Does any system of organs show segmentation?

Make a drawing to show the internal anatomy.

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MOLLUSCA

Unsegmented. Usually provided with a calcareous protecting shell and a ventral foot.

CLASS 1. Pelecypoda. (Lamellibranchiata.)

Bivalve shell. Gills adapted for gathering food as well as for respiration. Foot usually adapted for burrowing. No hard mouth parts.

Order 1. Protobranchia.

Gills composed of a series of transverse plates. Foot apparently split at the end. Two adductor muscles, posterior frequently the smaller. (Nucula, Yoldia.)

Order 2. Filibranchia.

Gills lamelliform. Filaments united by modified cilia. Anterior adductor muscle, frequently greatly reduced. (Mytilus, Modiolus.)

Order 3. Pseudolamellibranchia.

Gills lamelliform. Interfilamentar junctions usually not very extensive, may be either ciliary or vascular. Only one adductor muscle. (Pecten, Ostrea.)

Order 4. Eulamellibranchia.

Gills lamelliform. Interfilamentar junctions extensive and vascular. Adductor muscles of nearly equal size. (Venus, Unio, Mya.)

Order 5. Septibranchia.

Gills reduced to a horizontal partition. Two adductor muscles. Deep sea forms. (Silenia, Cuspidaria.)

CLASS 2. Amphineura.

Bilaterally symmetrical, elongated. Nervous system not concentrated. Radula sometimes present. Shell, when present, composed of eight transverse pieces.

Order 1. Placophora.

Dorsal shell, composed of eight transverse pieces. Foot broad. Gills simple, lateral. (Chiton, Chaetopleura, Trachydermon.)

Order 2. Aplacophora.

Body elongated, covered by a mantle. Adult without shell but with spicules. No true foot. Gills posterior. (Neomenia, Dondersia.)

CLASS 3. Gastropoda.

Body unsymmetrical, usually covered by a spiral shell. Foot usually flattened and adapted for creeping. Radula usually present.

Subclass 1. Streptoneura.

Nervous system twisted into the form of a figure 8. Sexes separate.

Order 1. Aspidobranchia.

Nervous system not concentrated. Gills usually present and paired. Auricles paired. (Acmaea, Patella, Haliotus.)

Order 2. Pectinibranchia.

Nervous system somewhat concentrated. Single gill. Single auricle. (Buccinum, Busycon, Crepidula.)

Subclass 2. Euthyneura.

Nervous system not twisted into the form of a figure 8. Hermaphroditic.

Order 1. Opisthobranchia.

Aquatic respiration. Shell when present rather delicate. (Bulla, Aeolis.)

Order 2. Pulmonata.

Air breathers. Live on land or in fresh water. Aperture to mantle cavity narrow and contractile. (Limax, Limnaea, Helix.)

CLASS 4. Scaphopoda.

Bilaterally symmetrical. Shell tubular, elongated dorsoventrally and open at both ends. Foot conical. (Dentalium.)

CLASS 5. Cephalopoda.

Bilaterally symmetrical. Shell chambered or reduced and internal. Distinct head with arms bearing suckers.

Subclass 1. Dibranchiata.

Arms forming a circlet around the mouth. Funnel a complete tube. Shell usually internal. Two gills.

Order 1. Decapoda.

Ten arms, two of which are elongated, suckers on stalks. (Loligo, Sepia, Spirula.)

Order 2. Octopoda.

Eight arms, suckers sessile. (Octopus, Argonauta.)

Subclass 2. Tetrabranchiata.

Tentacles numerous. External chambered shell. Funnel open along one side. Only one living genus. Four gills. (Nautilus.)

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PELECYPODA

VENUS MERCENARIA (Quahog)¹

Animals of this species wander around over muddy bottoms in rather shallow water, keeping the siphon end, at least, above the surface of the mud. If possible, you should find specimens in their native places and watch their movements. Specimens placed in water and left undisturbed for some hours are likely to protrude the siphons, and the foot may be protruded in some cases.² Allow powdered carmine to settle slowly past the openings of the siphons and determine the direction of the current of water for each. Touch

¹ Points in which the fresh-water mussel differ have been noted, so the directions may be used for that form.

² Other species of lamellibranchs are more satisfactory than Venus for studying movements, as they expand quickly after being disturbed. Among the common ones that may be mentioned are Ensis, Cumingia, Yoldia, and Mytilus.

portions of the animal and find what parts are most sensitive.

Shell.—Note its general shape, and that it is composed of two symmetrical parts, the valves. For each valve notice:

1. The outline.

2. A swelling, the *umbo*, ending in a point, the *beak*, from which growth has proceeded.

3. The *lines of growth*. Were the valve cut off along one of these lines, the shape would not be changed. Why are the lines arranged in this manner? How were they formed?

The two valves are joined by the *ligament*. The margin bearing the ligament is dorsal, and that toward which the beaks point is anterior. Which valve is right and which is left?

Draw a valve, showing the points observed.

Pry the two valves apart and insert a knife blade between the mantle and one valve of the shell. Notice that the lobes of the mantle are loosely attached to the shell along their margins, and more firmly attached a half inch or more from the margins.

Separate the mantle from one valve, and cut the adductors where they are attached to this valve. Why do the valves gape now? Press them together, and notice that they stay closed only while held. Remove a valve and study its interior.

1. Find the large scars where the anterior and posterior *adductor muscles* were attached.

2. Find smaller scars where the anterior and posterior *foot muscles* were attached. The anterior scar is dorsal and a little posterior to the corresponding adductor muscle scar. (Not the position for *Unio*.) The posterior scar connects with the dorsal portion of the corresponding adductor muscle scar.

3. The ventral borders of the adductor muscle scars are connected by a distinct line, the *pallial line*. What forms it? The posterior end of this line is indented to form the *pallial*

sinus. (Not true for *Unio*.) What is the meaning of this *sinus*?

4. Along the dorsal margin of the valve notice prominences, the *teeth*. There are two kinds of teeth. The anterior, cardinal, consist of short elevations. The posterior, lateral, are not very prominent, but are comparatively long and extend along the dorsal margin. Notice that the teeth on the two valves interlock. What is their function?

Draw a valve as seen from the inside.

5. By examining the inside of a shell of *Unio* or *Mytilus* near its margin, the typical three layers of which it is composed can be seen. How is it possible for all three layers to be secreted by the mantle, which lines the inside of the shell? Can you find any reason for more than one layer?

Mantle.—This consists of a dorsal covering and two lateral lobes (one of which is applied to the inner surface of each valve of the shell).

1. The free border of each lobe is thickened, and contains muscles that were attached to the shell along the pallial line. What function do these muscles perform?

2. The posterior portions of the lobes of the mantle are thickened and united to each other so as to form two tubes (in *Unio* the ventral tube is formed by contact only), the *siphons*, through which water passes into and out of the shell.

3. See how the muscles of the siphons are arranged and attached. Does the attachment bear any relation to the pallial sinus in *Venus*?

Visceral Mass and Foot.—These portions form the large median mass. The viscera are contained in the dorsal portion.

1. The ventral portion is hard and muscular, and forms the *foot*.

2. Besides the crossing muscle fibers of which the foot is largely composed, it is supplied with two pairs of muscles that are attached to the shell. The cut ends of these muscles, the anterior and the posterior *foot muscles*, may be seen pro-

truding through the lobe of the mantle.¹ They correspond in position to the scars on the shell.

Do you understand by what means the foot is protruded?

Gills.—These consist of two pairs of thin, striated, somewhat brownish organs, a pair lying on each side of the visceral mass, between it and the lobes of the mantle.

1. Each gill extends from the wall that separates the two siphons, anteriorly and dorsally to a point nearly opposite the beaks of the shell, and is attached by its dorsal margin only.

2. Each outer gill is attached along its dorsal border to the corresponding mantle lobe on the outer side. The inner gills, besides being attached to the dorsal margins of the outer gills, are on their inner sides attached to each other and to the visceral mass. (For some distance the inner side of the inner gill lies against the visceral mass, but is not attached to it.)

By this arrangement the space between the lobes of the mantle, which is known as the *mantle chamber*, is divided into a ventral and a dorsal portion. The ventral portion is much the larger, communicates with the ventral siphon, and because the gills hang into it, it is known as the *branchial chamber*. The dorsal chamber is known as the *cloacal chamber*. The siphons are frequently referred to by names corresponding to the chambers with which they communicate. The minute structure of the gills will be studied later.

3. Place a little powdered carmine on the gill of a specimen that is submerged in sea water and see what becomes of it.

Labial Palps.—These consist of a pair of rather small triangular flaps on each side of the visceral mass.

1. The two outer palps are united above the mouth, which is situated just posterior to the dorsal border of the anterior

¹The anterior foot muscles are sometimes called protractors, and the posterior foot muscles retractors. Both are actually used to retract the foot. The greatly retracted foot may be pulled slightly forward by the anterior muscles, but the mechanism of protruding the foot is very different.

adductor muscle, and form a small fold that corresponds in position to an upper lip.

2. The two inner palps likewise unite to form a fold corresponding in position to an under lip.

Make a drawing showing the arrangement of the soft parts.

Structure of a Gill.—Cut off a piece of the edge of a gill, put it on a slide with a drop of sea water, and examine with a low power of the microscope.

1. Notice the *cilia* on the edge and surface of the gill.

2. The surface is marked by a series of parallel ridges, the *filaments*, with grooves between them.¹

The filaments are joined together laterally by series of bridges (you will see them later), the *interfilamentar junctions*, with the pores, *inhalant ostia*, between them. Each side of the gill is thus composed of a single layer of united parallel filaments, which together form what is known as a *lamella*. Each gill is composed of two such lamellae, one on each side. These lamellae are united at intervals by bridges that run the whole width of the gill (dorsal to ventral), parallel to the filaments, and at right angles to the interfilamentar junctions. These are called the *interlamellar junctions*. By means of the interlamellar junctions, the space between the two lamellae is divided into a series of *water tubes*. The openings of these tubes into the cloacal chamber may easily be seen after the cloacal chamber has been cut open.

3. Separate a small piece of one lamella from the other. This can be done most readily by catching the free dorsal border of the inner lamella of an inner gill with the forceps, and either tearing off a piece or freeing it by cutting with scissors while it is being pulled with the forceps. Mount this piece, with the outer surface up, under a cover glass in a drop of sea water and observe, with a lower power, the following:

¹The general surface features are especially easily seen in *Pecten*, where the interfilamentar junctions are small and well marked, and the inhalant ostia are correspondingly large and distinct.

(a) Filaments. Run the width of the gill.

(b) Interfilamentar junctions. Form bridges connecting the filaments.

(c) Inhalant ostia. The openings bounded by filaments and interfilamentar junctions.

(d) The position of the torn interlamellar junctions, appearing as indefinite dark stripes running in the same direction as the filaments.

With a high power observe:

(a) The chitinous rods which lie inside of the filaments and stiffen them.

(b) The cilia on the sides of the filaments. These are of two kinds: (1) surface cilia that form currents of water along the filaments. These will be seen waving back and forth, or if still moving rapidly, apparently moving along the sides of the filaments. (2) Deeper cilia that are down between the filaments and can be seen by changing the focus. These move at right angles to the others, and apparently become longer and shorter. Explain.

Draw a surface view of a piece of a lamella.

Examine a piece of the gill of *Mytilus* for the above structures. In this form the interfilamentar junctions are small and composed of modified cilia only, and the inhalant ostia are correspondingly large. By pressing the gill the interfilamentar junctions can be pulled apart.

Study prepared sections of the gill of Venus and notice:

1. Lamellae.
2. Interlamellar junctions.
3. Water tubes.
4. Filaments.
5. Interfilamentar junctions.
6. Cilia.
7. Inhalant ostia.
8. Blood spaces.
9. Chitinous rods.

Draw.

Understand the direction taken by water in passing from the branchial to the cloacal siphon. What makes the water move?

Labial Palps.—The positions of these organs have already been noted.

1. Examine a piece of the palp with a microscope, and notice that the side turned toward the adjacent palp is thrown into ridges and grooves, and is densely ciliated.

2. The space between each outer and inner palp is continuous with the "corners" of the mouth. The free margins come close to the borders of the gills and normally inclose them.

Understand how food is gathered and carried to the mouth.

Circulatory System.—The pericardium, in which the heart lies, is a somewhat triangular space that appears clear, through the mantle. It lies just anterior to the posterior adductor muscle. Open the pericardium, and notice the beating of the heart. The heart consists of three parts:

1. A central portion, the *ventricle*, which surrounds the intestine and gives rise to a blood vessel at each end.

2. Two triangular portions, the *auricles*, which receive blood from the gills and open into the sides of the ventricle.

Notice the sequence and power of the contractions.

Just posterior to the pericardium is an enlarged portion of the alimentary canal. This has no relation to the heart, for which it is sometimes mistaken.

Excretory and Genital Systems.—The excretory system consists of a pair of dark colored glandular organs that lie beneath the pericardium. Each communicates with the pericardium by a small opening that is not easy to demonstrate in dissections, and with the cloacal chamber by another small opening.

By turning the two gills (of Venus) dorsally a very small papilla may be seen, just beneath the free border of the inner gill, lying in the cloacal chamber. On the tip of this papilla

are two openings. The inner one is the opening of the excretory organ. The outer one is the opening of the genital duct.

The genital glands are light colored organs that, during the breeding season, extend through the principal part of the visceral mass. Neither the genital nor the excretory systems can be profitably studied in a general dissection of this form. In *Unio* the excretory organs are more satisfactory for study. Do you understand the supposed significance of their connection with the pericardium?

Nervous System.—1. Carefully remove the body wall along the side of the esophagus and notice the *cerebral ganglion* of the corresponding side. This is a rounded, slightly yellow organ, about the size of a pin head, lying just posterior to the dorsal border of the anterior adductor muscle. (In *Unio* it is more ventral in position.) The cerebral ganglia of the two sides are united by a commissure that passes anterior to the esophagus. Two connectives leave each cerebral ganglion. One passes posteriorly to join the visceral ganglion of the corresponding side. The other passes into the foot to join the pedal ganglion of the corresponding side.

2. Cut the united lamellae of the inner gills ventral to the posterior adductor muscle. This will expose the *visceral ganglia*. They are pear-shaped bodies lying just beneath the posterior adductor muscle, connected with each other by a short commissure, and connected with the cerebral ganglia by connectives that may be traced a short distance forward without dissection. A large nerve leaves the posterior end of each ganglion and supplies the posterior end of the corresponding lobe of the mantle. Smaller nerves go to the posterior adductor muscle and gills.

3. With a razor or sharp scalpel make a median sagittal section of the foot, extending it some distance into the visceral mass. This will expose the *pedal ganglia*, that lie just anterior to a loop of the intestine, and dorsal to the muscular

portion of the foot. The pedal ganglia are connected with each other by a broad commissure and with the cerebral ganglia by connectives.

By careful dissection it is possible to trace the connectives and many of the nerves. The razor clam, *Ensis*, is especially favorable for dissections of the nervous system, as the ganglia, connectives, and many important nerves lie very near the surface and can be seen without cutting the tissues above them.

Make a drawing, indicating the positions of the ganglia.

Digestive System.—This may be traced by following a guarded bristle that has been inserted into the mouth of a specimen that has been killed in hot water (not boiling), or by very carefully picking off the tissue from one side. The intestine where it penetrates the heart has already been seen, and may easily be followed to the anus.

The general arrangement of the alimentary canal is well shown by a median sagittal section of a preserved specimen.

The brownish digestive gland, commonly called the "liver," will be seen surrounding a portion of the stomach.

The enlargement on the intestine in the posterior portion of the pericardium is of unknown function. In some forms a special diverticulum from the stomach bears a transparent cylindrical rod, the *crystalline style*. This can easily be found in *Mya*. Probably all lamellibranchs have similar structures more or less well developed, but many do not have special pouches for their formation.

Draw the alimentary canal. (This may be included with your sketch of the nervous system.)

Cut a preserved specimen into transverse sections about a quarter of an inch thick, and place the sections in their proper order and position. (They should be placed in a dissecting pan in very little water.)

Study these sections for the arrangement of organs. The relation of the gills to the branchial and the cloacal chambers should be understood.

Make drawings of sections that pass through the heart and through the posterior adductor muscle.

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If time permits, it will be desirable to become acquainted with some of the structures of theoretic importance and with some of the adaptations of pelecypods for the lives they live. For this purpose a few forms have been selected, and directions for the study of the particular parts in question are given.

YOLDIA LIMATULA

This form belongs to the order Protobranchia, and is supposed to be one of the most primitive of living pelecypods. It lives in soft mud, such as is found in quiet coves and bays. (It is abundant in the Eel Pond at Woods Hole.) Although it burrows in the mud, it lives near the surface, and frequently has the posterior end above the mud.

1. Place a specimen in a dish of sea water, and notice the movements and shape of the *foot*. See if the movements are always alike. What would happen if such movements were made by a specimen lying on soft mud? Place a specimen on mud and watch the results.

2. Leave a specimen in an aquarium in which two inches of bottom mud has been placed, and see if it is feeding in the morning.

3. Place a young, transparent specimen in a watch glass

of sea water and study the parts. The foot has already been observed. Its motions will probably be seen again here. It has been considered a creeping organ. Do you find evidence that confirms or opposes the view? With a lower power of the microscope study:

(a) *Palps*. These are very large. The outer palp on each side is provided with a long appendage that may be protruded from between the valves of the shell. This is the feeding appendage.

(b) *Gills*. These are quite small and are composed of parallel plates. They are situated behind the palps, are attached dorsally by muscular membranes to the body wall, and posteriorly to the wall that separates the siphons. They illustrate what is supposed to be the most primitive type of lamellibranch gill. Watch their movements and see if you can determine how they cause the jets of water to leave the cloacal siphon. What reason is there for forming such strong jets of water?

(c) *Heart and Ganglia*. Nicely shown in such a specimen.

4. Remove one of the shell valves of an adult specimen and examine the organs. An elongated *sense tentacle* occurs on one or the other side of the base of the branchial siphon, between the wall of the siphon and the corresponding mantle lobe.

A drawing of the organs will prove profitable.

Drew: The Anatomy, Habits, and Embryology of *Yoldia limatula*. Mem. Biol. Lab. Johns Hopkins Univ., 4, 1899.

—: The Life-History of *Nucula delphinodonta*. Quart. Jour. Mic. Sci., 44, 1901.

Mitsukuri: On the Structure and Significance of some Aberrant Forms of Lamellibranchiate Gills. Quart. Jour. Mic. Sci., 21, 1881.

MYTILUS OR MODIOLUS (Mussels)

These animals belong to the order Filibranchia, and show comparatively simple gills, as well as interesting modifications for their manner of living. They live attached to

stones, shells, piles, or even to sand grains, sometimes in moderately deep water, but frequently between low- and high-tide marks. The two forms may easily be distinguished by the positions of their beaks. The beaks of *Mytilus* form the anterior end of the shell. Those of *Modiolus* are placed a short distance posteriorly. You should visit "mussel beds," and see where and how they are attached and on what they must depend for food.

1. Place young specimens in dishes of sea water and see if they will attach themselves by their *byssal threads*. (They will generally require some hours.) If you can get them to attach to slides, the attachment may be microscopically examined.

2. Test the strength of the byssal threads of a rather old specimen. Are they elastic? How would elasticity aid the animal in remaining attached?

3. Leave specimens in sea water for some hours, and see if they change their positions.

4. Notice the margins of the *mantle*. Are they fused? Why are siphons not necessary? See if you can find where water passes in and out.

5. Wedge the valves of a specimen apart, cut the adductor muscles (take note of their relative size), and examine the arrangement of organs.

6. Find where the *byssal threads* are attached. Where secreted?

7. Notice the relatively small *foot*, and compare it with the powerful foot muscles. Why are such powerful foot muscles necessary? How does the foot function in attaching byssal threads?

8. See how the *gills* are attached to the body. The filaments of the gills of this form are very loosely attached to each other by modified clumps of cilia, that represent the interfilamentar junctions. Cut off a piece of a gill, mount it in sea water under a cover, and examine with low and high powers. Find places where filaments are attached by the

bunches of cilia. Find places where the cilia have pulled apart. Notice the size and shape of the ostia and find the two kinds of movable cilia.

9. This form usually shows the way food is gathered especially well. Place powdered carmine on the surface of a gill and see what becomes of it.

10. Notice the thickened condition of the mantle. The *gonads* extend into it, and the thickening is due to sexual products.

Drawings of the arrangement of the organs, and especially of the microscopic structure of the gill, will prove profitable.

Meisenheimer: Entwicklungsgeschichte von *Dreissensia polymorpha*.
Zeit. f. wiss. Zool., 69, 1900.

PECTEN GIBBUS BOREALIS (Scallop)

This species belongs in the order Pseudolamellibranchia and lives on muddy or sandy bottoms, generally where the water is from a few inches to several fathoms deep. It has the power of swimming well developed. At rest on the bottom it always lies on the right valve of the shell.

1. Do the valves of the shell differ in color or shape?

2. On each side of the beak of each valve is a flattened projection frequently called an "ear" or "wing." The posterior wing slopes backward; the anterior, especially the one on the right valve, is somewhat separated from the body of the shell by a notch.

3. Place specimens in dishes of sea water, and when they have opened their shells notice:

(a) *Mantle*. See if it is sensitive. How far can it be drawn back into the shell? What muscles are used in withdrawing it? Why is it necessary to withdraw it? What is peculiar about the shape of the margin? What reason is there for this structure?

(b) The pallial *eyes*, bright specks along the margins of the mantle. Are they placed in any order?

(c) The arrangement of the *tentacles* on the margins of

the mantle. Why should sense organs be placed in this position?

4. Specimens in aquaria will often swim. If possible, notice how this is accomplished.

Wedge the valves of a specimen apart and notice the single large *adductor muscle*. What need has Pecten for such a large adductor? Notice the *foot* and compare it with the foot of Venus.

How are the *gills* attached to the body? What would be the effect on the gills if they were attached to the mantle and to each other, as in most forms, when water is ejected in swimming?

Examine the structure of the gill and notice how much larger the interfilamentar junctions are near the interlamellar junctions than elsewhere. Near the margins of the gills the junctions are frequently simple bunches of cilia, as in *Mytilus*. Observe the muscular movements of the gills. The gills of this form are muscular and can be drawn together when the animal swims.

Drawings to show the arrangement of the organs and the structure of the gill are desirable.

Belding: The Scallop Fishery of Massachusetts. Mass. Fish and Game Com., 1910.

Drew: The Habits, Anatomy, and Embryology of the Giant Scallop, *Pecten tenuicostatus*. Univ. of Maine Stud., No. 6, 1906.

OSTREA VIRGINICA (Oyster)

This also belongs to the order Pseudolamellibranchia. It forms a good example of adaptation for a sedentary life. It occurs, fastened to rocks and other shells, in positions where it is much exposed to attacks of the enemies of lamellibranchs.

1. Notice the difference in the size and shape of the valves. Why is this desirable?

2. Notice the thickness of the valves and the completeness with which they come in contact when the shell is closed. Would such a heavy or tight-closing shell be satisfactory for the scallop or the razor-shell clam?

3. Open the shell by breaking the edge, inserting a knife blade through the opening, and cutting the adductor muscle away from the flattened right valve of the shell and notice the single adductor, extensive gills, and the absence of a foot. The larval oyster has a foot, but this is lost early in life.

Brooks: The Oyster.

Grave: Maryland Shell-Fish Commission, 4, Rep., 1912.

Horst: On the Development of the European Oyster (*Ostrea edulis*, L.). Quart. Jour. Mic. Sci., 22, 1882.

Nelson: The Attachment of Oyster Larvæ. Biol. Bull., 46, 1924.

SOLEMYA

This form, a member of the order Protobranchia, with much the same structure as *Yoldia*, shows an interesting method of swimming that should be compared with *Pecten*, and with the jets of water formed by *Mya*. Specimens may be dug at low tide from mud or sandy mud, placed in a dish of sea water, and observed. Does the posterior opening in the mantle chamber correspond to typical siphons? See if you can find how the animal swims. Is the movement continuous or jerky? Does the animal move forward or backward? Is the foot active? Are jets of water thrown from the shell? Is the animal adapted to forming jets of water?

Examine a specimen that has the valves closely drawn together and see how rounded the margins appear. Examine a shell from which the animal has been removed by maceration and see the relation of the shell cuticle and the calcareous portion of the shell. What becomes of the marginal cuticle when the shell is closed? Can this have anything to do with throwing jets of water from the shell?

Drew: Locomotion in *Solenomya* and its Relatives. Anat. Anz., 17, 1900.

Stempell: Zur Anatomie von *Solemya togata*. Zool. Jahrb., 13, 1899.

MYA ARENARIA (Long Clam)

This animal belongs to the order Eulamellibranchia, as does *Venus*, and is introduced because of adaptation for its manner of living. It lives buried in the mud, in which as an

adult it remains stationary. You should find a "clam bed" along the shore, and after noticing the pits in the surface of the mud, and the jets of water that are sometimes thrown from the pits, dig down and see how the animals are placed. If the water is calm, find, at the surface of the mud, the openings of the siphons of specimens that are still covered by water. You will need to walk very carefully so as to disturb mud and water as little as possible, as the siphons are otherwise closed and withdrawn.

1. Why does this animal not need a shell that is as heavy and closes as tightly as that of *Venus*? Does it show the same points regarding the valves (umbos, beaks, lines of growth, and ligament)? Later, when the shell is removed, the large cartilage pit on the left valve will be seen.

2. The ventral borders of the mantle lobes are united except near the anterior end, where there is a space through which the foot may be seen.

3. The siphons are large and muscular and may be retracted, as in the specimen that you are handling, or may be greatly extended, as may sometimes be seen in aquarium specimens. Why does *Mya* need larger siphons than *Venus* does?

4. Pick up a specimen that has the siphons extended and notice the powerful ejection of water. Is it ejected from one or both openings? How is this accomplished? Of what service can such jets be to the animal? Why are powerful jets of this nature of more service to *Mya* than to *Venus*?

Notice the cartilage in the cartilage pit on the left valve. What function does it perform? Why is there no need for a large and powerful foot? It is much easier to trace the alimentary canal and the ganglion connectives in this form than in *Venus*.

Belding: The Mollusk Fisheries of Massachusetts. Mass. Fish and Game Com., 1909.

Kellogg: Life-History of the Common Clam, *Mya arenaria*. Bull. U. S. Fish Com., 1899.

Mead and Barnes: Observations on the Soft-shell Clam. Rhode Island Com. Inland Fish., 20 to 24, 1900 to 1904.

Yonge: Studies on the Comparative Physiology of Digestion. I. The Mechanism of Feeding, Digestion, and Assimilation of the Lamelli-branch Mya. Brit. Jour. Exp. Biol., 1, 1923.

ENSIS DIRECTUS (Razor-shell Clam)

This species is another representative of the order Eulamellibranchia and is introduced because of its adaptation for a burrowing habit, and because of the great ease with which its nervous system can be studied. Individuals are not uncommon on mud or sand flats from which the water flows at low tide. They may sometimes be seen protruding above the surface of the mud, but are hard to approach because of their great sensitiveness. Upon being disturbed they quickly disappear beneath the surface of the mud. These animals are sometimes used for food. They are frequently collected in Japan by placing a little common salt in the opening of the burrows. Within a few seconds an animal so treated energetically backs out of its burrow.

1. Notice the shape of the shell, the way it gapes at both ends, and the way the lobes of the mantle are fused.

2. With a pencil point or seeker stroke the tentacles around the siphon openings, while the animal is being held anterior end downward. This will cause it to perform the burrowing movements. Study the movements carefully and see what the effects would be were they performed in mud. Thrust the anterior end of the shell in mud and watch the result of the movements.

3. Water is ejected by the sides of the foot to aid in burrowing or to enable the animal to swim, but observations on its method of ejecting it are not easily made, and are sure to take much time. Notice the way the anterior margins of the lobes of the mantle scrape mud from the foot when the foot is being withdrawn.

4. With a scalpel separate the united margins of the mantle throughout their length. Slowly pry the valves apart, lift up the free end of the foot and pull it posteriorly.

The *cerebral ganglia* are plainly visible without further cutting. They lie just posterior to the anterior adductor muscle and in front of the mouth, and are widely separated. They are connected by a narrow commissure, and each gives rise to a cerebrovisceral and a cerebropedal connective and to a number of nerves. The nerves that supply the anterior part of the mantle and the anterior adductor muscles are especially easily seen.

5. If the specimen is one that is nearly or quite dead, it is easy to dissect out the *cerebropedal connectives* and the *pedal ganglia*, which are not far from the base of the foot and not deeply embedded.

6. Allow the foot to return to its normal position and cut along the line of union of the inner gills. Without further cutting the *visceral ganglia* may be studied. Their connectives may be followed easily as far forward as the palps. The posterior pallial and the branchial nerves may also be seen.

A drawing of the nervous system should be made.

Drew: The Habits and Movements of the Razor-shell Clam, *Ensis directus*. Biol. Bull., 12, 1907.

—: The Physiology of the Nervous System of the Razor-shell Clam, *Ensis directus*. Jour. Exp. Zool., 5, 1908.

CUMINGIA TELLINOIDES

In separate bowls of sea water place several *Cumingia* which have been kept in moist sand for a few hours. Watch the shedding of eggs (pinkish) or sperm (whitish) which ordinarily will require at least thirty minutes. Transfer 30 to 50 eggs to a Syracuse dish containing clean sea water. Then fertilize them by adding not more than a very small drop of sperm suspension. Observe the developing eggs from time to time, noting when the following stages occur: (1) Blastula. Is this ciliated? (2) Gastrula. (3) Trochophore larva. (4) Veliger larva.

AMPHINEURA

CHAETOPLEURA (Chiton)

It will be profitable to study only external features, unless time is to be had for cutting and studying sections, as the species is small and difficult to dissect. Its apparently generalized structure, and its adaptations, make it desirable for students to understand from descriptions and figures the main features of its anatomy.

1. Examine specimens that are attached to stones and shells and see how nicely they adapt their shapes to the shapes of the objects to which they are attached. How is this possible?

2. Remove a specimen and *quickly* transfer it to a clean glass slide, applying its ventral side to the glass. Put your finger in its back and prevent it from curling for a minute. It will then generally remain attached to the slide and may be studied from both sides.

3. How many plates are there? What is the shape of each? Do they apparently join edge to edge or do they overlap? Do the plates extend clear to the margin of the animal? What reason is there for having plates instead of a solid dorsal shell?

4. Notice the thickened margin of the animal, and see that dorsally it bears spicules, while ventrally it is smooth and is applied closely to the slide.

5. Notice the flattened elliptical *foot*. Do you understand how the animal creeps and adheres?

6. In front of the foot is the *head fold* in which the *mouth* can be seen.

7. In the furrow bordering the foot are the *gills*.

8. Remove the animal from the slide and see how it curls up. Try to unroll it. Explain.

9. If you care to see the *radula*, the organ that especially indicates affinity to the Gastropoda, it can be pulled out by grasping just behind the mouth with pointed forceps and

pulling forward. When removed it may be mounted on a slide with water and studied with the microscope.

Haller: Die Organisation der Chitonen der Adria. Arb. Zool. Inst. Wien, 4, 1882; 5, 1884.

Heath: The Development of Ischnochiton. Zool. Jahrb., 12 (Anat.), 1899.

GASTROPODA

A majority of the Gastropoda have the body protected by a spirally wound shell, and crawl around by means of a flattened muscular foot which forms the ventral portion of the body.

Examine specimens of such active forms as *Alectrion obsoleta*, *A. trivittata*, and *Melampus*, and notice:

1. The relation of the animal to its shell when retracted and when extended.

2. Movements. Any cilia on the foot? Any rhythmic waves passing from end to end? What is the mechanism of foot locomotion?

3. The movements of the tentacles and proboscis. What do the movements accomplish?

Touch a specimen and see what positions the parts take when it retracts into the shell. If the animal has an operculum see where it is borne, and how it fits into the aperture of the shell.

BUSYCON (FULGUR, SYCOTYPUS) (Whelk)

This large gastropod lives in comparatively shallow water and depends largely on other Mollusca for its food. Examine a retracted specimen and see how the shell is closed by a horny lid, the *operculum*. Examine expanded specimens in aquaria, and see where the operculum is placed. What position must the animal assume in the shell to bring the operculum in position?

Shell.—A somewhat conical tube, spirally wound, somewhat like a spiral stairway. Observe the following parts:

1. The *apex*, forming the closed end of the tube.

2. The *spire*. How many whorls are there? Do they

differ in number in different specimens? In what direction are the whorls wound? (Hold the apex toward you in determining this point.) Examine old and young specimens and see if there is evidence that the apex is worn off.

3. The *body whorl*. The one that opens to the outside.

4. The *columella*. The axis around which the whorls are wound. This is best studied in a broken shell.

5. The *aperture*, which is bounded by the *inner lip* on the columellar side and by the *outer lip* along the free edge.

6. The *siphonal canal*, which forms the spoutlike prolongation of the shell.

7. The *lines of growth*. What do they represent? Do they show evidence of injuries that have befallen the shell during the life of the individual?

8. The three layers of which the shell is composed. In a broken shell notice: (a) the *cuticle*, worn away from the greater portion of the shell; (b) the *nacre*, smooth and lining the inner surface of shell; (c) the *middle layer*. How can three layers be secreted by the mantle?

Soft Parts.—Examine an animal that has been removed from its shell and killed while more or less expanded¹ and see in what position it was placed in the shell. Compare the number of whorls made by it to the number in the shell. Understand which is right and which is left for the coiled part of the body. Which side was applied to the columella? In determining the position of organs, constantly keep the sides in mind.

¹This can be accomplished by breaking the shell away with the blade of a hatchet, and when enough of the shell has been removed, loosening the muscle from the columella with the thumb, and then pulling and twisting the animal out. When free from the shell place the animal in sea water to which has been added about one tenth its volume of alcohol and a little turpentine (about 10 cc. of turpentine to each 100 cc. of alcohol) and leave for several hours. An animal treated in this way will usually die with its proboscis extended. For the method we are indebted to Mr. Geo. M. Gray, Curator at the Marine Biological Laboratory, Woods Hole, Mass.

Before beginning the dissection, note the following parts:

1. The *visceral dome*. The portion that extended into the spire of the shell.

2. The *mantle*, which is thin and closely applied to the visceral dome, and raised to form a thickened collar that extends entirely around the body along a line that corresponds to the aperture of the shell.

3. The *siphon*, which is a spoutlike prolongation of the collar. Into what portion of the shell does it fit?

4. The *mantle chamber*. This can be seen by raising the edge of the collar of the mantle.

5. The *head*, which forms an anterior prolongation.

6. The *tentacles*, forming two triangular projections on the head.

7. The *eyes*, pigmented spots on the outer edges of the tentacles.

8. The *proboscis*, which, when extended, protrudes from beneath the portion that bears the tentacles. What is its size, shape, and general appearance? It may be retracted entirely into the body.

9. The *mouth*, at the end of the proboscis. The end of the *odontophore* may frequently be seen protruding from the mouth.

10. The *foot*. What is its position, consistency, color, and shape? Is it slimy?

11. The opening of the *pedal gland*, on the sole of the foot. Is the pedal gland well developed in both sexes? Do you know its function? (See *Buccinum* by Dakin, 1912.)

12. The *operculum*. Notice its position and attachment.

13. If the specimen is a male, the large, somewhat flattened and bent *penis*, a little to the right and posterior to the right tentacle.

A number of organs may be seen through the somewhat transparent mantle. These are:

14. The *liver*, which forms the first two whorls of the spire. Notice its color.

15. The *gonad*, which is borne on the dorsal surface of the liver, and differs in individuals from red and brown to yellow.

16. The *stomach*, which lies on the left (external) surface of the liver. It is curved and light colored and is frequently rather indistinct.

17. The *kidney*, which lies on the dorsal surface, and a little to the left side, on the anterior end of the liver. It is somewhat rectangular in shape and differs in color from a yellowish brown to a chocolate color. The kidney is composed of two parts, the large *acinous* portion, and the smaller *tubuliferous* portion. The latter lies along the left side of the former, by the side of the pericardium.

18. The *pericardium* lies to the left of the anterior end of the kidney. Through its dorsal wall the yellowish *heart* can generally be seen.

19. The *columellar muscle*, which attached the animal to its shell and enabled it to withdraw, can be traced to the foot.

20. If the specimen being examined is a female, the large yellowish *nidamental gland* will be seen near the right side.

21. The large, brownish *gill* lies to the left of the nidamental gland in the female and anterior to the heart.

22. The *osphradium* is a small, brownish organ to the left of the anterior end of the gill and at the base of the siphon.

23. The *hypobranchial gland* is a glandular portion of the mantle, to the right of the gill (between the gill and the nidamental gland, in the female).

Make a drawing of the animal as a whole, showing as many of the observed points as possible.

Open the mantle chamber by cutting the mantle along the right side of the gill to the limit of the cavity, reflect the flaps, and notice the position and structure of the gill, osphradium, hypobranchial gland (cut in opening the mantle cavity), and, if the specimen is a female, the nidamental gland. The opening of the *rectum* will be seen at the end of a short papilla in the right side of the mantle cavity. The opening of the nidamental gland will be seen on an elevation

a little to the right and anterior to the anus. If possible, insert a guarded bristle into this opening and see what becomes of it. Trace the *oviduct* from the ovary along the columellar side of the liver. See what becomes of it. Examine the inside of the nidamental gland and see its relation to the oviduct.

If the specimen is a male, follow the *vas deferens* from the testis to the base of the penis.

Circulatory System.—Remove the thin membrane that forms the roof of the pericardial chamber.

1. The heart consists of: (a) the large, rounded *ventricle*; (b) the smaller, conical, thin-walled *auricle*.

2. The auricle receives blood by two vessels. One, returning blood from the gill, runs along the left side of the gill to its posterior end, where it bends abruptly to the right along the margin of the pericardial cavity, and enters the auricle. The other returns blood from the tubuliferous portion of the kidney and follows the right side of the pericardium to the auricle.

3. The gill receives its blood through a vessel that borders its right side. This vessel receives the blood from a portion of the mantle, and from the large, acinous portion of the kidney.

4. The blood leaves the ventricle by a single vessel, the *aorta*, that almost immediately gives rise to the *visceral artery* which supplies the visceral hump. Trace its distribution.

The aorta makes an abrupt turn downward and forward and enlarges to form the *secondary heart* which lies alongside the esophagus. The course of this vessel can be studied best after the completion of the work on the nervous system.

The course of general circulation is, beginning with the heart, (a) system, (b) kidney, (c) gill, and (d) back again to the heart. What is the advantage of such a course of circulation over the reverse?

Draw a figure showing the vascular system.

Excretory System.—The two portions of the kidney have

already been noticed. Cut along their common line of union and examine the inner surface of each part.

1. Notice the parallel lines of tubules that form the substance of the *tubuliferous* portion, and the lobules that form the comparatively thick walls of the *acinous* portion.

2. Find the slitlike opening that leads from the kidney to the mantle cavity. It is at a point between the two portions of the kidney and is easily found from the mantle chamber. A small opening leads into the pericardium, but it is hard to find it in dissections.

Digestive System.—1. Remove part of the integument at the base of the proboscis and find the muscles that retract it. How many are there and how are they attached? Do you understand how the proboscis is extended?

2. With a pair of scissors open the extended proboscis along the ventral line, pin it open, and notice that the exposed muscular mass, the *buccal mass*, is attached to the wall of the proboscis in the region of the mouth, at its base, and by means of fibers along its sides.

3. Push the muscular mass slightly to one side and notice the *esophagus*, which is closely applied to the dorsal wall of the proboscis. Notice the muscle fibers that extend from it to the proboscis. What is their function?

4. The odontophoral apparatus consists of a forked cartilage, the *odontophoral cartilage*, that is surrounded by muscles and cannot be seen until these are removed, a *radula* which is for most of its length enclosed in a sac, the *radular sac*, and is exposed only in the region of the mouth, and the muscles for moving the cartilage and the radula.

(a) The strands of muscle which run forward from the odontophoral cartilage to be inserted on the walls of the proboscis are the *cartilage protractors*.

(b) Attached to the ends of the two horns of the cartilage and running posteriorly to be attached to the walls of the proboscis near its base are the flat *cartilage retractors*.

(c) Running lengthwise of the buccal mass, on its ventral

side, are three pairs of slender muscles, one pair median and the others covering the horns of the odontophoral cartilage that has just been observed. Find to what the muscles are attached anteriorly and posteriorly. If the animal is fresh, pull on the muscles or stimulate the nerves by pinching gently with forceps and see what moves. These are the *radula protractors*.

(d) Beneath the radula protractors observe the sheet of cross fibers that bind the horns of the odontophoral cartilage together.

Make a drawing showing the ventral side of the buccal mass.

(e) A portion of the *radula* is visible near the anterior end of the proboscis. Introduce a bristle into the esophagus and determine its relation to the exposed radula.

(f) Loosen the anterior end of the buccal mass from the wall of the proboscis, turn it back and see how the radula passes around the odontophoral cartilage. With a hand lens notice the teeth on the open radula, ventral to the cartilage, and see how the radula is folded as it passes over the dorsal side of the cartilage so the teeth are turned in. What function does this serve?

(g) Cut the cartilage protractors and reflect the buccal mass. It is attached to the wall of the proboscis at its posterior end by strong muscles, the *radula retractors*. These may be studied after cutting the sheath of cross fibers that hold the mass together. Determine how they are attached to the sides of the radula. Why do they need to be so powerful?

Make a drawing of the buccal mass as seen from the dorsal side.

(h) Pull away the muscles and examine the shape of the odontophoral cartilage and its relation to the radula.

(i) Remove the radula, unfold it, and examine it microscopically. Do the teeth differ in any way at the two ends? Why is the radula so long?

Draw a portion.

5. In a living *Busycon* removed from its shell and partially anesthetized (by the addition of chloroform to the sea water), observe the activity of the radula. Any student who has already completed the dissection of the radula in the injected specimen may make a special dissection of the odontophoral apparatus, utilizing this fresh material. Follow directions as given in section 4, page 148. After exposing the various muscles and nerves these may be stimulated by pinching gently with forceps or by pulling the muscles. In this way a clear idea of the function of the muscles may be obtained.

The radula is the organ upon which most gastropods depend for getting food. You should understand how:

1. The proboscis is protruded and retracted.
2. The odontophoral cartilage is protruded and retracted.
3. The radula is protracted and retracted. By means of a binocular dissecting microscope note its action in a living *crepidula*.
4. The radula is folded by the cartilage and spread for action.

5. The food is torn off and taken into the mouth.

Near the base of the proboscis is a pair of large, yellow *salivary glands*, the ducts from which extend on either side of the esophagus to the mouth. Further back, on the right side of the esophagus, is the small *pancreas*.

After studying the nervous system, trace the esophagus to the *stomach* and the *intestine* to the anus.

Nervous System.—Most of the ganglia are grouped around the esophagus, about three quarters of an inch posterior to the base of the proboscis. They are all brown and accordingly conspicuous. Carefully cut around its base so the proboscis may be turned back, and the ganglia on the ventral surface of the esophagus may be seen. Carefully pick away the tissue that covers the ganglia and notice on the ventral side of the esophagus:

1. The small but conspicuous *buccal ganglia*. These are

united with each other and with the cerebral ganglia and send nerves to the mouth apparatus.

2. The large *pedal ganglia*, fused together but distinctly paired, lying posterior to the buccal ganglia and sending nerves to the two sides of the foot. Each is united by connectives with the corresponding cerebral and pleural ganglia.

From the dorsal side a number of ganglia may be seen, more of which lie to the right than to the left of the median line.

1. On the left side there are two ganglia that are in rather close union with each other. The most anterior, the *left cerebral*, is the larger of the two. The *left pleural* joins it posteriorly and ventrally and extends nearly to the ventral side of the esophagus.

2. On the right side four ganglia may be distinguished. The *right cerebral* and *right pleural* are fused to form one mass, but there is a marked constriction between them. Posteriorly and dorsally the right pleural is connected by a connective with the *right parietal (supra-intestinal)*, which lies very close to it. The remaining ganglion, the *left parietal (subintestinal)*, which is almost in contact with the right pleural and right pedal ganglia, lies ventrally and to the right of the right parietal ganglion. It is connected with the left pleural ganglion by a connective that runs behind the pedal ganglia. There seems also to be a connection with the right pleural ganglion, but this must be considered a secondary connection. Do you understand how this ganglion comes to have this position?

3. Another ganglionic mass, the *visceral ganglion*, possibly formed by the fusion of two ganglia, lies just below the external opening of the kidney, where it can be seen as a brown mass through the body wall. It lies on the elongated commissure that connects the two parietal ganglia. The commissure may be followed by dissection.

The cerebral ganglia are the most centralized. Besides being connected with each other by a commissure dorsal to

the esophagus, and being intimately connected with the pleural ganglia, each cerebral ganglion is connected with the corresponding buccal and pedal ganglion and, through the pleural, with the parietal ganglion. The parietal ganglia are connected with each other by a long commissure on which the visceral ganglion is placed. Each pedal ganglion receives connectives from the cerebral and from the pleural ganglion of the corresponding side.

*Draw figures of the nervous system and compare them with the clay model.*¹

The dissections of the circulatory and digestive systems may now be completed.

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¹ Instructors will find that a model prepared by sticking lumps and strands of modeling clay on a cylindrical graduate to illustrate the positions of the ganglia and connectives on the esophagus will greatly aid the students.

CEPHALOPODA

LOLIGO PEALEI (The Squid)

Specimens of this or closely related species are rather common along the Atlantic coast of the United States. They are active swimmers, but may occasionally be seen in shallow, quiet water near the shore. The movements and positions of adult specimens in aquaria should be studied. Do you know what they eat and how they capture their food?

Study a small living specimen in a jar of sea water and notice:

1. Its general shape and distinct head.
2. Its position in the water. For convenience, the lower surface may be referred to as ventral, but this is not to be considered as morphologically the same as the ventral surface of other Mollusca. What parts are kept moving? Why is water pumped when the animal is not swimming?
3. In what direction can it swim best? Can it swim in the other direction? How does it swim? Is the funnel movable? How does it guide its movements?
4. Its color. Irritate it and see what happens. What purpose does the change in color serve? What is the ink for?
5. What happens when the end of a finger is placed within the circlet of tentacles of an animal about two inches long that is being held firmly?

Using an adult specimen, observe:

1. The arrangement of the *arms* on the head. Are they arranged in any definite order? Are they all alike?
2. The suckers of the arms. Do they follow the same order on all of the arms?
3. The structure of a *sucker*. Notice the peduncle, outer thin margin, horny ring, and piston. Is the horny ring smooth? What is its function? How does the sucker work? Split one and draw the cut surface.
4. The *mouth*. Where is it placed? Notice the tips of the horny beak. Which jaw is the longest?
5. The *eyes*.

6. The fold of tissue behind each eye. These have been called the *olfactory organs*, but there is no experimental evidence of function.

7. The small pore in front of the eye, the *aquiferous pore*. With what does this communicate?

8. The attachment of the head and the extent of the mantle opening around the neck.

9. The *funnel* protruding from beneath the mantle on the ventral surface. Notice the position and character of its opening.

10. The median dorsal projection of the mantle.

11. The *tail fin*, its position and shape. What is its function?

Draw the animal as seen from the ventral side.

Carefully open a specimen by cutting through the mantle a little to one side of the midventral line.

Notice:

1. The thickness and character of the mantle and its relation to the rest of the body. Why does it need to be so muscular?

2. The arrangement of the funnel. Why does it have a thin posterior edge? How is it held in position against the mantle? Does it have a valve? Is the funnel movable in the living animal? Is there any provision for movement?

3. The free edge of the mantle and its relation to the folds beneath the eyes. Do you understand how the water gets into and out of the mantle cavity?

4. The large *retractor muscles of the funnel*. How many are there? How can the funnel be pointed in different directions? What need is there for such a provision?

5. The *retractor muscles of the head*. How many are there? Are they used in swimming in any way?

6. The *rectum*, opening near the base of the funnel between two small lateral flaps of tissue.

7. The *ink bag*, dorsal to the rectum and opening into it near the anus.

8. The *gills*, extending from a point about midway of the body toward the free edge of the mantle. How many are there? How are they attached? Why does an animal that is not swimming continually pump water through the mantle chamber?

9. The *branchial hearts*, at the bases of the gills, rounded, light colored organs than can be seen through the membrane covering them.

10. The median ventral *mesentery*.

If the specimen is a male, notice:

1. The slender, tapering *penis*, to the left of the rectum.

2. The *kidneys*, white organs to be seen through the membranous covering, between the bases of the gills. From this position they taper anteriorly for half an inch or more and send small lobes posteriorly.

3. The openings of the kidneys near their anterior ends, on small papillae.

4. The conical posterior portion of the viscera. This is composed of a large visceral sac and portions of the sexual organs.

Draw the animal, showing the points observed.

If the animal is a female, notice:

1. The pair of large, white *nidamental glands* that cover a portion of the rectum and the greater part of the ink bag.

2. The openings of these glands at their anterior ends. Do you know the function of these glands?

3. The small *accessory nidamental glands* just anterior to the nidamental glands. These have large ventral openings. Just before egg laying they become brilliantly red.

4. The opening of the *oviduct* dorsal to, and a little to the left of, the left nidamental gland.

5. The rounded swelling, the *oviducal gland* on the oviduct.

6. The mass of eggs that fills the posterior portion of the body. These are in the ovary and oviduct.

Draw the animal, showing the points observed.

Excretory System.—If the animal is a female, remove the

nidamental glands, and the kidneys will be seen in the position described for the male. The kidneys consist of:

1. The white, somewhat triangular, glandular portions already noticed, extending from the region of each branchial heart anteriorly, and forming a portion of the walls of the precavae.

2. The cavities of the organs lying ventrally, and at the sides of the glandular portions.

3. The external openings, at the ends of small papillae, on either side of the rectum near the anterior ends of the kidneys.

Digestive System.—Remove the funnel and its retractor muscles and carefully lay the head open, along the ventral side.

Find:

1. The *buccal mass*. This is a rounded, muscular organ, with a double ring of tissue, the *buccal membranes*, at its anterior end, that surrounds the horny jaws. Examine the jaws and see which is the larger.

2. Behind the buccal mass are the *paired salivary glands*.

3. Trace the narrow *esophagus* from the posterior end of the buccal mass backward. At the base of the head it enters the *liver*, a large, white organ that lies between the retractor muscles of the head, and extends from the base of the head to a point dorsal to the external openings of the kidneys. Lying close to the esophagus and covered by the anterior end of the liver is an elongated *median salivary gland*, the duct from which follows the esophagus into the head. The esophagus leaves the liver about midway of its length, and follows along the ventral surface nearly to the stomach. Before entering the stomach the esophagus passes the *pancreas*, a white, lobed organ that lies just above the glandular portion of the kidneys, and the *systemic heart*, a roughly diamond-shaped organ that lies between the branchial hearts.

The *stomach proper* is a rather small, thick-walled sac that lies on the right side of the body, dorsal and posterior to the right branchial heart. From the left side of the stom-

ach a rather large opening leads into a thin-walled blind sac, the *visceral sac*. The latter, when filled with partly digested food, as it frequently is, extends posteriorly to the end of the body and occupies a considerable part of the conical portion of the body. When empty, it is quite small and inconspicuous.

The *intestine* leaves the stomach very near the point where the esophagus enters, and just anterior to the opening that leads into the visceral sac. It passes ventrally, and becomes visible from the surface, where its position has already been noted.

Draw a figure showing the digestive system.

Cut a median sagittal section of the buccal mass, and notice the mouth cavity, the jaws, the muscles that move the jaws, the tongue, and the position of the radula. Is the radula arranged in the strap-over-pulley manner that it is in *Busycon*?

Draw a figure of the section.

Male Reproductive System.—1. The *testis* (morphologically the left) is large, white, and flattened, and lies far back in the pointed end of the body. It is enclosed in a sheath which serves to collect the liberated sperm.

2. Just anterior to the testis is a small rounded vesicle, the *ampulla*, which is the point of origin of the vas deferens.

3. The *vas deferens* is a plaited slender tube, which being packed with sperm is opaque white. It extends from the ampulla along the right side of a large sac to be referred to later, the spermatophoric sac, to and beneath (dorsal to) the spermatophoric organ, where it joins a portion of the organ on its left side about one third the length of the organ back from its anterior end. Remove the left gill and branchial heart and strip away the thin tissue that covers the vas deferens and spermatophoric organ, being careful not to injure either. Carefully lift the right ventral side of the spermatophoric organ and see where the vas deferens enters it. Drop the spermatophoric organ into position again.

4. The *spermatophoric organ* lies almost on the left side, but a little ventrally. In it the spermatophores are formed. It consists of a series of glands and mechanical arrangements that secrete and wind materials into spermatophores. It consists of a number of parts.

(a) On the right dorsal side, the part joined by the vas deferens, is the *mucilaginous gland*. This consists of two parts.

(b) The *ejaculatory apparatus gland* extends from the mucilaginous gland posteriorly and then ventrally. Both ends of this gland are marked by constrictions.

(c) From this gland forward to a narrow duct is the *middle tunic gland*.

(d) The narrow duct leads into a large blind pouch and is the *outer tunic gland*.

(e) The large blind pouch extends posteriorly nearly the length of the organ and is the *hardening gland*.

(f) A branch leaves the narrow duct just before it enters the hardening gland that leads to the curved anterior extremity of the gland. This is the *finishing duct*.

(g) The finishing duct leads to the curved gland mentioned, the *finishing gland*.

This completes the spermatophoric organ, although some minor structures have not been mentioned. A section across the ejaculatory apparatus gland will show a large ridge with a groove along one side through which the spermatophore travels and rotates while being formed.

5. From the last part of the spermatophoric organ, the finishing gland, a straight duct leads posteriorly by the side of the vas deferens to join the spermatophoric sac about the length of a spermatophore from its posterior end. This is the *spermatophoric duct*.

6. The *spermatophoric sac* is somewhat spindle shaped, usually filled with spermatophores, and joins the base of the penis.

Make a drawing of the system.

Open the spermatophoric sac and remove some of the spermatophores. If the specimen has not been preserved place them immediately in 10 per cent formalin or stronger to keep them from ejaculating. Mount under a cover and examine. The specimens may be stained with dilute Ehrlich's triacid stain and mounted in glycerin jelly if preferred. Notice:

1. The spermatophore is covered by a transparent elastic *outer tunic* which has a *cap* and *cap thread* at the smaller, oral end.

2. Inside this is the somewhat granular *middle tunic*, which is much thicker at the aboral end.

3. The contents consist of the aboral *sperm mass*, the oral *ejaculatory apparatus*, and the *cement body*, which lies between them.

4. The sperm mass may be seen to consist of a closely wound thread. It is actually covered by a very thin *inner tunic* which is separated from the outer tunic by a space filled with liquid.

5. The cement body is attached to the sperm mass by a narrow core and is covered by a continuation of the inner tunic. It is flask shaped, with the narrow end pointing orally.

6. The neck of the cement body is covered by parts of the ejaculatory apparatus and the bulge of the body is joined and possibly covered by the *outer membrane*, which with the inner tunic forms the outer covering of the ejaculatory apparatus that appears as a double membrane. Inside the outer membrane is the rather thick middle membrane, the aboral end of which encloses the neck of the cement body and ends against the bulge of the cement body, and the oral end of which is thrown into bends and loops and is finally attached to the cap end of the outer case. Inside the middle membrane is the thin *inner membrane* and the *spiral filament* which enclose a narrow lumen.

7. In ejaculating, the ejaculatory apparatus turns wrong

side out and the cement body and sperm mass are crowded down the tube thus formed by the elastic force of the outer tunic and the elastic and osmotic action of the middle tunic.

The sperm mass is forced into a sac composed of the inverted inner tunic and outer membrane, which remain attached to the bulge of the cement body; the cement body is ruptured and the cement spread over the closed end of this sac. The reservoir is now ready to stick in position.

Studying the method of ejaculation is time consuming. Fresh specimens placed in about one-fourth saturated solution of magnesium chloride for ten minutes or more will be slowed in action so the process can be followed more readily. Remove a specimen from this solution to sea water, grasp the cap thread with forceps, and shake the spermatophore. This should start ejaculation. Ejaculation can be stopped promptly by squirting full strength formalin on the ejaculatory apparatus.

Make a drawing of a spermatophore.

Spermatophores are carried into position by the action of the left ventral arm of the male. Examine its tip and notice the modification of the suckers.

Female Reproductive System.—The opening of the oviduct has already been noticed. Observe:

1 The large, swollen portion, the *oviducal gland*, that lies on the oviduct dorsal to the left branchial heart.

2. The long convoluted *oviduct* extending posteriorly from the oviducal gland. It is frequently filled with eggs for the greater part of its length.

3. The lighter colored, greatly lobulated *ovary*, also frequently filled with eggs, lying dorsal to the oviduct and visceral sac and extending from the region of the stomach to the end of the body. The ovary is inclosed in a capsule from which the oviduct leads.

4. The *nidamental* and *accessory nidamental glands* have been studied and removed.

5. On the median line of the inner surface of the outer

buccal membrane of the female is the *sperm receptacle*. During the summer this is usually filled with sperm, and is, accordingly, white and conspicuous. Below the receptacle is a modified area for the attachment of sperm reservoirs as they are delivered from the spermatophores.

Draw a figure of the female reproductive system.

Circulatory System.—An injected specimen is desirable. The blood that has been supplied to the body in general is collected by veins and carried to the branchial hearts. The vessels that collect the blood are:

1. The *precavae*. A single vessel carries the blood from the head to the anterior ends of the kidneys. Here the vessel divides into right and left precavae that are intimately connected with the kidneys. The precavae diverge near the posterior ends of the kidneys and enter the corresponding branchial hearts.

2. The *postcavae*. A pair of very large vessels that return blood from the posterior end of the body. They join the corresponding precavae near the anterior borders of the *branchial hearts*.

3. The *mantle veins*. These return blood to the branchial hearts from the anterior portion of the mantle.

The blood that is received by each branchial heart is sent into the corresponding gill through a *branchial artery* that leaves the heart near the opening of the mantle vein, and runs along the side of the gill that is attached to the mantle.

The blood is collected from each gill by a large *branchial vein* that runs along the ventral side of the gill, and enters the systemic heart.

Draw a figure showing the vessels connected with the branchial hearts.

Expose the *systemic heart* by carefully removing the superficial tissue between the branchial hearts, and notice that it is not symmetrical. Its lateral angles receive the branchial veins and it gives rise to an artery from each of the other two angles.

1. The *posterior aorta* divides almost immediately into three large vessels. These are:

(a) The *median mantle artery* which follows the edge of the ventral mesentery to the mantle.

(b) A pair of *lateral mantle arteries* which diverge posteriorly and supply the two sides of the mantle. Besides these large vessels there is a small vessel that runs anteriorly over the ventral surface of the heart and supplies the ink gland and rectum, and another one that runs dorsally and posteriorly to supply part of the reproductive system.

2. From the dorsal surface of the heart, near its anterior end, a small vessel passes over the anterior and dorsal surfaces of the stomach and finally passes into the gonad.

3. The *anterior aorta* is larger than the posterior aorta. From the anterior angle of the heart, which is to the right of the median line, it follows a straight course alongside the esophagus to the head. A number of small vessels are given off along its course, and it is finally distributed to the head and arms.

Draw the vessels connected with the systemic heart, into the figure you have just made.

Nervous System.—The *stellate ganglia* may be seen through the transparent lining of the mantle, on either side of the neck, where the body joins the mantle. They send nerves to the mantle and are joined to ganglia in the head (the infra-esophageal) by connectives. Why does the mantle need such large special ganglia? Other small ganglia are situated in the body, but the large and important ones are grouped in the head, where they are supported and protected by cartilages.

With a razor make a median sagittal section of the head of a squid and notice:

1. Dorsal to the esophagus a rounded mass, the *supra-esophageal ganglion*, which is supposed to represent the fused *cerebral ganglia*.

2. Ventral to the esophagus the elongated *infra-esophageal*

ganglion, which is supposed to represent the fused pedal and visceral ganglia and (together with the masses that connect the supra- and infra-esophageal ganglia around the esophagus) the pleural ganglia.

3. The anterior prolongation of the infra-esophageal ganglion to form the *propedal portion*, which supplies nerves to the arms.

4. The small *suprabuccal ganglia*, lying dorsal to the esophagus, and a little further anterior than the ends of the propedal portion. These are joined by connectives with the supra-esophageal ganglia.

5. The *infrabuccal ganglia*, about the same size as, and lying ventral to, the suprabuccal ganglia, and joined with them by connectives that run around the esophagus.

Draw a figure of a sagittal section of the head.

Two large ganglia, the *optic ganglia*, lie against the eyes and will be seen in cross sections of the head that will be studied later. A dissection of one side of the head will show one.

Open the animal along the mid-dorsal line and find the *pen* which is embedded in the mantle. After exposing it for its full length, turn the flaps aside and see that it lies in a pocket. It probably represents a modified shell that has become entirely inclosed by the mantle. What is its function?

Pull the pen out of the mantle and draw it.

With a razor make cross sections of a squid, a quarter of an inch thick, and arrange them in order, in a little water, as they are made. Identify the parts you have found in dissection.

Make drawings of the sections that pass through the infra-esophageal ganglion, through the eyes, through the liver, and through the heart.

If time permits, study prepared sections that have previously been made. The structure of the eye and the positions of the parts of the nervous system should receive special attention.

Specimens of other cephalopods, such as *Octopus* and *Nautilus*, should be compared with the squid and the adaptations that fit them for their particular lives noted.

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ARTHROPODA

With segmented bodies which are provided with segmented appendages.

CLASS 1. Crustacea.

Usually aquatic. With a more or less hardened outer covering and many thoracic appendages.

Subclass 1. Branchiopoda.

Crustacea with compound eyes; mandibular palp usually absent or vestigial; 4 or more pairs of trunk limbs usually broad and lobed. Some orders of this subclass are: Anostraca (*Artemia*); Notostraca (*Apus*); Cladocera (*Daphnia*, *Simocephalus*).

Subclass 2. Ostracoda.

Free swimming with the body inclosed in a bivalve shell; mandibular palp usually biramous; not more than 2 pairs of trunk limbs. (Cypris.)

Subclass 3. Copepoda.

Free or parasitic; no compound eyes or carapace; mandible with biramous, uniramous or no palp; typically 6 pairs of trunk limbs, first pair uniramous, next 4 pairs biramous and sixth pair often uniramous. (*Cyclops*, *Argulus*, *Lernaea*.)

Subclass 4. Cirripedia.

Comparatively large and usually attached; no compound eyes in adult; forms that are not parasitic covered with calcareous plates; usually 6 pairs biramous, thoracic limbs. (*Lepas*, *Balanus*, *Chthamalus*.)

Subclass 5. Malacostraca.

Usually of considerable size; with compound eyes, usually stalked; mandibular palp, if present, uniramous; thorax of 8 segments; abdomen typically with 6 segments. Some orders of this large subclass are: Leptostraca (*Nebalia*); Hoplocarida (or Stomatopoda) (*Chloridella*); Decapoda (*Homarus*, *Cam-*

barus, Crago, Pagurus, Emerita, Callinectes, Cancer, Uca); Isopoda (Idothea, Erichsonella); Amphipoda (Talorchestia, Gammarus, Caprella).

CLASS 2. Arachnida.

Body divided into two principal regions, cephalothorax and abdomen; cephalothorax bears sessile eyes, 4 pairs of walking legs, chelicerae and pedipalpi; no antennae; respiration usually by tracheae or lung sacs. Some of the important orders are: Scorpionida (Buthus); Xiphosura (Limulus); Pseudoscorpionida (Chelifer); Pedipalpida (Phrynus); Solpugida (Galeodes); Phalangida (Phalangium); Araneida (Epeira, Agalena); Acarina (Sarcoptes, Dermacentor).

Supplementary to the Arachnida.

Pycnogonida. (Pantopoda.)

(Doubtfully referred to the group.) Body composed of segmented cephalothorax and vestigial abdomen. Legs very long, angular, and containing portions of the viscera. No special respiratory organs (Anoplodactylus, Pallene, Phoxichilidium).

CLASS 3. Onychophora.

Elongated bodies with some annelid-like characters. Appendages short, numerous, and creased rather than jointed. Respiration by means of tracheae. (Peripatus.)

CLASS 4. Myriapoda.

Generally elongated bodies with numerous jointed appendages. A distinct head bearing ocelli, antennae, and jaws is present. Respiration by means of tracheae.

Order 1. Symphyla.

With not more than twelve leg-bearing trunk segments. A single pair of branching tracheae. (Scolopendrella.)

Order 2. Chilopoda.

With numerous trunk segments, each with a single pair of legs. First pair of trunk appendages forming poison jaws. Body dorso-ventrally compressed. (Lithobius.)

Order 3. Diplopoda.

With numerous trunk segments, each with two pairs of legs. No poison jaws. Body not compressed. (Julus.)

Order 4. Pauropoda.

With ten trunk segments and nine pairs of legs. (Pauropus.)

CLASS 5. Insecta.

Body divided into head, thorax, and abdomen. Three pairs of thoracic legs and generally one or two pairs of wings. Some of the important orders are: Thysanura, Orthoptera, Neuroptera, Hemiptera, Diptera, Lepidoptera, Coleoptera and Hymenoptera.

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CRUSTACEA

HOMARUS AMERICANUS (Lobster¹)

These animals are not generally found where they can be readily observed in nature, but many valuable observations can be made on specimens confined in aquaria. If other animals are present in the aquarium notice the position of defense that is taken. In nature the animal spends considerable time under rocks with the anterior end of the body turned toward the opening. In this position both sense organs and weapons are in the proper position for attack or defense. Notice how the appendages are used. Are the sense organs moved frequently? What is the advantage of having the

¹These directions may be used for the crayfish without much modification. The smaller size of these animals will make it more difficult to trace some of the nerves and blood vessels.

eyes on stalks? What appendages are used in walking? Are all of these appendages used in just the same way? Does the animal move equally well in all directions? Perhaps you can make the animal swim; if so, observe the method. Feed a specimen with portions of a clam or fish, and see how food is torn to pieces and transferred to the mouth, and determine, if possible, how the mouth appendages are used.

Appendages may be missing. If any are, notice at what point they are broken. Possibly small appendages may be growing from the old stubs. Autotomy may be studied by crushing a claw or a leg of the fiddler crab, *Uca*. Other forms will respond, but sometimes not promptly. What is the importance of this reaction?

External Anatomy.—As in *Nereis*, the body is segmented. The five segments of the head and the eight segments of the thorax, however, are immovably fused to form a *cephalothorax*. This is covered dorsally by a single piece, the *carapace*.

1. Note, on the carapace, the *cervical groove* between the head and thorax, and the beak or *rostrum* forming an anterior spine. The ventrolateral edge of the carapace is not attached. A flat object thrust between it and the body passes into the gill chamber. This free plate of the carapace is called the gill cover. Notice the hairlike spines along its free border. What purpose do these serve?

2. The abdomen is composed of seven movable segments, each bearing a pair of jointed appendages except the last, which is sometimes not considered a true segment and is called the *telson*. Each abdominal segment consists of a dorsal piece, the *tergum*, which is continued as a free plate laterally (the *pleuron*), and of a ventral piece, the *sternum*. Move the abdominal segments and see where they are hinged. How are the terga and sterna arranged to allow free movement? In the thorax the sterna, though fused, can be distinguished.

3. **Appendages.**—Aside from the stalked eyes, whose ho-

mology with true appendages is doubtful, there are nineteen pairs. These are, counting from before backward: *antennules* (or *first antennae*), *antennae*, six pairs of *mouth appendages*, five pairs of *walking legs* (*pereiopods*), of which the first are the claws or *chelae*, and six pairs of swimmerets (*pleopods*). In the male, the first two pairs of pleopods are modified to form copulatory organs. The first pair is greatly modified and the second pair bears a special portion.¹

(a) Turn one of the fifth pair of pleopods forward and examine its posterior aspect. It consists of a basal piece, the *protopod*; a lateral branch, the *exopod*; and a median branch, the *endopod*. This branched type of appendage is designated as biramous. What is its use? Compare with this the modified sixth pair of pleopods, called the *wropods*.

Make a drawing of one of the fifth pleopods.

(b) In front of the chelae will be seen the sixth pair of mouth appendages, the third *maxillipeds*. Remove that of the right side and compare it with the fifth pleopod. In addition to the protopod, exopod, and endopod, it bears a long blade, the *epipod*, which extends into the gill chamber. The protopod is composed of two segments, *coxopod* and *basipod*; the endopod of five segments, *ischipod*, *meropod*, *carpopod*, *propod*, and *dactylopod*. The exopod is composed of one long and many short segments. How is the appendage modified to serve in feeding?

Make a drawing of the third maxilliped.

(c) Remove the remaining five mouth appendages and compare each with the third maxilliped. These are, beginning posteriorly, the *second maxilliped*, *first maxilliped*, *second maxilla* (with a broad paddle, the *scaphognathite*, the use of which should be understood), *first maxilla*, and the *mandible*. Just back of the mandibles are two small flaps, the *paragnatha*, which are not true appendages. Do you understand the use of each of these appendages? Most of the appendages have parts that may be compared with the

¹The crayfish has the first two pairs, both greatly modified.

typical biramous appendage, but they are much modified to serve special functions, and the exact homologies are not important. Between the mandibles note the mouth, bounded in front by the *labrum*.

Drawings of these appendages may be made if time permits.

(d) The *second antennae* are biramous. Notice on the ventral side of the basal joint of an antenna the opening of the *green gland* or nephridium.

(e) The *first antennae*, though branched, are not considered to be of the biramous type. Do you know why? Remove one and note on the dorsal surface of the basal joint a groove at whose median extremity is a small hole, the opening into the *statocyst*. Do you know the probable function of the antennules and of the statocyst? What reason is there for having both first and second antennae?

(f) Compare the *pereiopods* with the third maxilliped. Which is lacking, endopod or exopod? Examine each of the joints of one of these appendages and see in what directions the appendage may be moved. Are there any ball-and-socket joints? Compare the chelae with the other pereiopods and see how they differ. To what part of a chela does the last segment of the last pereiopod correspond? What reason is there for having these appendages different? Do you think the arrangement of the appendages would aid the lobster in climbing over rough bottom?

Open one of the large chelae and determine how the muscles are arranged to control its opening and closing. Which muscles are strongest? Find how the muscles are attached to the "thumb."

Find the openings of the sexual ducts on the basal joints of the pereiopods; the fifth pair in the male, the third pair in the female. In the female there is an opening into a *seminal receptacle* through a triangular elevation on the ventral side of the thorax.

4. **Gills.**—Remove the gill cover of the left side, being

careful not to injure the gills. Extending up into the gill cavity are seven *epipods* belonging to the three maxillipeds and the four anterior pereopods. They separate the gills into groups. Each group will be seen to correspond to a segment. The gills show three sorts of attachments: (a) to the appendages themselves (*podobranchs*), (b) to the articular membranes between appendages and body wall (*arthrobranchs*), and (c) to the body wall itself (*pleurobranchs*). There are two arthrobranchs in some segments, one behind and above the other. How is the current of water forced through the gill chamber? What is the function of the epipods? What direction must the water take through the gill chamber? Examine the structure of a gill. Move one of the appendages to which a gill is attached and see the effect on the gill.¹

Internal Anatomy.—Remove the carapace (beginning at the middle of the posterior margin and cutting forward, holding the cartilage knife parallel with the surface) as far laterally as the upper limits of the gill chambers and anteriorly to the base of the rostrum. What is the pigmented membrane for? Dissect it off so underlying organs may be seen.

1. The chitinous *stomach* lies near the anterior end with the *ophthalmic artery* running along its mid-dorsal line. Beside and behind the stomach are two masses of muscle which you have severed from the carapace. These are the *mandibular muscles*, and each is divided into an anterior and a posterior bundle. Lateral to these muscle masses are the yellow-green *digestive glands*, commonly called *liver*. Between and in front of the posterior mandibular bundle note the *gonads*, and follow one forward by pressing aside the muscle mass. In the male the *testis* is a slender, white, convoluted cord, which ends blindly against the side of the stomach. The extent and position of the far thicker yellow *ovary* is much the same (unless the animal be mature, in which case it will be found greatly enlarged and orange).

¹The crayfish differs slightly in gill arrangement.

2. The *heart* extends through the posterior third of the thorax. Remove the upper part of the delicate *pericardium* surrounding it, cut its arterial and other connections, and place it in water. Note the shape, the origin of the arteries, and the three pairs of *ostia*. Do you understand how the heart receives blood?

3. Trace the gonads as far as the abdomen, noting the single anastomosis between those of opposite sides just in front of the heart. Beneath the heart the sexual ducts are given off—*vasa deferentia* in the male, *oviducts* in the female. Trace one outward and downward to its opening by removing a portion of the body wall and of the basal joint of the proper leg.

4. Remove the posterior lateral body wall forward to a position opposite the anterior third of the stomach. Pull the anterior lobe of the liver, which extends beneath the stomach, outward and backward. The liver will be seen to be attached to the pyloric end of the stomach (*i. e.*, the smaller part, where the stomach passes into the intestine). Cut this attachment and note that it is really where the liver opens into the stomach. Just back of this point the right and left lobes of the liver are connected by a cross branch passing beneath the intestine. Remove one liver lobe back to the abdomen. After having the *circumesophageal connectives* pointed out, remove the stomach by cutting the esophagus, the intestine, and the bands of muscles attached to the stomach. Examine it in water, noting the cardiac and pyloric parts, the chitinous grinding and straining apparatus in the interior, and the muscles and plates that cause the movements of the grinding apparatus. Why does a lobster with chelae and six pairs of mouth appendages need a gastric mill?

5. Between the *circumesophageal connectives* medially and the large antennary muscles laterally, note the oval excretory organs, called the *green glands*. They are covered by a very delicate membrane. Poke a small hole in one of

the membranes and with a blowpipe show that it is really a thin bladder. Its opening on the antenna has already been seen.

6. Remove the dorsal wall of the abdomen and trace the posterior portions of the gonads, liver lobes, and intestine. In the sixth abdominal segment the intestine swells to form the chitin-lined *rectum* and gives off the blind *intestinal caecum*.

Circulatory and Nervous Systems.¹—Remove the carapace of an injected specimen as before, also the gill cover and gills on one side.

1. There can generally be seen, through the transparent body wall, *efferent branchial veins*, which return the blood from the gills. These unite into six large ones which open into the *pericardium* at the side. Find these openings if possible. Do you understand how blood gets into the heart?

2. Note, at the anterior end of the heart, the *ophthalmic artery* and the two *antennary arteries*. Trace the former forward to the rostrum, cut it on the stomach and turn it forward for future study. Trace the antennary arteries to the mandibular muscles and cut them near the heart. Press the front end of the heart back and note the two small *hepatic arteries*. Each branches immediately, one division passing between the gonads, and the other laterally.

3. Remove the muscles on one side of the heart and examine it from the side, noting the great *sternal artery* extending downward, and the smaller *dorsal abdominal artery* running back above the intestine. Follow the latter through the abdomen.

4. Cut all arteries and remove the heart. Trace the an-

¹The circulatory system of a fresh specimen may be satisfactorily injected with starch mass by inserting the needle of a hypodermic syringe into the pericardium from the posterior margin of the carapace. The operation is easily performed when the distance to the pericardium is understood. The carapace may be cut away and the needle inserted directly into the heart if preferred.

tennaries through the mandibular muscles, noting the branch to the stomach.

5. Remove the thoracic viscera as before, follow the *circumesophageal connectives* forward and identify the *cerebral ganglia* in order not to destroy them.

6. Follow one antennary artery to the green gland, antennary muscle, eye muscle, etc.

7. Follow the distribution of the ophthalmic artery.

8. Remove the intestine and muscles of the abdomen, and find and trace forward the ventral *nerve chain*. Notice the position of the ganglia and the nerves that leave them and the connectives. In the thorax the ventral nerve chain passes beneath a system of chitinous plates (*the endophragmal skeleton*) and lies in a cavity, the *ventral blood sinus*. Note the enlarged *subesophageal ganglion*, the cross commissure just back of the esophagus, the nerves to the mouth appendages, nerves from the cerebral ganglia, and nerves from the other ganglia. What indication is there that the subesophageal ganglia represent more than a single pair?

Draw the nervous system.

9. The *sternal artery* passes through the ventral nerve chain and then extends backward and forward as the *ventral longitudinal artery*. Remove the nervous system and follow this artery.

Draw a diagrammatic cross section through the thorax, putting in one drawing the circulation from the heart through the sternal artery to the limbs and back through the gills to the heart.

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CALLINECTES SAPIDUS (Blue Crab)

Crabs may be found in shallow water along shore, where they may be easily observed on quiet days. In what direction does the animal normally move? How are the legs used? What is the attitude of defense? Determine how the blue crab swims. What do crabs apparently use for food? Do they conceal themselves, are they protectively colored, or do they depend entirely upon their weapons for defense?

In studying the anatomy of the crab, constant comparisons should be made with the lobster.

External Anatomy.—1. The body is composed of *cephalothorax* and *abdomen*. Dorsally note the shape of the *carapace* and the position of the abdomen. The size of the abdomen differs in male and female. To what use is the larger abdomen of the female adapted?

2. Note the *first antennae*, *second antennae*, and *eyes*, and see how they are packed away in recesses in the carapace. In the living animal see if any of these are frequently moved.

3. The third maxillipeds are flattened and cover the other mouth appendages.

4. Straighten the abdomen and note the *anus*. Compare the abdomen of a male with that of a female and both with that of the lobster. The dorsal side of each segment is

covered by a tergum. The covering between each pair of *pleopods* is the *sternum*, the immovable flap lateral to them is the *pleuron*. Compare the abdominal appendages or *pleopods* of a male and a female.

5. The ventral side of the cephalothorax is covered by the *sternal plastron*. Note the eight sterna and six pairs of lateral *episterna*, the anterior pair of which is very small.

6. In the female find the openings of the *oviducts* in the sixth sternum.

Make a drawing of the ventral side.

7. Expose the gill chamber and compare the gill distribution with that of the lobster.

8. Remove the left third maxilliped entire, and compare it with the same appendage of a lobster. The protopod is composed of two segments (*coxopod* and *basipod*). The endopod has five pieces (*ischipod*, *meropod*, *carpopod*, *propod*, and *dactylopod*). The exopod has two large and many small segments. Attached to the coxopod laterally is an *epipod* which extends into the gill chamber.

9. Remove the remaining mouth appendages on the left side and compare them with the third *maxilliped*. They are: *second maxilliped* bearing epipod and two small gills; *first maxilliped* with an epipod; *second maxilla* with a flattened exopod, called the *scaphognathite*, which is made up of both exopod and epipod and which has a special function that should be understood; *first maxilla*, thin and leaflike; *mandible* with two hard rods for the attachment of muscles.

10. Detach and examine one each of the *eyes* and *first* and *second antennae*. On the flat side of the basal joint of each first antenna note a dark suture—the scar of the former opening into the *statocyst*. Do you understand what function is performed by the statocysts? Near the base of the second antenna find the opening of the renal organ (green gland).

11. Compare each of the five walking legs (pereiopods) with the third maxilliped. What part, endopod or exopod, is lacking? Which bears forceps or chelae? Note in the

male the openings of the sperm ducts on the coxopods of the fifth pair.

Internal Anatomy.—Remove the entire dorsal part of the carapace.

1. Posterolaterally are two firm prominences, the *flanks*, containing muscles. What are these muscles for? Anterior to these are the gill chambers covered by a thin cuticle. Remove this and note the gills with their tips converging medially.

2. Between the gill chambers and flanks is the delicate *pericardium*. Remove this and find the heart with its *ostia*. Anteriorly it sends out an *ophthalmic artery* and two *antennary arteries*. Just anterior to the heart are muscles which were attached to the shell. What organs do they supply? The antennary arteries pass through the heads of a pair of the muscles.

3. In front of the gill chambers are the gonads. *In the female* the orange *ovary* will be seen lying on the yellow liver. *In the male* the slender, wavy, white cord, the *testis*, lies in approximately the same position.

4. The heart is attached to the pericardium by muscular strands. Cut these, and the three anterior arteries, and remove the heart, noting the two *hepatic arteries* beneath the antennary arteries, the great *sternal artery* passing downward from the under side, and the small *abdominal artery* just behind the last.

Draw dorsal and ventral views of the heart to show the ostia and the origins of arteries.

5. Cut across a gill and notice its *afferent* and *efferent vessels*. The latter is continuous with one of the sinuses which empty into the pericardial cavity. Can you determine how many sinuses there are? Do you understand how the heart receives blood?

Reproductive System.—Beginning anterolaterally, on one side, dissect out the reproductive organs, noting at the same time the distribution of arteries.

(a) *Female Reproductive Organs*.¹—Each ovary passes inward and backward, anastomoses with the one of the other side behind the stomach, and extends back to the abdomen. On a level with the posterior part of the stomach a branch passes downward and outward and is continuous with a dense, white organ, the *seminal receptacle*. Leave this receptacle in place, but remove the entire ovary.

(b) *Male Reproductive Organs*.—The usually slender testis which is large during the season of activity passes inward and backward, anastomoses with its fellow of the other side behind the stomach, and is continued as a thick, much-coiled tube, the *vas deferens*, to the median side of the flank. It then runs forward nearly to the stomach, turns back again, and enters the substance of the flank. By removing the top of the flank and the upper side of the coxopod of the swimming leg, it can be followed to its external opening.

Digestive System.—1. The *liver* is large and fills a large part of the body cavity. Remove the portion of it that is in the region of, and anterior to, the stomach, noting its connection with the alimentary tract.

2. The *stomach* is a chitinous box divided into a larger cardiac and a smaller pyloric portion. On each side find the duct from the liver, and a slender, white, coiled tube, the *pyloric caecum*.

3. Follow the delicate *intestine* back beneath the heart. Between the posterior edges of the flank is a white mass composed of a coiled tube, the *intestinal caecum*. Remove the terga of the abdominal segments, follow this caecum to its connection with the intestine, and follow the latter to the *anus*, noting its chitinous lining.

4. Cut out the alimentary tract, open the stomach, and examine the grinding and straining apparatus.

Make a drawing of the alimentary canal.

Excretory Organs.—Examine the *antennary gland* (green gland) on the inside of the carapace opposite the base of the

¹The specimen must be large and mature.

antenna. It consists of a thin *bladder*, and, anterior to this, a mass composed of a coiled tube which opens at the base of the second antenna.

Nervous System.—Find the ring of *ganglia* around the ventral end of the sternal artery.¹ Trace the nerves from this to the appendages and to the small abdomen. Trace the *circumesophageal connectives* around the gullet (they anastomose just behind it) to the *cerebral ganglia*. Along with the distribution of the ophthalmic and antennary arteries, trace the nerves from the cerebral ganglia to the eyes, antennae, etc. Why should the nervous system be more concentrated than it is in the lobster?

Make a drawing of the nervous system.

Brooks: Hand-book of Invertebrate Zoology.

Churchill: Life History of Blue Crab. Bull. U. S. Bur. Fish., vol. 36, 1919.

Gurney: Metamorphosis of Corystes. Quart. Jour. Mic. Sci., 46, 1902.

Hay: Life History of the Blue Crab. Rep. U. S. Bur. Fish., 1912.

PAGURUS (Hermit Crab)

Examine a living specimen and see how it moves, and how the aperture of the shell is closed by the two large claws when the animal withdraws.

With a hammer crack the shell away from the animal and examine the twisted abdomen.

1. Has it lost its symmetry in appendages as well as in shape?

2. How many of the appendages have been retained? What is the function of these appendages?

3. Remove several other specimens from their shells and place them in a dish of sea water together. Do they seem disturbed? Compare their actions with those in shells.

4. Place an empty shell in the dish and see what happens.

5. Put more empty shells in the dish, but be sure they are not quite large enough for the crabs. Then add some

¹In a fresh specimen the ganglia can be more easily studied after treating them with strong alcohol or Schaudinn's fluid for a moment.

larger shells and watch the crabs test them to determine which will serve best.

A drawing is desirable.

Thompson: The Metamorphoses of the Hermit Crab. Proc. Bost. Soc. Nat. Hist., 31, 1903.

EMERITA (Sand Mole)

On sand beaches, between low- and high-water mark, there may frequently be seen the shallow depressions that mark the places where these animals have burrowed. They may be dug out with a shovel, but they quickly disappear again.

1. Notice their shape and the ease and rapidity with which they burrow.

2. Place specimens in a dish containing sand and a little sea water and try to determine just how the burrowing is done. This may frequently be done by holding a specimen so it just touches the sand. Which end goes into the sand first? Notice the positions in which the appendages are held. Does this have anything to do with the direction in which it burrows? Does the animal jump or crawl? In what direction and how can it swim?

3. Examine the body and see if it is divided into *head*, *thorax*, and *abdomen*. In what way is the shape of the telson adapted to its function?

4. Examine the appendages.

(a) The stalked eyes.

(b) The biramous *first antennae* and the exceedingly long, feathery *second antennae*. What is the usual position of the antennae?

(c) The *mouth* appendages. Are strong, hard mandibles present? What must the character of the food be?

(d) The thoracic appendages. How many are there? Are they similar? Are there any chelae?

(e) The abdominal appendages. Are they all alike? What functions are performed by them?

Make a drawing.

CHLORIDELLA

Compare the animal carefully with the lobster, noting all of the important differences. The posterior three thoracic segments are free. The male possesses a copulatory organ on the basal joint of the last thoracic leg. In the female the opening of the oviducts is in the midventral line, on the next to the last thoracic segment. Examine the chelae and compare them with the chelae of a lobster. Are they homologous appendages in the two animals? If you have living specimens, study their movements while they are walking and swimming.

A drawing of a side or ventral view will be profitable.

Internal Anatomy.—1. Remove the top of the carapace and abdomen. Beneath the muscles note the elongated, white tube, the *heart*, which extends from the stomach to the fifth abdominal segment. The anterior end is slightly enlarged and gives rise to the *anterior aorta*. The posterior end gives rise to a *posterior aorta*. Note *lateral arteries* and *ostia*. Remove the heart.

2. Beneath the heart, in the male, is a whitish, pigmented, flattened mass which consists of two convoluted tubes, the *testes*. Cut this mass across between the second and third abdominal segments and force it posteriorly. The two testes are continuous posteriorly. Follow them anteriorly and find the slender, dense, coiled *vasa deferentia* passing outward and downward at the posterior end of the third thoracic segment. Cut them and lay them back where they can be dissected later. The testes extend forward to the region of the stomach. Remove the testes.

3. Beneath the heart, in the female, are the two *ovaries*. Trace them forward and backward, and find the very slender *oviduct* that extends from each outward and downward in the region of the antepenultimate thoracic segment. Remove the ovaries, deferring the tracing of the oviduct.

4. Beneath the reproductive organs is the granular *liver*. This consists of two lobes which extend from the stomach to

the end of the telson. They form saccular diverticula between segments and in the telson. Where do they open into the alimentary tract?

5. Free the *intestine*, which is between the lobes of the liver. The rectum is in the sixth abdominal segment.

6. Pull back the anterior end of the *stomach*, identify the circumesophageal connectives, in order not to destroy them, and free the stomach by cutting the esophagus and intestine. Examine the stomach under water.

7. Trace the *nerve chain*. What ventral ganglia are fused? The *cerebral ganglia* are most easily exposed by slicing away, very superficially, the dorsal surface of the rostrum and pressing the eye muscles apart.

A drawing of the nervous system will be profitable.

8. Trace the genital ducts to their external openings.

MICHTHEIMYSIS (OR HETEROMYSIS)

If living specimens are to be had, watch them swim, and determine what parts are used in swimming. Does the animal swim in one direction or in both?

1. Compare the body with that of a lobster.

2. Are appendages present on each of the divisions of the body? Compare them with the appendages of the lobster? How do the thoracic appendages differ?

3. Notice the *statocysts* in the tail fin.

4. The living animal is transparent, and many internal organs, such as heart, gills, and portions of the alimentary canal, can be seen.

If time permits, make a drawing.

Bergh: Beiträge zur Embryologie der Crustacea. Zool. Jahrb. (Anat.), 6, 1893

TALORCHESTIA (Beach-flea)

These active little animals inhabit sand beaches, where they burrow in the sand near high-water mark. Turn over some of this sand and notice the activity of the animals that are disturbed. (In the decaying vegetable matter which

accumulates along such beaches along high-water mark, smaller animals of a closely related genus, *Orchestia*, may be found. The movements of both species are much the same.) How far can a specimen leap? Are the leaps of an individual continuously in one direction, so it may get away from the point of danger? Is each leap straight forward or does the animal whirl in the air? What purpose may be served by the leaping? Try to catch a specimen. Determine how the leaping is accomplished. Determine how the specimens burrow.

If you will walk along a beach some quiet night with a lantern you will probably see something of the night activities of these animals.

1. Select a large specimen and count the number of segments. Is the body divisible into head, thorax, and abdomen?

2. The *eyes* are not stalked. Are they compound?

3. The *second antennae* of the male are very large. Compare them with the first antennae and with the antennae of a female.

4. Around the mouth are the *labrum*, forming an upper lip, the *first maxillipeds* (fused), forming a lower lip, and between them the *mandibles*, *first maxillae*, and *second maxillae*.

5. Examine the appendages behind the mouth. How many are there? How many bear claws? Compare these claws with those of a lobster, and see how they differ. Which appendages are used in crawling? Some of the appendages are arranged so they can be twisted around by the sides of the animal. What is their function? What are the remaining appendages used for?

6. Spread the appendages apart and find the gills, which are attached to the bases of the appendages.

Make a drawing of the animal.

Kunkel: The Arthrostraca of Connecticut. Conn. State Geol. and Nat. Hist. Survey, Bull. 26, 1918.

Smallwood: The Beach Flea: *Talorchestia longicornis*. Cold Spring Harbor Monogr., 1, 1903.

PORCELLIO OR ONISCUS (Sow-bug)

These animals occur in damp places, such as under stones, logs, etc., and in cellars. They live for the most part on decaying vegetable matter. To what class of the Arthropoda do they belong?

1. Notice the shape. Is this an adaptation?

2. Is the body divisible into head, thorax, and abdomen? Count the number of segments. Is there any evidence of fusion at the posterior end of the body?

3. Examine the appendages.

(a) Are the *eyes* stalked or sessile?

(b) Only one pair of antennae is well developed, the first pair being rudimentary.

(c) The mouth appendages are small. They consist of *mandibles*, two pairs of *maxillae*, and one pair of *maxillipeds*.

(d) How many *walking legs* are there? Are these all alike?

(e) Notice the character and number of the *abdominal appendages*. On the posterior surface of all but the last pair, which are modified to form *anal feelers*, are gills. These are the only respiratory organs. Why must these animals live in damp places?

Make a drawing of the animal from the ventral side.

CAPRELLA (Goat Shrimp)

These animals are very common on hydroids, but because of their peculiar shape and slow motions are rather inconspicuous. Watch the animals and see how they move. Is the body kept at rest and moved by the action of the appendages, or how is movement from place to place effected? Are the appendages adapted for grasping? Watch specimens and see if you can determine on what they feed.

The form is of interest because of its extreme modification to suit it to the needs of its life. There is some difference in the structure of the male and female.

1. Count the segments of the body. Do they differ in

number and shape in male and female? The first represents the head with two fused thoracic segments. The abdomen forms a minute protuberance at the posterior end of the body.

2. At the anterior end of the body are the *eyes*, two pairs of *antennae*, a pair of *maxillipeds*, and a pair of *legs*.

3. At the hinder part of the body are three pairs of *legs*.

4. Near the middle of the body of the female, and near the anterior end in the male, is another pair of *legs*.

5. On two of the segments which do not bear legs are *gills*.

If time permits, make a drawing.

BRANCHIPUS (Fairy Shrimp)

These animals may be found in pools of fresh water in the early spring, just as the ice is leaving. Their method of swimming by means of the large, expanded appendages should be observed.

1. Into what parts does the body seem to be divided? Do all of these parts show segmentation?

2. Find the following organs.

(a) The stalked, prominent *eyes*.

(b) The *antennae*. In the female the first are slender and the second vestigial. In the male the first are slender and the second are enormously enlarged to form a clasping organ.

(c) The *labrum* forms an upper lip.

(d) The *mandibles*, beneath the labrum and by the sides of the mouth. Do they have cutting edges?

(e) Vestigial *maxillae* behind the mouth.

(f) *Swimming appendages*. How many are there? Notice the fringe of hairs on each. What are these for? Remove one and examine it with a microscope. The lobes have been described as exopod and endopod, but their exact relationship is not certain.

A drawing is desirable.

DAPHNIA

This small fresh-water form frequently occurs in large numbers in small pools and brooks. Determine how it swims. Being small and transparent, it may be satisfactorily studied with a compound microscope.

1. Notice the shape and extent of the protective covering. To what part of other crustaceans does this correspond? Are the appendages and the abdomen capable of being thrust out? Are there any signs of segmentation of the body?

2. Determine what parts are used in keeping a current of water passing through the shell. Why is such a current needed?

3. If the animal carries young, notice how they are kept in the brood chamber by a spine that extends up from the dorsal portion of the base of the abdomen.

4. Notice the beating of the *heart*.

5. Are the *eyes* stalked or sessile? They frequently show a peculiar reaction to light. If the light is cut off from the microscope, the eye will be seen to rotate on its axis. If the light is admitted again, the eye rotates back to its original position.

6. The *first antennae* are very small and project ventrally. What is the chief function of the *second antennae*?

7. Several appendages will be seen inside of the shell, but it is hard to determine their exact relation. The functions of some of them may be apparent.

A drawing is desirable.

CYCLOPS (Water-flea)

Almost any free-swimming copepod, either fresh water or marine, will answer quite as well as the fresh-water Cyclops.

Cyclops may be found in almost any pool of fresh water and the marine forms are among the most abundant of the animals of the sea. Surface skimming of the sea, made with a net composed of cheese cloth or silk bolting cloth, will yield an abundance of material.

1. Watch the animals and see how they swim. With a pipette try to catch a certain individual and see whether the jerky movements probably aid these animals in escaping enemies. Determine what organs are used in swimming.

2. With a microscope examine specimens that have been confined under a cover glass, and notice the shape of the body. Into what parts is it divided? Count the number of segments. Look for evidence of fused segments. Notice how the spines on the abdomen are arranged.

3. Do you find *eyes* that are equivalent to the usual arthropod eyes? Do you find an eye spot? If such a spot is found, determine its position and shape.

4. Which pair of *antennae* is larger? Why are the large *antennae* fringed with spines?

5. Are there *thoracic* or *abdominal* appendages? Are any appendages other than the first antennae used in swimming?

6. The mouth parts consist of *mandibles* and two *maxillae*.

7. If the specimen is a female it may have two large egg sacs attached to the sides of the base of the abdomen. The female has two of the abdominal segments fused. In the male the segments are free.

A drawing of the specimen is desirable.

Fish: Seasonal Distribution of the Plankton of the Woods Hole Region.

Bull. Bur. Fisheries, vol. 41, Doc. 975, 1925.

Heath: The External Development of Certain Phyllopoets. Jour.

Morph., vol. 38, No. 4, 1924.

Sharpe: Notes on the Marine Copepods and Cladocera of Woods Hole and Adjacent Regions, Including a Synopsis of the Genera of the Harpoeticoida. Proc. U. S. Nat. Mus., 38, 1910.

Wheeler: Free-Swimming Copepods of the Woods Hole Region. Bull. U. S. Fish Com., 19, 1899.

ARGULUS (Fish-louse)

These animals may be found on many species of fresh-water and marine fish. Notice their shape and determine how they cling to their host. Are they able to crawl? Can they swim?

Find:

1. Into what regions can the body be divided?
2. The *eyes*, the *eye spot*, and the two pairs of small *antennae*.
3. The sucking *proboscis*, composed of *mandibles* and *maxillae*, which lies between the suckers.
4. The *suckers*, which are the modified *second maxillae*.
5. The *posterior* (third) *maxillipeds* just behind the suckers.
6. Four pairs of biramous thoracic appendages. What is their function?

Make a drawing of the animal.

Wilson: The Fish Parasites of the Genus *Argulus* Found in the Woods Hole Region. Bull. U. S. Bur. Fish., 24, 1904.

LEPAS (Goose Barnacle)

If possible, examine a cluster of specimens as they naturally occur attached to floating timber.

1. Account for the fact that the peduncles are much larger in some specimens than in others. Are they contractile so the body may be moved into different positions? Would such movements be of value?

2. Notice the thoracic appendages. Can they be thrust from the shell? What is their character? What are their characteristic movements? Drop a small piece of clam meat on these appendages of a living specimen and see what happens. What kind of food would they naturally collect?

3. Examine the portions of the shell. The portion on the closed margin is the *carina*, laterally and near the base of the peduncle are the *scuta*, and near the extremity the *terga*. Why are there so many pieces? Notice the lines of growth and determine the direction of growth of each piece.

Draw the animal as seen from one side.

Carefully remove the *carina* and with a scalpel or razor cut a preserved specimen into right and left halves, extending the cut through the peduncle.

4. The mouth will be seen at the end of a rather thick

prolongation which extends to near the bases of the abdominal appendages. On the margin of this prolongation are the small scalelike *mandibles*, *first maxillae*, and *second maxillae*. The *stomach* is rather large and the small *intestine* leads to the posterior end of the abdomen, where it opens between the abdominal appendages.

5. The *nervous system*, consisting of a large pair of cerebral ganglia and a short ventral chain of ganglia, should be seen in such a section.

6. The animal is hermaphroditic. The *testes* lie dorsal to the stomach and communicate with a conspicuous coiled *vas deferens* that is continued to the elongated *penis* at the end of the abdomen. What need is there for such a long penis? The *ovary* occupies the interior of the peduncle. The *oviducts* are inconspicuous and hard to follow. They open near the bases of the anterior thoracic appendages. An *ovigerous mass* may sometimes be found lying between the body and mantle. Remove some of this, place on a slide, cover, and examine with the compound microscope.

7. Examine the appendages carefully and be sure that you understand the relation of parts. Remove the *thoracic* appendages one by one on one side only. What part must the peduncle represent? Note the beautiful adaptation of the animal for its life.

A drawing showing the organs is desirable.

Bigelow: Early Development of Lepas. Bull. Mus. Comp. Zool. Harvard, 40, 1902.

Delage: Evolution de la Sacculine. (Sacculina carcini.) Arch. Zool. Exp. et Gen., 2° Series, 11, 1884.

ARACHNIDA

LIMULUS (Horseshoe Crab)

Notice the way in which the animal crawls upon the bottom. Is it well protected from enemies? Examine it carefully for parasites and for animals that are attached to it. Disturb it and see if it will swim. The animals are usually

quite active in the evening, and if you visit a car in which they are kept, at this time of the day, you are likely to find them crawling up the sides, falling over and swimming on their backs. In this position it is easy to determine how they swim. The animals are very hardy and will stand even complete removal from the water for days at a time. During the spring and early summer, eggs are deposited in the sand; the male holding to the edge of the abdomen of the female with claws modified for the purpose, is dragged after her. If possible, the method of egg deposition and fertilization should be observed.

1. The animal consists of a hoof-shaped *cephalothorax*, an *abdomen*, and a *caudal spine*. How are these joined? Is there any indication of segmentation of any of them?

2. Examine the *eyes* with a lens and see that they are compound.

3. On the lower side of the cephalothorax notice the appendages. Are they all built on the same plan? Compare them in male and female. Do you know what the modifications are for? Compare the pincers with those of a lobster. The first pair of appendages is called the *cheliceræ*. Between the bases of the last pair of walking legs are the *chilaria*. Behind the chilaria is the broad flat *operculum*. Does this show evidence of being modified appendages? What is its function?

4. Between the bases of the cephalothoracic appendages is the *mouth*. Do the bases of the appendages show any modifications that may serve as teeth? Can the pincer-bearing appendages be so bent as to be used in feeding?

5. Along the sides of the abdomen notice the movable spines. How many are there?

6. Under the operculum are the *gills*. How many groups are there? Are they arranged in pairs? How are they attached to the body? Are they movable? What reason is there for moving them? Examine a bunch of gills, frequently called a *gill book*, and see how it is formed.

7. At the base of the caudal spine notice the *anus*.

Make a drawing of the ventral surface.

Internal Anatomy.—If the *Limulus* is alive the simplest method of killing it in preparation for dissection is to cut off the legs and allow the blood to drain off from the large blood sinuses which extend into each leg.

In order best to remove the heavy *carapace* it is desirable to make several cuts in the exoskeleton with a hacksaw or a fine-toothed saw. (For an outline showing a good method of making the cuts, see Cole.) With cuts properly made lift a corner of the carapace slightly, and with scalpel cut away the adhering tissue. Use short cuts and keep the cutting edge close to the inner surface of the exoskeleton. With the carapace raised in the region of the *compound eyes* it is possible to see the nerves leading to these organs. When the piece of the cephalothoracic exoskeleton has been cut free from adhering tissue, the *ligament* along its posterior border should be cut and the entire piece removed. In cutting these ligaments be careful not to cut too deep for the heart lies close to the surface at this point. It is best to remove the abdominal exoskeleton by beginning at the posterior end. Cut the *interarticular membranes* at the base of the telson and lift the abdominal carapace slightly. The two rows of depressions seen on the surface of the latter indicate the position of chitinous infoldings of the exoskeleton, called *entapophyses*. The *heart* lies, in large part, between these rows. Slip the scalpel (or a small pair of bone forceps) under the exoskeleton and cut the entapophyses one at a time. The exoskeleton may now be separated from the soft tissues underneath and removed. The animal should now be placed in a large crystallization dish and the rest of the dissection carried out under sea water.

The Circulatory System.—Remove such portions of the *gonads* (orange masses) and *liver* tissue (yellow masses) as may cover the *heart* and major *blood vessels*. The heart will now be seen as a long tubular organ lying enclosed in

the *pericardium*. Locate the eight pairs of *ostia*, which are transverse, slitlike valves opening from the pericardium into the heart. Note the median and the two lateral *cardiac nerves* lying on the pericardium. From the anterior end of the heart extend the *median frontal artery* and the two lateral *aortic arches*. The former extends forward and downward and branches into two *marginal arteries*. The aortic arches pass down on either side of the *proventriculus* and follow the *esophagus* to the *nerve collar*. Here the vascular system widens to enclose the *collar*, then extends posteriorly, enclosing the *ventral cord* (*vascular ring* and *ventral artery*). Branches from the ventral artery supply the telson.

Along the sides of the anterior half of the heart may be found four pairs of *lateral arteries*. These are valved tubes leaving the heart just beneath the first four pairs of *ostia*. The lateral arteries on either side lead into a *collateral artery* extending in a posterior direction. Each of the collateral arteries gives off branches going to the muscles and the *hemal surfaces* of the body, and other branches (directed toward the median line) to the digestive tube. The two collateral arteries unite just behind the heart to form the *superior abdominal artery*. Branches of this artery anastomose with branches of the *ventral aorta*.

Five pairs of *branchiocardiac canals* or sinuses bring blood from the gills to the *pericardial sinus*. The first of these receives blood from the *operculum* and the first gill; the remaining four receive blood from gills 2, 3, 4, and 5 respectively.

The remaining portions of the circulatory system are not so easily seen, but will be mentioned for the sake of completeness. The *vascular ring* supplies blood to the appendages of the cephalothorax. The ventral artery supplies the gills. Blood from all parts of the body collects in a pair of *longitudinal sinuses* which lead to the gills.

The Digestive System.—Remove the heart and such gonad and liver tissues as lie in the way. Observe the large sac-like proventriculus or *stomach*, extending into the anterior

part of the cephalothorax. If this organ be pushed to one side it is possible to see the *esophagus* leading from the mouth forward to the under side of the stomach. Turning now to the more posterior parts of the digestive tube, it will be seen that the stomach leads directly into the long, straight *intestine*. The latter ends in a muscular *rectum*, which opens to the exterior through the *anus*. If the esophagus, stomach and pyloric valve be split open, the strongly ridged chitinous lining may be seen. The intestine, on the other hand, has a glandular lining. On each side of the intestine may be found a pair of *hepatic ducts* which convey fluids from the liver to the lumen of the intestine.

The Nervous System.—Sever the digestive tube at the rectum and again at the junction of the esophagus and stomach. Carefully remove this section of the tube. The *endocranium* (a cartilaginous shelf supposed to be homologous with the cartilaginous brain case of certain vertebrates) is in clear view. This should be removed carefully to expose the underlying *brain* or *nerve collar*. The esophagus is completely encircled by the brain. Note an anterior enlargement, the *frontal lobe* or *forebrain*. Three *olfactory nerves* and a nerve to the *median eye* are given off from its anterior end. Arising from the hemal surface of the forebrain are a pair of *lateral eye nerves* and a pair of smaller lateral or *first hemal nerves*. Behind the latter are seven pairs of *hemal nerves* radiating from the nerve ring. A pair of *stomodéal nerves* arise from the inner anterior edge of the nerve ring.

The nerves so far described are those the origins of which may be seen when the brain is *in situ*. All nerves should be fully exposed and traced to the organs which they supply. Schaudinn's fluid may be used to whiten the nervous tissue. (Note: this solution is poisonous.)

It is desirable to draw the nervous system in situ.

Further study of the nervous system may be made by removing the brain and nerve cord. To remove the brain and

cord, dissect the esophagus from the nerve ring; free the nerve cord from the cartilaginous bridges that extend across it at intervals; sever the main nerves at some distance from the brain and cord; lift the entire central nervous system out and place it in a crystallization dish.

Before this system is further studied it should be hardened and whitened. First arrange the brain, cord and nerves in a normal position and cover the system with a piece of dry newspaper. Hold the paper in place with the fingers and slowly pour over it enough 5 per cent formalin just to cover the paper. In about half an hour the nervous tissue will be hard and white. The preparation may then be turned to expose the neural surface of the brain and cord, and the origins of the neural nerves.

Note the *preoral commissure* extending across the nerve ring anterior to the position occupied by the esophagus. The commissure gives off three *rostral nerves* directed caudad. Find four *postoral commissures*, the last three of which are fused. Just behind the forebrain arises a pair of cheliceral nerves. Five pairs of large nerves are given off from the nerve ring to the next five thoracic appendages. Each of these gives off a *neural mandibular* branch and several *hemal entocoxal* branches. Two pairs of small nerves are given off from the posterior part of the nerve ring at points neural to the origin of the nerve cord. These are the *chilarial* and *opercular* nerves.

The double nerve cord extends into the abdomen. Here may be found 5 pairs of ganglia, and a terminal knot consisting of 3 pairs of ganglia closely fused. Each of the first five pairs of ganglia gives off a pair of *hemal nerves* directed cephalad, and a pair of *neural nerves* directed caudad. Usually the neural nerves are lacking in the last 3 pairs of ganglia. The 3 pairs of hemal nerves are spread fan-wise. The pair nearest the median line innervates the telson.

Make a drawing of the nervous system from the neural aspect.

The Excretory System.—The *coxal glands* lie one on each side of the endocranium. They may be recognized by their brick-red color. Lobes extend into the bases of the second, third, fourth and fifth pairs of appendages. A duct leads from each of the last pair of lobes, opening on the posterior face of the fifth appendage.

Cole: Preliminary Dissection of *Limulus*. Collecting Net, 9, 1934.

Lankester: *Limulus* an Arachnid. Quart. Jour. Mic. Sci., 21, 1881.

Packard: The Anatomy, Histology, and Embryology of *Limulus polyphemus*. Mem. Bost. Soc. Nat. Hist., 1880.

Patten and Redenbaugh: Studies on *Limulus*, Jour. Morph., 16, 1899.

Zittel: Text-book of Paleontology. Macmillan and Co., Ltd., 1913.

BUTHUS (Scorpion)

Living specimens of these animals are not usually available for laboratory study. They live for the most part concealed during the day under old bark and in crevices and holes and are active at night. Their food is largely spiders and insects which are seized by the claws and killed with the abdominal sting.

1. Into what parts is the body divided? How many segments are recognizable? Which are the most freely movable?

2. Look for *eyes*. Do you find any besides the large pair?

3. Find four pairs of slitlike openings on the ventral side of the pre-abdomen. These are the *stigmata*, the openings of the lung books.

4. Find the following appendages:

(a) The *chelicerae*. What is their structure and where are they placed?

(b) The *pedipalpi*. Compare them with the *chelicerae* and count their segments.

(c) Four pairs of *walking legs*. Count their segments and see if they are armed with claws.

(d) The comb-shaped *pectines*. Are they on the thorax or the abdomen? Their function is doubtful.

5. Examine the *mouth*. Are there any jaws? Is a labrum present?

6. Find the position of the *anus*. The terminal spine is provided with a poison gland and serves as a *sting*. In the living animal, the postabdomen is habitually carried over the back.

Make a drawing of the under side of a specimen.

EPEIRA (Round-web Spider)

Examine the webs of different species of spiders and see how they are constructed. Do all of the webs have places for the concealment of the owners? Do all spiders seem to construct definite webs for the capture of insects? How do spiders entangle insects in their webs? Do different kinds use different methods? What parts of insects are eaten?

By destroying webs that are occupied by spiders that are in convenient places for observation, the construction of new webs may be observed. Notice how the framework of a round web is laid and then how the threads are attached to the framework. Are any of the legs used in handling the thread? Are spiders equally active at all times of the day?

Spiders' webs may frequently be seen floating in the air, especially in the late summer or autumn. By watching spiders that are on fences and bushes the formation of these threads may be observed. Watch such a spider and see if you can determine the use to which the thread is put.

Capture a spider and watch it descend by a thread. Where is the thread formed? Does the spider hold to it with its legs? Keep taking the thread up so that the spider cannot reach the ground, and see if there is a limit to the amount that can be formed. When the spider starts to climb the thread see how this is done, and whether the thread is taken up as the animal climbs or is allowed to float free.

Find where spiders lay their eggs. Some carry them. If you can find a specimen with an egg sac, see how it is carried and whether it will drop its eggs when frightened. Remove the egg sac and see if the spider will accept it again. Open

several egg sacs and see if the eggs all appear to be in the same stage of development.

Study the movements of the animal and see how many of the appendages are used in locomotion. Are any of the appendages used sometimes for locomotion and sometimes for feeling?

Examine the external structure of *Epeira*.

1. Into what parts is the body divided? Do both parts bear appendages?

2. Look for *eyes* on the anterior end of the body. How many are there? Do they seem to be simple or compound? Determine whether a specimen can see.

3. The following appendages should be found:

(a) The *chelicerae* or *mandibles*. Notice their structure and see that each ends in a sharp claw. The poison gland discharges at the tip of this claw.

(b) The *pedipalpi* or *palpi*. How many segments have they? Examine their tips for claws. What are they apparently used for?

(c) Four pairs of *legs*. Are they all alike? Count the segments and examine their tips for claws.

(d) On the abdomen, three pairs of *spinnerets*. Notice their positions and see if they are segmented. Understand their function and whether they are all used at the same time. They are probably true abdominal appendages.

4. On the lower surface of the abdomen, near its anterior end, are two slits, the openings into the *lung sac* or *lung books*. They are respiratory in function.

5. Just in front of the spinnerets is a minute median pore, the *spiracle*, that is often very hard to find. It is the external opening of a series of abdominal tracheae.

Make a drawing of a ventral view.

Baerg: *The Black Widow; Its Life History and the Effects of the Poison.* Sci. Mo., vol. 17, 1923.

Comstock: *The Spider Book*, 1913.

Emerton: *New England Spiders.* Trans. Conn. Acad. Many papers, 1882-1915.

- Montgomery: Studies on the Habits of Spiders, Particularly Those of the Mating Period. Proc. Acad. Nat. Sci., Philadelphia, 1903.
- : On the Spinnerets, Cribellum, Colulus, Tracheæ and Lung-books of Araneads. Proc. Acad. Nat. Sci., Philadelphia, 1909.
- : The Development of Theridium, an Aranead, up to the Stage of Reversion. Jour. Morph., 20, 1909.
- : The Significance of the Courtship and Secondary Sexual Characters of Araneads. Am. Nat., 44, 1910.
- Peckham: Observations on Sexual Selection in Spiders of the Family Attidæ. Occas. Papers Nat. Hist. Soc., Wisconsin, 1 and 2.
- Wood: Autotomy in Arachnida. Jour. Morph., vol. 42, No. 1, 1926.

PHOXICHILIDIUM

The exact affinities of the pycnogonids to other forms is not known, but they have certain characters that have suggested a possible relationship to the Arachnida. They are frequently found in considerable abundance on the material that is attached to piles. Notice their movements and see how they cling to the material on which they are moving.

1. The body is very slender and is composed of a number of free segments that form the *head* and *thorax* and a small, vestigial *abdomen*. How many free segments are there? At the anterior end is a rather prominent *proboscis*, with the mouth at its end.

2. The following appendages will be found:

(a) The *chelicerae*. What is their structure? Are they armed with pincers?

(b) Four pairs of long *walking legs*. How many segments have they? The viscera extend into the bases of these appendages.

(c) The male is provided with a pair of ventral appendages called the *ovigerous legs*, by means of which the eggs are collected as they are laid by the female. These appendages are not present in the female.

Make a drawing of the under side of a specimen.

Cole: Pycnogonidia of the West Coast of North America. Harriman Alaska Exped., 10, 1904.

MYRIAPODA

LITHOBIUS (Centipede, Earwig)

These animals may frequently be found under stones, logs or boards, or about rubbish or manure heaps. They live largely on insects, larvae, and small worms, and are very active.

1. Notice the shape of the body and count the number of segments. Is there a distinct head? Are the segments very movable?

2. How many appendages does each segment possess? Are all of the segments provided with appendages? Allow the animal to run and see how the legs are used. Do those of a side all move in the same direction at the same time? Are all of the legs alike? Notice the pair of appendages just behind the head and see how they differ from the others. These appendages are organs of prehension that are used in grasping the prey. They are provided with poison glands that open on their inner sides near their free ends.

3. Examine the head and find the *eyes*, *antennae*, and *mouth parts*. The latter consist of a *labrum*, a pair of *mandibles*, and two pairs of *maxillae*, the last pair of which is united to form a *labium*.

4. Understand how the animal breathes. The *stigmata* are situated near the bases of the legs, but are hard to see except in favorable specimens.

Make a drawing of the animal.

JULUS (Thousand-legs)

These animals are frequently very abundant under the dead bark of logs or stumps, in decaying wood, and in decaying heaps of grass. In the autumn they frequently congregate under boards and in corners. They feed largely on decaying vegetable matter, but may become pests in gardens, destroying tomatoes and fallen fruits and many vegetables.

1. Disturb a specimen and see how it rolls up. Can this

be protective? See if there is any odor when it is disturbed. What purpose can such an odor serve?

2. What is the shape of the body? Is it hard or soft? How many segments are there?

3. How many appendages are borne on a segment? Do all of the segments bear appendages? Does the animal move rapidly? Does the first pair of appendages behind the head show modifications for prehension? Does this animal need such an organ?

4. Compare the organs of the head with those of the preceding form.

Make a drawing of the under side of one segment.

Williams: Habits and Structure of *Scutigerebella immaculata*. Proc. Bost. Soc. Nat. Hist., 33, 1907.

INSECTA

ACRIDIDIUM (Grasshopper)

Study grasshoppers as they occur in nature and determine as far as possible the following points:

1. Do they see or hear? Are they equally sensitive to touch on all parts of the body? Are these animals well provided with sense organs?

2. What is their food? Are all plants eaten or are some avoided? See how the mouth parts are used in feeding.

3. What are the important enemies of grasshoppers? How do they escape their enemies? Do they hide? Are they protectively colored? How does jumping serve them better than crawling? How many times its length can a grasshopper jump?

4. During late summer and autumn you may find individuals depositing eggs. See if you can determine how the end of the body is worked into the ground.

For study it is desirable to use a rather large, freshly killed or alcoholic specimen.

The body is divided into three well-marked regions.

1. **The Head.**—Is it movable? Does it need to be as movable as your own head? It bears several organs.

(a) The *compound eyes*. Examine one with a lens or remove its outer covering and examine it with a compound microscope. You should understand the structure of the whole eye and how it gives a single visual image.

(b) The *ocelli*, three in number, one near the middle of the front part of the head and the others placed near the bases of the antennae.

(c) The *antennae*. Why are they so flexible? Examine one with a microscope and notice the spines. What are these for?

(d) *Mouth parts*. These should be studied later.

2. **The Thorax.**—Why should it be large and comparatively firm? This portion is more or less distinctly divided into three parts, each of which carries a pair of legs.

(a) Compare the three legs on one side. Do they have the same number of segments? Do all of the joints of the leg move in the same plane? The five divisions of a leg are, beginning with the basal end: *coxa*, *trochanter* (immovably joined to the coxa in the leaping legs), *femur*, *tibia*, and *tarsus*, which is composed of four movable pieces. Do the femurs of the leaping legs differ from the femurs of the other legs? Account for this. Determine how the foot is arranged to hold to objects. Have you noticed a grasshopper settle its feet preparatory to jumping? Examine the joint between the femur and tibia.

(b) Examine the wings and notice their size, shape, places of attachment, and general character. Do they apparently have different functions to perform? Notice how the posterior wings are folded so that they may be covered by the anterior. Does this seem greatly to reduce their strength?¹

3. **The Abdomen.**—Count the number of segments. Each one is covered dorsally by a *tergum* and ventrally by a

¹You should examine the posterior wing of a beetle and see how it is folded.

sternum. Is the abdomen more movable than the other portions? Of what advantage is this condition? The posterior ends of the abdomens of male and female differ. This portion of the female is modified to form the *ovipositor*, which consists of two large pairs of plates that inclose a smaller pair of plates. It is between these plates that the oviduct opens. What advantage lies in the fact that the larger plates possess hard tips? Along the sides of the abdomen notice the *stigmata*, the external openings of the respiratory system. Do you find *stigmata* on other parts of the body?

Draw an enlarged side view of a grasshopper, placing the appendages in their proper positions.

Mouth Parts.—It has already been noticed that the mouth parts serve to cut off pieces of leaves, which are then passed directly into the alimentary canal. For such a purpose there should be holding as well as cutting parts.

1. Pass a needle under the *labrum*, which forms the upper lip, and notice that it is hinged and that the end is lobed. It is not supposed to be homologous with usual arthropod appendages. With fine scissors remove it and place it in a watch glass containing water.

2. Immediately behind the *labrum* is a pair of hard, dark-colored organs, the *mandibles*, that are used in cutting the food. Their position should be carefully noted, but it will be better to leave them in position until the other mouth appendages have been removed.

3. Situated by the side of the mouth and just behind the *mandibles* are the *maxillae*. With a needle push one to one side and notice that it consists of a somewhat flattened portion with a jointed *maxillary palp* at one side. Carefully determine the positions of the *maxillae* with relation to other parts. What possible uses are served by the two parts? Remove them with scissors and place them in the watch glass with the *labrum*, in approximately their relative positions and study carefully.

4. Pass a needle behind the remaining appendage, the

labium, and see that it is hinged and forms the lower lip. Remove it with scissors and place it in position in the watch glass. You will find that it bears a pair of *labial palpi*, and that there is a deep cleft along the middle line. These are indications that the appendage is the result of the fusion of a pair of appendages (the second maxillae).

5. Remove the mandibles and examine their cutting margins. Place them in position in the watch glass.

Make a drawing showing the structure of each of these appendages. Arrange your figures as nearly as possible in the relative positions of the parts.¹

Internal Structure.—Remove the wings, and before opening the body notice the rather large, somewhat transparent *tympanum* on each side of the first abdominal segment, very near the base of the leaping leg. The structure of the auditory organ may be easily studied by staining, clearing, and mounting in balsam. (See Packard's "Text-Book of Entomology" or Brooks's "Hand-book of Invertebrate Zoölogy.") Remove the dorsal portion of the wall of the abdomen and thorax, and notice:

1. The *heart*, which will be found attached to the portion of the wall of the abdomen that has been removed, by means of numerous radiating muscle fibers. You probably will not be able to determine the structure of the heart in the dissection. Read this up, and determine what the radiating muscle fibers are for.

2. The space between the muscles and the viscera is filled more or less completely by the *fat body* and the *tracheae*. With a lens notice how the tracheae connect with the spiracles and how they branch. Remove a portion of the tissue in which you can see tracheae, mount it in water under a cover, and examine it microscopically. Each tracheal tube is

¹The mouth parts of insects that depend on biting off portions of plants for food are similar. Directions for the study of the mouth parts of the honey-bee are given further on, but the mouth parts of other forms, such as the fly, butterfly, and bug, should be studied.

marked by striations wound around it. Do you know what causes this appearance and what the arrangement is for? Do you understand how the tracheal system is arranged? What is the distribution of this system and how is the air made to go in and out?

3. Near the dorsal surface of the posterior part of the abdomen, surrounded by the tissues already mentioned, are the *gonads*. These differ in size and shape according to the sex. In the male the *vasa deferentia* may be seen leaving the lobulated testes. In the female the *oviducts* pass around the sides of the intestine. They may be followed later.

4. Loosen the anterior ends of the gonads and turn them posteriorly to expose the hinder part of the alimentary canal.¹

(a) The *esophagus*, which bends backward from the mouth, gradually enlarges as it enters the thorax.

(b) The *crop*, which is not sharply separated from the esophagus, gradually narrows posteriorly.

(c) Following the constriction posterior to the crop is the elongated *stomach*, frequently called the *ventriculus*. Surrounding the anterior end of this portion is a series of rather large diverticula, the *gastric caeca*, that extend both anteriorly and posteriorly from the points where they open into the stomach.

(d) Some distance behind the posterior ends of the hepatic caeca, quite concealed by the mass of small *uriniferous tubes*, is a slight constriction and hardening of the alimentary canal that marks the division between the stomach and *intestine*. It is at this point that the uriniferous tubes join the alimentary canal.

(e) Behind the intestine the alimentary canal becomes much smaller and is known as the *hind intestine* or *colon*.

(f) Behind the colon, forming the hinder portion of the alimentary canal, is the slightly enlarged *rectum*. The rectum

¹There is great diversity in the parts of the alimentary canals of different insects. This is correlated with the great differences in feeding habits.

cannot be seen until the ovary is removed, which should be deferred until the ducts have been seen.

Make a drawing showing the position of the parts of the alimentary canal in side view.

Cut the intestine and turn the alimentary canal posteriorly and anteriorly.

5. Notice the muscles:

(a) That move the abdominal segments.

(b) That move the legs (those that supply the wings have been destroyed).

(c) That move the jaws.

Do you understand now why the thorax needs to be comparatively large and firm?

6. The *nervous system* is directly comparable to that of the lobster, but the connectives between the ganglia will be found to be distinctly double and the ganglia to be somewhat differently arranged.¹

The ventral chain will be found to consist of a pair of *subesophageal*, three pairs of *thoracic*, and five pairs of *abdominal ganglia* with the connectives between them. Which of these are largest? Why is this the case? Trace the nerves from them and see what organs they supply.

Trace the connectives forward from the subesophageal ganglia and see that they pass around the esophagus, thus forming the *circumesophageal connectives*. Cut away the dorsal portion of the head and expose the *cerebral ganglia*.

Add the nervous system to the figure that shows the alimentary canal.

7. Trace the oviducts down around the sides of the body and notice that they unite with each other ventral to the nervous system, to form the *vagina*. This may be traced to its opening between the plates of the ovipositor. Dorsal to the vagina, opening to the exterior very near it, is a small sac, the

¹The arrangement of the ganglia in insects is very variable, showing many gradations in concentration.

spermatheca, which serves to store the spermatozoa received from the male until the eggs are laid.

The reproductive organs may also be added to your figure showing internal anatomy.

Lang: Handbuch der Morphologie der wirbellosen Tiere. Bd. 4, 1921.
Brooks: Hand-book of Invertebrate Zoology.

APIS MELLIFICA (Honey-bee)

The life of this form is so different from that of the grasshopper that, should time permit, a study of its complete anatomy would be profitable, but attention will here be confined to a few of the more general adaptations that fit it for its life.

Bees at work on flowers should be examined and the methods of getting honey and pollen noticed.

1. Catch by the wings a bee that has been gorging itself and bend the abdomen forward with your thumbnail until the bee disgorges. Notice where the fluid comes from and how much there is of it. When the abdomen is released watch the bee as it swallows the drop it has disgorged.

2. Notice where the pollen is carried, and see if you can determine how it is attached. Examine bees working on different flowers, or watch them as they enter their hives, and see if the pollen is always of the same color. Do you understand what the pollen is and what the bees use it for?

3. You may find bees gathering pitch from buds, knots, boards, or freshly varnished furniture, and fastening it on their legs. Do you know what this is used for?

4. Watch the entrance of a beehive and see if the bees going in are ever challenged. Perhaps you may see the method of defense. If so, you will notice that the stranger simply tries to get away. You may also see how dead bees and foreign materials are removed.

5. It is desirable to see something of the activities in the hive. This can be most satisfactorily done with a glass-walled observatory hive, by means of which comb-building, honey-

storing, egg-laying, brood-rearing, etc., can be very satisfactorily studied.

Directions for the study of the mouth parts and the sting are all that seem necessary, but the wings should be examined microscopically to see how those of a side are joined together, and a hind leg should be examined to see how the hairs on the tibia form a pollen basket.

Mouth Parts.—1. With a lens notice the pair of hard jaws, the *mandibles*, situated on the sides of the head at the base of the tongue. These mandibles are directly homologous with the mandibles of the grasshopper. Between the bases of the mandibles is a *labrum*, and extending from beneath the end of the labrum is a small *epipharynx*.

2. With scissors remove the *tongue*, which is normally carried against the lower surface of the thorax, and transfer it to a watch glass. It may now be dehydrated, passed into oil of cloves, placed in position on a slide, and mounted in balsam, when it can be studied best, or it may be immediately spread under a cover or between slides in glycerin.

3. The central portion is the hairy, segmented *labium* (the hypopharynx of some authors), bearing at its end a little pad called the *spoon*. The labium is folded lengthwise so as to form a pair of fine ducts which run from tip to base. The arrangement is such that the bee may, through blood pressure, unfold the labium. This probably is an adaptation for cleaning it. Attached to a median rod, the *mentum*, which forms the base of the labium, is a pair of flattened appendages, the *labial palps*, that are hinged so that they may be drawn together to inclose the labium and thus form a rather large tube, which is made more complete by means of the remaining pair of flattened appendages, the *maxillae*. On the outer margin of each maxilla is a small protuberance, the *maxillary palp*. When sipping from an abundance of liquid the extemporized tube formed by the labial palps and maxillae around the labium is used, the liquid being drawn in by means of the sucking stomach. When the liquid is in very

small quantities it is apparently lapped up by the spoon and transferred through the labium.

*A figure of the mouth parts is desirable.*¹

Sting.—The sting is to be regarded as a modified ovipositor that is no longer concerned in depositing eggs, but has become a weapon of offense and defense. It is accordingly present only in the female. The queen never uses her sting except on other queens.

Remove the dorsal integument of the abdomen of either a fresh or preserved specimen, and find the dark brown shaft of the sting, near the posterior end. Grasp the shaft with a pair of fine forceps and forcibly remove it. A considerable mass of tissue will be removed adhering to the base of the shaft, but this consists for the most part of accessory organs that must be understood. Spread the sting upon a slide, and either dehydrate and mount in balsam, or mount in glycerin. The balsam mount will prove more satisfactory, but the cover must be clamped down until the balsam hardens.

1. The shaft consists of three parts:

(a) A heavy support, called the *awl* or *sheath*, pointed at its extremity and sending a pair of arms or arches from its base, which normally bend ventrally, but are here forced to the sides. At its extremity each of these arches enlarges to form a rather large flattened plate, the *sheath plate*, to which strong muscles are attached.

(b) A pair of *lancets* which are fastened to the dorsal surface of the sheath and the sheath arches by tongue and groove joints (each tongue is enlarged along its inner margin so that it is held firmly in the groove). Each lancet is pointed at its free extremity, and its sides near the point are set with *barbs* that point toward the base of the sting. The arch of each lancet is continued past the end of the corresponding sheath arch, and is there articulated to one corner of a somewhat *triangular plate*. The remaining corners of each are

¹The comparative study of the mouth parts of a butterfly, horse fly, house fly, and mosquito will prove valuable.

articulated respectively to the large sheath plate and to another plate, the *oval plate*. Determine the attachment of the muscles to the plates and find what movements of the lancet the contraction of the different sets of muscles would cause. Note that the lancets are elastic and bend easily.

The large muscles attached to the sheath plates were attached to the wall of the abdomen and function to give the thrust that sets the sting. After the sting is drawn from the body of the bee the muscles attached to the plates continue active, and the sting works deeper and deeper in. Understand why it works in instead of out.

2. Lying near the base of the shaft is a large *poison sac* or *reservoir*, which is very muscular. It receives its poison from the *poison gland*, a long and narrow coiled tube that is bifurcated near its free end. It discharges the poison by means of the contraction of the muscles of its walls through a rather large, short duct into the space inclosed by the sheath and the two barbs. Each barb bears a prominence that serves as an injector, which moves backward and forward with the barb to which it is attached, in an enlargement of the basal portion of the sheath. It may be seen in the preparation. In this way poison is forced into the wound. Poison may also be admitted to the cavities of the lancets, which are hollow, and escape through minute pores near the barbs.

3. Lying near the base of the shaft of the sting, sometimes covered by the poison sac, may nearly always be found the last pair of abdominal ganglia, from which nerves may be traced to the muscles that are attached to the plates.

Understand the whole mechanism, how it is operated and its use.

4. Catch a living bee by the wings and press the end of the abdomen against a piece of soft leather, such as a leather-covered book. Pull the bee away and with a lens watch the movements of the sting, which will remain caught in the leather. Observe the spasmodic contractions of the poison sac. See how long and how energetically the movements are

continued and how deep the sting is worked in. This should remind you that a sting should be removed immediately, and that it should not be *pulled out*, as grasping the poison sac will aid in injecting the poison, but scraped off with a fingernail or rubbed off.

A drawing showing the mechanism of the sting is desirable.

Field: A Study of an Ant. Proc. Acad. Nat. Sci., Philadelphia, 1901.

Philips: A Review of Parthenogenesis. Proc. Am. Phil. Soc., 42, 1903.

Root: A, B, C and X, Y, Z of Bee Culture.

ECHINODERMATA

Radially symmetrical animals, with calcareous plates in the integument. Water-vascular system always present.

CLASS 1. Asteroidea.

With radiating arms not sharply defined from the central disk. Ambulacral feet in grooves on the oral side.

Order 1. Phanerozonia.

With large marginal ossicles. (Astropecten.)

Order 2. Cryptozonia.

Marginal ossicles inconspicuous. (Asterias.)

CLASS 2. Ophiuroidea.

With slender radiating arms sharply defined from the central disk. No ambulacral grooves.

Order 1. Ophiurida.

Arms not branched. (Ophiura.)

Order 2. Euryalida.

Arms branched. (Astrophyton.)

CLASS 3. Echinoidea.

Globular, or somewhat disk-shaped, spiny bodies. Shell or test composed of close-fitting plates.

Order 1. Regularia.

Nearly globular test. Spines rather large. Mouth and anus polar. Jaws present. (Arbacia, Strongylocentrotus.)

Order 2. Clypeastroidea.

More or less flattened test. Spines very small. Anus not polar. Jaws present. (Echinarachnius.)

Order 3. Spatangoidea.

Somewhat flattened and elongated. Spines very small. Neither mouth nor anus polar.

CLASS 4. Holothuroidea.

Bodies soft, elongated and cylindrical. Mouth and anus polar, the former surrounded by a circlet of large oral tentacles.

Order 1. *Elasipoda*.

Well-marked bilateral symmetry. Tube feet on ventral and papillae on dorsal surface. Deep sea only.

Order 2. *Pedata*.

Ambulacral feet in rows or scattered. (*Thyone*, *Cucumaria*.)

Order 3. *Apoda*.

Without tube feet. Wormlike. (*Synaptula*.)

CLASS 5. *Crinoidea*.

Temporarily or permanently attached by a stalk. With five branching arms radiating from a small disk.

Order 1. *Neocrinoidea*.

Characters as above. (*Antedon*, *Pentacrinus*.)

Berry: Metamorphosis of Echinoderms. *Quart. Jour. Mic. Sci.*, 38, 1905.

Coe: Echinoderms of Connecticut. *State Geol. and Nat. Hist. Surv.*, 19, 1912.

Grave: Occurrence among Echinoderms of Larvæ with Cilia Arranged in Transverse Rings. *Biol. Bull.*, 5, 1903.

Newman, H. H.: Experimental Analysis of Asymmetry in Starfish—*Patiria miniata*. *Biol. Bull.*, vol. 49.

ASTEROIDEA**ASTERIAS (Starfish)**

Starfishes are rather common along most coasts and are among the worst enemies of oysters, mussels, clams, and barnacles. They occasionally capture fish in aquaria. They can generally be most satisfactorily examined on shallow-water mussel-beds or on rocks covered with barnacles. Places where starfish occur should be visited, and the conditions under which they live studied.

1. How do they feed?

2. What enemies do they have?

3. How are their arms repaired when injured? Do you find specimens that are growing new tips to injured arms or are such arms apparently replaced? When an arm is injured how must the animal proceed to repair it?

4. Do specimens ever conceal themselves? See if speci-

mens can be found with pieces of grass and weeds covering them. Try picking these pieces off to see if they adhere.

5. Do the animals have other means of protection?

Examine a specimen and notice that:

1. The surface by which the animal clings, the *oral surface*, is different from the other, *aboral surface*, and both surfaces are covered with short spines. What is the use of the spines?

2. The animal consists of a central *disk* and radiating *arms*.

3. On the aboral surface of the disk, near the junction of the two arms, is a small, frequently conspicuously colored, circular body, the *madreporic plate*. The two arms adjacent to this plate are sometimes referred to as the *bivium*, and the remaining three as the *trivium*. The radial symmetry of the animal is disturbed externally only by the madreporic plate. Examine this plate with a lens and determine its structure.

4. Radiating from the mouth situated on the oral surface are the *ambulacral grooves*, one on each arm. In these grooves are *ambulacral* or *tube feet*. Do they have a definite arrangement? Along the sides of the grooves are slender spines that differ from the general body spines in being movable.

5. Scrape the tube feet from a portion of an ambulacral groove of a dried specimen and notice the *pores* through which the feet are attached to organs inside the arm. Notice also the exposed *ambulacral plates* and determine their relation to the pores.

Draw figures of the aboral and oral surfaces of a starfish, and a diagram to show the relation of the ambulacral plates and pores.

Place a living starfish in a dish of sea water.

1. Study its method of locomotion. How are the tube feet used? Does each foot act independently, or is there any evidence of coordinated movement?

2. Place the starfish on its aboral surface and analyze the method of righting.

3. Tear the starfish quickly from the substratum upon which it is crawling. Are any of the feet torn from the animal? (See Paine¹ for a study of the adhesive power of the tube feet.)

4. Find the threadlike *dermal branchiae* projecting through the body integument on the aboral surface. They serve as respiratory organs and probably also have an excretory function. The phagocytic nature of the cells of the coelomic fluid may be studied by simple methods reported by Kindred. (See reference below.)

5. Stroke the starfish with a camel's-hair brush and notice how the hairs are caught. Can you determine by what and how they are held? With a hand lens examine around the bases of the spines, and see the arrangement of the *pedicellariae*. Their function is obscure, but they enable the starfish to hold small objects firmly and they may be of service in dealing with possible surface parasites.

6. Remove some of the *pedicellariae* with a scalpel and examine them under the microscope. Do you find more than one kind?

Draw a pedicellaria.

Internal Structure.—*Make the dissection under water, and in cutting through the integument be careful not to injure the underlying soft parts.*

With strong scissors cut through the aboral body wall near the tips of the rays of the trivium. Carry the cuts forward along the sides of the rays to the disk. The cavity thus opened is the coelom or body cavity.

Lift up the integument at the tip of each arm and carefully snip away the *mesenteries* which attach the organs to it. Cut the membranes that extend into the disk opposite the junctions of the arms, and, cutting as close as possible to the

¹ Paine, V. L.: Adhesions of the Tube-feet in Starfish. Jour. Exp. Zool., vol. 46, No. 2, 1926.

madreporite, but leaving this in place, remove the three-rayed flap of integument thus freed.

Irving, L.: Ciliary Currents in the Starfish. Jour. Exp. Zool., vol. 41, 1924.

—: Regulation of the pH Concentration and Its Relation to Metabolism and Respiration in the Starfish. Jour. General Physiology, November 20, 1926.

Kindred, J. E.: The Cellular Elements of the Perivisceral Fluid of Echinoderms. Biol. Bull., vol. 46, 1924.

Digestive System.—In studying this system you should constantly bear in mind the peculiar method by which the animal feeds, as the digestive system is highly modified to suit this method.

1. The short, cone-shaped *intestine* and the *intestinal caeca* were probably removed with the integument. The intestine probably does not function, and may be regarded as a vestige. It opens near the center of the disk, on the aboral side, by a very minute *anus* that is very hard to see.

2. The *stomach*, which occupies the greater part of the space in the disk, is composed of a small aboral portion, the *pyloric* division, that receives the ducts from the hepatic caeca, and a larger, lobed, *cardiac* division, into which the mouth opens. The cardiac portion may be everted through the mouth, thus being turned wrong side out. Five pairs of muscles, which draw this portion of the stomach back into place, may be seen attached to the ridges formed by the ambulacral plates in each arm. How is it possible for the stomach to be everted? What reason is there for two divisions?

3. In each arm is a pair of long, glandular organs, the so-called *hepatic caeca*. The ducts of each pair unite and join the pyloric division of the stomach by a common duct. These are digestive glands. What reason is there for having ten enormous digestive glands? Does this have anything to do with the method of feeding?

Make a drawing of the digestive system of the disk and one arm.

Reproductive System.—Turn the hepatic caeca to one side and notice the *ovaries* or *testes*. The sexes are separate, but the organs have the same general appearance in both sexes. They vary in size according to the season of the year, sometimes being so small that they are not easily found, and again being nearly or quite as large as the hepatic caeca. With a pair of forceps lift up one of these organs and see where it is attached. It is at this point that the reproductive cells reach the exterior. How many gonads are there?

Draw the gonads into another arm of your figure.

Water-vascular System.¹—1. Carefully remove the side of the stomach next to the bivium, being very careful not to disturb the *stone canal*, which runs from the madreporic plate to the margin of the membrane around the mouth. By the side of the stone canal is a thin band of tissue formerly supposed to be a heart. It is generally referred to as the *axial organ* of the *hemal system*. See Chadwick's monograph on *Asterias* for a discussion of the theories concerning the nature of the hemal system.

2. The *circular canal*, which is joined by the stone canal at the outer margin of the peristomial membrane, follows the margin of the membrane and so encircles the mouth. Originating from it at points very near the ampullae of the first tube feet are nine small vesicles, *Tiedemann bodies*. They are smaller than the ampullae and project in toward the mouth. The position where the tenth Tiedemann body might be expected, is taken by the stone canal.

3. Leaving the circular canal are five *radial water tubes*, one for each arm. These tubes lie along the oral surfaces of the ambulacral plates, and are accordingly not visible on the inside of the animal. The position of the tube can best be understood by making a transverse section of an arm. It will then be seen either in injected or uninjected specimens, lying

¹ This may be injected in fresh specimens, either with gelatin or fine starch mass, by picking up one of the radial canals with a hypodermic syringe and injecting toward the disk.

immediately below the ambulacral plates. In injected specimens it may be followed by dissecting from the oral side, from the circular canal to the extremity of the arm, where it ends in a small tentacle.

4. Along the sides of the ambulacral ridges, within the body cavity, are rows of little saclike *ampullae*. Determine their relation to the ambulacral pores. If the specimen is fresh, press a few ampullae and see if the corresponding tube feet are affected. Can you determine their function? In a dissection it is hard to find the *connecting tubes* that join the radial tubes to the tube feet, but they can sometimes be seen in sections of arms of injected specimens. They can readily be seen in microscopic preparations.

The water-vascular system is very distinctive for the Echinodermata, and you should understand perfectly:

(a) How the tube feet are extended.

(b) What causes them to adhere.

(c) The connection between tube feet, ampullae, connecting canals, radial water tubes, circular canal, stone canal, and madreporic plate.

(d) How it is possible to extend one foot without extending others.

Make a drawing showing the arrangement of the water-vascular system.

Nervous System.—This is not easily studied by dissection. It consists of a *nerve ring* which encircles the mouth and lies just ventral to the circular water canal, and five *radial nerves* that extend down the arms just beneath the radial water tubes, to end at the tips of the arms in pigment spots, the *eye spots*. The whole central nervous system is superficial and forms a portion of the outer covering of the body. The radial nerves can be seen by separating the rows of ambulacral feet, but it is much more satisfactory to study them in prepared sections.

Muscular System.—Examine the walls of the starfish for its muscular system. If time permits, it will be desirable to

macerate a portion of an arm to see the skeleton to which these muscles are attached.

Study prepared sections of the arm of a small starfish and determine the relation of organs.

1. The hepatic caeca. How are they supported? What is their structure?

2. The radial canal, connecting tubes, tube feet, and ampullae.

3. The thickened, deeply stained, radial nerve between the tube feet and below the radial water tube.

4. The perihemal canal, divided by a thin partition, that lies between the radial water tube and the radial nerve.

Make a drawing of a section of an arm that will show these points.

Understand how a starfish can open an oyster or a mussel and how it digests it when open. How can it digest a barnacle or small snail? How does it respire?

Chadwick, H. C.: Memoir No. 25. Asterias. Liverpool Marine Biol. Committee, 1923.

Cole: Experiments on Coordination and Righting in the Starfish. Biol. Bull., 24, 1913.

—: Direction of Locomotion in Starfish, *Asterias forbesi*. Jour. Exp. Zool., 14, 1913.

Field: Larva of the *Asterias vulgaris*. Quart. Jour. Mic. Sci., 34, 1892.

Gemmill, J. F.: The Development and Certain Points in the Adult Structure of the Starfish, *Asterias rubens*. Phil. Trans. of the Roy. Soc., London, Series B, vol. 205, 1914.

Hopkins, A. E.: On the Physiology of the Central Nervous System in the Starfish, *Asterias tenuispina*. Jour. Exp. Zool., vol. 46, No. 2, 1926.

Jennings: Behavior of the Starfish *Asterias forreri*. Univ. Calif. Pub. Zool., 4, 1907.

MacBride: Development of *Asterias gibbosa*. Quart. Jour. Mic. Sci., 38, 1896.

Mead: The Natural History of the Starfish. Bull. U. S. Fish Com., 1899.

Tennent and Hogue: Studies on the Development of the Starfish Egg. Jour. Exp. Zool., 3, 1906.

OPHIUROIDEA

OPHIURA (Serpent-star)

These animals live more or less concealed in crevices, shells, eel grass, etc., and may be obtained either by dredging or by pulling a dip net through eel grass. They are not conspicuous objects along the shore, as are starfish, and they differ essentially from starfish in their method of locomotion and their method of feeding.

Examine a specimen and notice:

1. The appearance of the disk and arms. Are the spines similar to those of Asterias? The arms are more flexible. In what direction do they bend easiest?

2. The five buccal plates, one of which bears a madreporic opening that is not easily seen.

3. The size and shape of the mouth.

4. The ambulacral grooves. Are they distinct?

5. The ambulacral feet. Do they have suckers? How are they arranged?

6. The openings to the bursae, near the bases of the arms. Most ophiurans have five pairs of these openings, one for each bursa, but Ophiura has ten pairs, two for each bursa.

Draw an oral view of a specimen.

Place a living specimen in a dish of sea water and watch its movements.

1. Compare the rate and method of movement with Asterias.

2. Are all of the arms used in progressing in the same way?

3. See if the arms can be used interchangeably or if a certain one is always directed forward.

4. Are the ambulacral feet of any service? Do they adhere? The internal structure shows that the stomach is not eversible and that the hepatic caeca do not extend into the arms. Is there any correlation between these two facts?

The nervous and water-vascular systems are very similar to those of Asterias, but here the former lies within instead of

on the surface of the arm, the entire arm being encased with four or more rows of shields. They can be studied best in sections.

Grave: *Ophiura brevispina* I. Mem. Biol. Lab. Johns Hopkins Univ., 4, 1900. Mem. Nat. Acad., 8, 1899.

—: *Ophiura brevispina* II. An Embryological Contribution and Study of the Effect of Yolk Substance upon the Developmental Process. Jour. Morph., 27, 1916.

ECHINOIDEA

ARBACIA OR STRONGYLOCENTROTUS¹ (Sea Urchin)

In some localities sea urchins can be found in tide pools or near low-tide mark, where they may be very abundant. In other localities they can be obtained only by dredging. When possible they should be observed in their native places and the conditions noted.

1. What apparently serves as food for the animal? Can you determine how this is obtained?

2. Do you find attempts at concealment?

3. Are the animals able to climb?

Put a living sea urchin in a dish of sea water and study its movements.

1. When placed on its back, how does it turn over?

2. What is the normal method of progression?

3. How are the spines arranged when the animal is creeping on the bottom?

4. What difference do you note between the *spines* on the lower and upper surfaces?

5. How long are the *tube feet*? Are they used with the spines in moving or do both sets of organs act independently?

6. Grasp a spine with your forceps and see if neighboring spines respond. Do they form defensive armor?

7. In what directions may a spine be moved? Remove a spine from a preserved specimen and determine how it was attached and how the muscles that moved it were attached to the spine and to the test.

Make a diagram showing the arrangement.

¹These directions will serve for any of our common sea urchins.

8. Do the spines have any definite arrangement?

9. By means of the tube feet, notice that there are five *ambulacral* areas, between which are five *interambulacral* areas.

10. Notice an area on the aboral surface which is free from spines. This is the *periproct*.

11. Notice the membrane around the mouth, the *peristome*.

12. Look for *pedicellariae* on the peristome. In what other places are pedicellariae found? Do they differ from those of the starfish?

Draw one.

13. Notice the *tentacles* (modified tube feet) on the peristome.

14. The *dermal branchiae* are shrublike appendages at the outer edge of the peristome. They are situated opposite each interambulacral area.

Skeleton.¹—Examine the aboral surface of a cleaned "test."

1. The periproct has scattered plates which cover the anal opening. (Four triangular ones in Arbacia.)

2. Around these *anal* plates are five large ones, that form the apices of the interambulacral series of plates. These are the *genital plates*, and each is perforated by a small opening, the *genital pore*.

3. One of the genital plates is larger than the others and is full of very minute pores. This is the *madreporite*, which is homologous with the madreporite of the starfish. Determine its structure with a lens.

4. Between the genital plates are five smaller *ocular plates*, also perforated, which form the apices of the ambu-

¹ If a preserved specimen of Strongylocentrotus be placed in a solution of nitric acid (about 15 per cent) from five to ten minutes, the plates of the test can be more easily seen, especially after drying. This is apparently due to the coloring matter in the animal itself. Arbacia is not helped by the treatment.

lacral series of plates. These plates and the genital plates together form what is known as the *apical system*.

5. In the ambulacral series of plates, note the arrangement of the openings (ambulacral pores) through which the tube feet protrude.

6. Do all of the plates bear balls to which spines were articulated? Are the balls of equal size? Do they have a definite arrangement?

Can you homologize the positions of the ambulacral, interambulacral, ocular, and genital plates in the sea urchin and starfish? What portion of the starfish is represented by the periproct of the sea urchin?

Make a drawing of the test, showing the ambulacral, interambulacral, and apical systems of plates.

7. Around the peristome, on the inside of the test, note the five *auricles* forming arches or bridges over the bases of the ambulacral areas. Their purpose will be seen later.

Cut around the equatorial region of an *alcoholic* specimen, taking care to *cut through the test only*. Break the aboral portion away bit by bit with forceps until near the genital plates, freeing the fragments from the internal organs without disturbing their positions.

Reproductive System.—How were the *gonads* (their appearance is the same in both sexes) attached to the test? How many are there? Opposite what areas of the test are they placed? Where do they open to the exterior? Without mutilating, find the narrow strip of tissue that connects the gonads to each other near their aboral ends. This is the *genital rachis*. Connected with the genital rachis and lying alongside the stone canal, which leads from the madreporite, is the *genital stolon*.

Digestive System.—Remove the gonads from the three areas farthest from the madreporic plate, lift the remaining aboral portion of the test slightly, examine the alimentary canal, and note:

1. The large and conspicuous *jaws*, frequently called the lantern. They will be studied later.

2. The *esophagus*, passing between the jaws, and bending over to one side to join the intestine.

3. The *intestine*. Notice its size and its shape. Do its loops have any relation to the positions of the gonads?

4. The *intestinal siphon*, lying along the intestine and attached to it at both ends.

5. The *rectum*, running from the end of the intestine to the *anus*.

6. The *mesenteries* which hold the various organs in place. *Make a drawing to show the reproductive and digestive organs.*

Water-vascular System.—1. The *stone canal* leads from the *madreporite* to the *circular canal*, which encircles the esophagus at a point just above the lantern.

2. From the circular canal *radial tubes* pass over the top and down the sides of the lantern, to pass through the auricles and up the ambulacral tracts, to the ocular plates. They can easily be seen along the sides of the test, but are difficult to see before they leave the lantern.

3. Along the course of each radial canal, the *ampullae*, which supply the *tube feet*, are to be seen. The relations of the tube feet and radial canals are practically the same as in the starfish except that the removal of the radial tubes to the inner sides of the ambulacral plates causes two perforations for each foot here, while the starfish has only one. One of these perforations is for the connection between the ampulla and the foot, the other is for the connecting tube between the radial canal and the foot. The connecting tube joins the foot outside of the plates (as in the starfish), while it joins the radial canal inside of the plates (different from the starfish).

Remove the intestine and study the lantern and its attachments.

1. The whole lantern is inclosed in a delicate membrane, the *peripharyngeal* or *lantern membrane* which contains the

lantern coelom. This space communicates with the five *radial perihemal canals*, which run along the ambulacral areas between the radial canals and radial nerves, and with the dermal branchiae. It is important in respiration.

2. The tip of the lantern is attached to the flexible peristoma, and muscles extending from various parts of it are attached to the hard parts of the surrounding test.

In shape the lantern is a five-sided, radially symmetrical pyramid. Each of the sides consists of a massive calcareous structure, the *alveolus*, which supports an elongated tooth the tip of which projects through the peristome. The base of the pyramid may be compared with a wheel, in which the ten *epiphyses*,¹ two of which are attached to each alveolus, are the tire, and the five radially directed *rotulae* are the spokes. Each rotula has a more slender bar, forked at the free extremity, the *compass* or *radius*, lying over it. Each of the five segments represents a jaw that is articulated to its neighbors at its base, near the esophagus. The points of the teeth can thus be separated and closed, and the jaws protruded and retracted by means of muscles.

3. Connecting adjacent *alveoli* from top to bottom are the *comminator muscles*, that by their combined action close the jaws.

4. To each of the arms of the radius fork a muscle is attached. Where is it attached at the other end?

5. A pair of *protractor muscles* pass down from each epiphysis. To what are they attached? They are used in protruding the jaws.

6. A pair of *retractor muscles* is attached to the tip of each alveolus. They can be used in opening the jaws or in retracting the jaws. Do you see how?

7. There are also *internal* and *external rotula muscles* that connect the epiphyses with the rotulae. Their contraction moves these plates upon one another and thus causes a rocking motion of the jaws.

¹In *Arbacia* the epiphyses form small hooks that do not unite across the base of an alveolus.

Understand how the jaws may be protruded, opened, closed, and retracted by means of these muscles.

8. The compasses are attached one to the other by the *elevator muscles*. Their contraction elevates all of the compasses and thus enlarges the lantern coelom.

9. Attached to the forked end of each compass is a pair of *depressor muscles*. By their contraction the lantern coelom is compressed.

Understand the action of this mechanism in respiration. (See Von Uexhüll or the Cambridge Natural History, Echinoderms, p. 527.)

Make a drawing to illustrate the arrangement of the muscles.

10. Remove the lantern by cutting the peristome, clear away the external tissues, and examine the construction of the lantern. With a scalpel cut the interalveolar muscles so the jaws may be separated. Find:

(a) The large V-shaped *alveoli* (a straight suture indicates that each is formed by the fusion of two parts). Notice the roughenings on their esophageal sides. What purpose can they serve? Why should the alveoli be so large and the interalveolar (comminator) muscles be so strong?

(b) The *epiphyses*, which are fused with the upper corners of each alveolus and extend in to form a bar over its base, thus being functionally a part of the alveolus itself. The sutures between them and the alveolus proper can usually be seen.

(c) The *rotulae*, one of which joins the ends of each epiphysis and extends to the position of the esophagus. The five rotulae of the lantern articulate with each other around the esophagus, and each rotula articulates with the epiphyses of two adjacent jaws. Do you understand how the jaws move on the rotulae?

(d) The *compasses*, lying over the rotulae, are slender and bifurcated at their outer ends.

(e) The *teeth*, one enclosed in each alveolus. Examine

both extremities of a tooth and determine why the inner end is soft.

Understand thoroughly how the jaws are used and why the animal needs them. Why does the sea urchin not need large hepatic caeca?

Gemmill, J. F.: The Locomotor Function of the Lantern in *Echinus* with Observations of the Allied Lantern Activities. Proc. Roy. Soc., London, vol. No. 85, 1912.

The Nervous System.—The nervous system is difficult to demonstrate in dissections, but is easy to trace in sections. It consists of:

1. A *nerve ring* that encircles the esophagus at a point just above the mouth.

2. Five *radial nerves* that pass from the ring, along the insides of the ambulacral areas of the test, to the ocular plates.

The radial water tubes will be found in sections adjacent to the radial nerves. The two are separated only by a narrow space, the *pseudohemal canal*. Between the radial nerves and the tissue of the test there is another narrow cavity, the *epineural sinus*.

If time permits, students will find a dissection of the sand dollar, *Echinarachnius*, valuable for purposes of comparison. Special notes will not be necessary. Its shape and restricted ambulacral areas should be studied in the light of its habits and food supply. How does the animal move?

Chadwick, H. C.: Memoir No. 3, *Echinus*. Liverpool Marine Biol. Committee, 1900.

MacBride: Cambridge Natural History, Echinodermata.

Tennent: Variation in Echinoid Plutei. Jour. Exp. Zool., 9, 1910.

von Uexhüll: Die Physiologie des Seeigelstachels. Zeit. f. Biol., 39.

—: Ueber die Function der Polischen Blasen am Kauapparat der regulären Seeigel. Mitth. Zool. Stat. Neapel, 12, 1897.

HOLOTHUROIDEA

THYONE (Sea Cucumber)

These animals may be found in protected and usually muddy places, concealed in eel grass. They are generally

so well concealed that they cannot be satisfactorily studied in their native places. It is desirable to visit places where they occur and to observe the parts visible above the mud. It is then possible to realize the life for which they are adapted.

Examine a living expanded specimen in an aquarium (taking care not to disturb it) and note:

1. How the *tentacles* are used. What kind of food would it get by this means? Compare the method of food-getting with the starfish and sea urchin. How many tentacles? Arrangement? To what structures in the sea urchin do they probably correspond?

2. The respiratory movements of the body. Notice the strength of the current of water ejected.

3. The general shape of the body when expanded. Does it seem to rest on a particular side?

Kill the specimen by catching it with strong forceps behind the mouth, when the tentacles are expanded, and holding it in hot water.¹ Note that:

1. The body is covered with papilliform *ambulacral feet*. It is possible in some cases to see that they are arranged in five broad, longitudinal bands.

2. The suckers are less abundant on the dorsal (upper) surface than on the ventral.

3. A small papilla is to be found on the dorsal surface, between the tentacles. On it is the *genital opening*. This will be referred to again.

Make a drawing of the animal as seen from the side, indicating all of the points of structure that have been seen.

With a pair of scissors, open the animal longitudinally along the middle of the ventral (lower) surface.

Digestive System.—Note: 1. The delicate, perforated *mesentery*, which attaches digestive tract to body wall.

¹Specimens that do not expand may be injected with a saturated solution of chloretone (saturated by heating). After the animal relaxes the tentacles may be pushed out. Then kill in hot water or dissect immediately.

2. The *esophagus*, leading from the mouth through a calcareous structure, which recalls the *lantern* of the sea urchin. Examine and see if the arrangement is similar to that of the sea urchin lantern. The muscles for the retraction of the lantern are frequently torn from their attachments at one end.

3. The thin-walled and enlarged *stomach*.

4. The coiled *intestine*, which leads to the *cloaca*.

Draw the alimentary canal in position.

Cut the alimentary canal just in front of the stomach, and close to the cloaca, and as you remove it notice the blood vessel that runs along the intestine.

Respiratory and Excretory System.—Arising laterally from either side of the cloaca are the two *respiratory trees*. They are branched and project far forward into the body cavity. Can you determine how they are filled with water and how the matter is expelled? With a pipette inject them with starch mass. The strong jets of water ejected by the living specimen were thrown from these tubes. Can you understand how they serve for respiration? The walls of the tubes composing the trees are glandular and may thus serve to excrete wastes. Notice the muscles that radiate from the walls of the cloaca to the body wall. What is their function?

Make a drawing of the cloaca and respiratory trees.

Reproductive System.—The single *gonad* (ovary or testis) occupies a median dorsal position in the anterior part of the body cavity. It is composed of a multitude of filaments, which join to make a brush. This brush projects backward into the body cavity. The duct of the organ lies along the dorsal midline, between the right and left dorsal muscle bands, and leads to the opening upon the small papilla near the mouth that has already been noticed.

Water-vascular System.—1. The *circular canal* can be found in favorable specimens, surrounding the deeper portions of the esophagus. It gives rise to one or two *Polian vesicles*, which are very large and hang down into the body cavity.

2. The five *radial canals* (homologous with the radial canals of the starfish and sea urchin) originate from the circular canal, pass forward and then backward, and end near the cloaca. The radial canals take the general course of the longitudinal muscle bands and lie between this muscle band and the body wall. The radial canal may be seen if the muscle band is carefully removed.

3. Ten forwardly directed canals, the *tentacular canals*, leave the radial canals near the circular canal and pass into the tentacles, which may be homologized with tube feet.

4. The *stone canal* and *madreporite* are much reduced in holothurians. The madreporite, except in larvae and very young specimens, is not found on the outer surface. The stone canal leads obliquely backward from the circular canal, toward the dorsal body wall, to join a small calcareous body, the madreporite, which lies in the body cavity and is not perforated. Does this give you a reason for the presence of large Polian vesicles? The liquid in the water-vascular system is not sea water. Notice its color. Are there cells in it? Examine under microscope.

Make a diagram of the water-vascular system.

Muscular System.—Besides the special muscles radiating from the cloaca which have been referred to in connection with the respiratory system, and the muscles of the lantern, there are five strong longitudinal bands, really pairs. In which areas do they lie? What function do they perform? Look for smaller circular bands. Are there many of them? What is their function? Can you explain the varied worm-like motions of the body by the action of these muscles?

Nervous System.—This cannot be satisfactorily studied in dissections. There are five radial nerves and a circular ring. The nerves are embedded in the body wall and are hard to find

The classes of the Echinodermata show exceptionally well how a general type of structure may be retained and still

modified in certain regards for special habits. Compare, for instance, the feeding habits of the starfish, sea urchin, and sea cucumber.

Crozier, W. J.: The Orientation of a Holothurian by Light. *Am. Jour. Physiology*, vol. 37, 1921.

Kille, F. R.: Regeneration in *Thyone briareus* Lesueur following induced autotomy. *Biol. Bull.*, 69, 1935.

van der Heyde: Hemoglobin in *Thyone briareus* Lesueur. *Biol. Bull.*, vol. 42, 1922.

CHORDATA

Bilaterally symmetrical coelomate animals with a notochord, dorsal and tubular central nervous system, and a pharynx perforated by branchial clefts (gill slits).

Subphylum 1. Protochordata.

CLASS 1. Hemichorda.

The notochord is poorly developed and restricted to the anterior end of the body.

Order 1. Enteropneusta.

Wormlike, with numerous branchial clefts, a straight intestine, and a terminal anus. Body divided into three regions—proboscis, collar, and trunk. Development usually with a metamorphosis, the larva being known as a tornaria. (*Balanoglossus* and *Dolichoglossus*.)

Order 2. Pterobranchia.

Tubicolous, with one pair of branchial clefts or none, a U-shaped alimentary canal, and a dorsal anus situated near the mouth. Proboscis flattened ventrally into a large "buccal disk," its base covered dorsally by the collar which is produced into two or more tentaculiferous arms. Trunk short, prolonged into a stalk. Reproduction by budding occurs. (*Cephalodiscus*, *Rhabdopleura*.)

Order 3. Phoronidea (doubtfully placed with the chordates).

Tubicolous with gregarious habits. The body ends in a plume of ciliated tentacles; the alimentary canal is U-shaped. There is a larva known as *actinotrocha*. (*Phoronis*.)

CLASS 2. Urochorda.

The adult body is enclosed in a tunic or test, and usually lacks a notochord; the central nervous system is reduced to a simple ganglion. With an atrial cavity and a pharynx perforated by from two to many gill clefts. There is usually a tadpole-shaped motile larva which possesses a tubular dorsal central nervous system and a notochord restricted to the caudal region.

Order 1. Larvacea.

Small pelagic tunicates swimming throughout life by means of a tail. With a persistent notochord and a single pair of gill slits. (Appendicularia, Oikopleura.)

Order 2. Ascidiacea.

Mostly fixed, solitary or colonial tunicates, which in the adult are never provided with a tail and have no trace of a notochord. The test is well developed, the pharynx large and perforated by many gill slits. In most ascidians the sexually produced embryo develops into a tailed larva; many ascidians reproduce by budding to form colonies. (Ciona, Molgula, Styela, Perophora, Botryllus, Amaroucium, Leptoclinum.)

Order 3. Thaliacea.

Pelagic tunicates which swim by forming currents in the water. The adult is never provided with a tail or a notochord. The pharynx has two or more gill slits. Alternation of generations occurs, and may be complicated by polymorphism. (Salpa, Doliolum.)

CLASS 3. Cephalochorda.

The notochord extends the entire length of the body including the head. The body is metamericly segmented. (Amphioxus.)

Subphylum 2. Vertebrata.

A brain is developed as an enlargement of the anterior end of the central nervous system; the notochord extends no further forward than the middle of the brain, and a vertebral column and cranium are present. (Cyclostomes, fishes, amphibians, reptiles, birds, mammals.)

Conklin: Organization and Cell Lineage of the Ascidian Egg. Jour. Acad. Nat. Sci., Philadelphia, 2nd Ser., 13, 1905.

—: Does Half of an Ascidian Egg Give Rise to a Whole Larva? Arch. f. Entwicklunqsm. d. Org., 21, 1906.

Metcalf: Notes on the Morphology of the Tunicata. Zool. Jahrb., 13, 1900.

ENTEROPNEUSTA

DOLICHOGLOSSUS (BALANOGLOSSUS) KOWALEVSKII

In the natural habitat, note the character of the bottom where *Dolichoglossus* is found. Is the sand clean or is here an admixture of organic material? Note the frail tube of sand particles fastened together with mucus, and the numerous "castings." The animal has a characteristic and unpleasant odor.

Note the division of the body into three general regions: (1) a yellowish-white conical *proboscis*; (2) the *collar*, which is brilliant orange-red, especially in males, with a white ring posteriorly; and (3) the *trunk*, which is mainly orange-yellow, shading to a greenish-yellow in the transparent posterior region, which is often broken off when the animal is collected.

The trunk may be divided into the following regions, which overlap: (a) an anterior *branchial* region, bearing on each side not far from the dorsal median line a row of transverse gill slits; (b) a *genital* region, bearing on each side of the body an irregular and broken fold or ridge containing the reproductive organs, which are gray in the female and yellow in the male; (c) a posterior *abdominal* region, of much smaller diameter than the rest of the body.

The mouth is situated on the ventral side at the base of the proboscis, and is concealed by the free anterior edge of the collar. The animal is unable to close its mouth, and in burrowing a continuous stream of sand passes through the alimentary canal, forming the "castings" which are abundant in the natural habitat of the animal. What must be the nature of its food?

Burrowing is effected partly by muscular contractions of the body wall, but mainly through the power of the proboscis and collar to become turgid. In burrowing and feeding, of what use to the animal is the collar?

Note the characteristic coiling of the genital region in this species. The anterior end, including the branchial re-

gion, is normally maintained in a vertical position. The posterior end is also kept upright, and can be moved up and down in a vertical shaft opening on the surface, thus enabling the animal to eject the residue of sand from the anus.

For the internal anatomy, the account in the Cambridge Natural History may be consulted. Important chordate characters are the notochord, the dorsal central nervous system, and the branchial clefts.

Agassiz: The History of *Balanoglossus* and *Tornaria*. Amer. Acad. Arts and Sci., 9, 1873.

Bateson: The Early Stages of *Balanoglossus*. Quart. Jour. Mic. Sci., 24, 1884.

—: The Late Stages of the Development of *Balanoglossus*. Quart. Jour. Mic. Sci., 25, 1885.

Morgan: The Growth and Metamorphosis of *Tornaria*. Jour. Morph., 5, 1891.

—: The Development of *Balanoglossus*. Jour. Morph., 9, 1894.

Ritter and Davis: Studies on the Ecology, Morphology and Speciology of the Young of Some Enteropneusta of Western North America. Univ. Calif. Pub. Zool., 1, 1904.

UROCHORDA

MOLGULA MANHATTENSIS

Specimens of this simple ascidian may be found attached to old piles, associated with many other forms. In some localities they may be so abundant as practically to incrust the piles, and crowd each other out of shape. Examine such a mass and see how different sized individuals are associated. Pull them apart and see if there is any tissue connection between them that would indicate a definite relation between neighbors. Do you understand how the individuals get started in the places where they are attached? With a glass-bottomed pail you can see the expanded individuals on the piles, but they can be more satisfactorily studied in small dishes of sea water.

1. Observe the contraction and closure of the *two siphons* when the animal is irritated.

2. Add a little powdered carmine to the water to determine which is the *incurrent* or *oral* and which is the *excurrent* or *atrial siphon*.

3. Ascertain the number of *lobes* at the extremity of each siphon. Are *pigment spots* present on the siphonal lobes?

Certain organs are distinguishable through the tough tunic which incloses the body. The *endostyle*, a ciliated groove looking like a white thread along the midventral line of the *pharynx* or *branchial basket*, will serve as a guide in orienting the animal. Determine dorsal, ventral, anterior, posterior, right, and left aspects.

Make a drawing of an expanded animal.

4. The *tunic* or *test* can be removed by cutting through it with scissors, taking care not to injure the *mantle* or *body wall*. Enlarge the opening made in the tunic and strip it from the body. Where is the tunic most firmly attached? Examine a small piece of the tunic microscopically. Are blood vessels visible in it? Does it contain any cells?

5. For further study use both fresh and preserved material from which the tunic has been removed. Identify as many organs as possible through the mantle. In a living specimen note the beating of the *heart* (the heart is on the right side) and the frequent reversal of the direction of the pulsations. The *endostyle*, *longitudinal pharyngeal folds*, *intestine*, *gonads*, *gonoducts*, *renal organ*, and *subneural glands* are also visible through the mantle.

6. Note the *muscle bands* of the mantle which serve to contract the body and especially the siphons. Where are the muscles best developed? Is there any definite arrangement of the muscle bands?

Fix a large specimen by pins through the siphons, and with scissors and fine forceps remove a large section of the mantle from the *left* side, between the digestive tract and the siphons, injuring the underlying *pharynx* as little as possible. The large space between the pharynx and the mantle, laterally and dorsally, is the *atrium*, or *peribranchial chamber*, which is formed as an ectodermal involution. Into this

atrial cavity open the *intestine* and the *gonoducts*, and also the numerous *stigmata* of the pharynx. Ventrally the pharynx is fused with the mantle in the region of the endostyle.

1. On each side of the upper part of the pharynx six *longitudinal pharyngeal folds* will be seen.

2. The endostyle is a ciliated groove along the midventral wall of the pharynx. In a very fresh specimen, cut out a large piece of the ventral and left lateral wall of the pharynx, preferably near the siphon, mount it inside up in sea water and examine with a microscope. Note the structure of the endostyle. At some distance from the endostyle, on each side of it, note the meshwork of blood vessels, and the curved openings or *stigmata* lined with cilia. Of what use are the cilia?

3. Anteriorly the endostyle is continuous with the *peripharyngeal ciliated bands*, which encircle the oral end of the pharynx. From the point where they unite dorsally the *dorsal lamina* extends backward along the mid-dorsal line of the pharynx. At its posterior end will be seen the small opening into the *esophagus*.

Do you understand how the animal captures its food and how the endostyle, peripharyngeal bands, and dorsal lamina are used?

4. In front of the anterior end of the dorsal lamina note the small, volute-shaped *dorsal tubercle*. This is the extremity of the *hypophysis*, a tube connecting the subneural gland with the oral cavity.

5. A ring of oral tentacles will be seen in the mouth, anterior to the peripharyngeal bands. Of what use are tentacles in the mouth? How many tentacles are there?

6. The very short *esophagus* opens into the *stomach*, which will be recognized by the brown digestive glands that cover it. From the stomach the intestine forms a loop on the left side, and is easily traced to the *anus*, which opens dorsal to the pharynx in the atrial chamber. A longitudinal

fold, the *typhlosole*, extends throughout the intestine. What is the use of such a fold?

Reproductive System.—On each side of the body, adherent to the inside of the mantle, is an elongate *hermaphrodite gland*. Each gland consists of a lighter part, the *testis*, and a darker part, the *ovary*. The gonoducts open on the outer wall of the atrial cavity near the base of the atrial siphon. Each consists of two ducts, *oviduct* and *vas deferens*. Microscopic examination of the oviduct may show the presence of eggs.

Excretory System.—The renal organ is a conspicuous, elongated sac on the right side. It contains a brownish fluid and usually some solid matter. It does not possess a duct.

Nervous System.—The *cerebral ganglion*, which in *Molgula* is almost completely surrounded by the *subneural gland*, lies close to the mantle, between the two siphons, and is thus dorsal to the mouth. Nerves can be seen passing from the ganglion to the two siphons. The *hypophysis*, a tube leading from the subneural gland, opens as the *dorsal tubercle* mentioned earlier.

Circulatory System.—1. The heart, which lies on the right side between the hermaphrodite gland and the renal organ, is inclosed within a *pericardium* which is a portion of the coelom. It should be studied in a living specimen, with the aid of a hand lens.

2. If a very small *Molgula* ($\frac{1}{8}$ of an inch in length) is studied alive in a watch glass with the microscope, the course of the circulation, and the frequent reversal of its direction, can be observed.

3. From the dorsal end of the heart, a *cardiovisceral vessel* runs to the visceral mass, where it divides into smaller vessels. These, reuniting, form the *viscerobranchial vessel* which extends along the dorsal surface of the pharynx above the dorsal lamina. Numerous small *branchial vessels* in the pharyngeal wall connect this vessel with the *branchio-cardiac*, which lies ventral to the endostyle and unites with

the ventral end of the heart. The frequent reversal of the current can be readily seen both in the heart and in the vessels.

The relation of the parts will be more clearly understood if a second large specimen is dissected as follows: with scissors cut off the *atrial siphon*, thus exposing the atrium; then similarly remove by a single cut the *oral siphon*, together with the *anterior end of the pharynx* (the piece thus cut off should contain the *ganglion*, *dorsal tubercle*, *peripharyngeal bands*, *oral tentacles*, *anterior portion of the endostyle*, *dorsal lamina*, etc.).

Make drawings that will show the structure.

Hunter: Notes on the Heart Action of *Molgula manhattensis*. Am. Jour. Physiol., 10, 1903.

Kingsley: Some Points in the Development of *Molgula*. Proc. Roy. Soc. Nat. Hist., 21, 1883.

van Name: Simple Ascidians. Proc. Bost. Soc. Nat. Hist., 34, 1912.

PEROPHORA

This ascidian occurs on piles and other submerged materials, and is commonly attached by branching stolons to seaweeds, simple tunicates, or other sessile animals. Material should be quite fresh for satisfactory study, and should be carefully handled to avoid crushing. Study in a watch glass of sea water (or support the cover glass) with a low power of the microscope.

1. Notice that the individuals are very much like miniature *Molgulas*. Identify as many of the organs that were seen in *Molgula* as possible, noting the differences.

2. The form illustrates the type (*Clavelinidae*) in which a colony is formed by budding from a stolon, but in which the individuals retain their identity to a great degree and have separate tunics.

3. Study the *stolon* with its flattened *epicardiac tube*. This tube is derived from the branchial sac and is accordingly endodermic.

4. Study *buds* of various sizes and see how the inner vesicles arise from the epicardiac tube.

5. Try to make out the entire course of the circulation of the blood. Notice especially the *heart*, *branchial* vessels, vessels of the *mantle*, and the circulation of the *stolon*. Watch the pulsations of the heart and see the reversal of the blood current. Is the heart beat synchronous in different individuals? What part of the blood is colored?

6. Study the action of the cilia in the gill clefts.

Drawings of a colony and of an individual are desirable.

Lefevre: Budding in Perophora. Jour. Morph., 14, 1898.

BOTRYLLUS

The small, radially arranged colonies of this composite ascidian are common on eel grass, from which they may be separated by means of a knife, and studied alive in a watch glass with a lower power of the microscope. The cleaner and more transparent colonies should be selected.

1. Note the character which makes the form a "composite" ascidian—the common *tunic* or *test*. Find the *mouths* and the *common cloacal cavity*. Would it be correct to say that a common *atrium* is present?

2. Find the *annular blood vessel* and its numerous *ampullae*. Do you observe any striking facts regarding the circulation? What functions have the ampullae?

3. With your knowledge of Molgula as a guide, identify as many of the organs as possible. (This is sometimes difficult because of pigment.)

4. Very young colonies, with only the first one or two generations of buds, may also be found on eel grass, appearing as transparent hemispherical lumps about a millimeter in diameter. These should be fixed and stained on the eel grass, and later mounted (either still attached or removed) in balsam. These will show very clearly the formation of buds of the "parietal" or "peribranchial" type. (In this type the outer vesicle arises from the integument, and the inner vesicle

from the parietal wall of the atrial cavity.) The inner vesicle may be seen partly constricted into three divisions—the *pharynx* and the two *atrial sacs*. From which “germ layer” then are these parts in the bud derived?

5. Look for the tailed larvae or “tadpoles” near the surface and on the side turned toward the light, in a dish in which *Botryllus* has been kept for an hour or two. Is this positive phototropism advantageous? Examine a larva under a microscope.

Drawings of the adult, the young colony, and the larva are desirable.

Grave and Woodbridge: *Botryllus schlosseri*, Pallas: The Behavior and Morphology of the Free-swimming Larva. *Jour. Morph.*, 39, p. 207, 1924.

Herdman, E. C.: *Botryllus*. Liverpool Marine Biol. Com. Memoirs, xxvi, 1924.

AMAROUCIUM (Sea Pork)

Different species of this composite ascidian live at different depths and show minor structural differences, especially in the tests. Colonies may be found abundantly on piles and they are frequently brought up with a dredge.

1. Compare the grouping of the individual in the colony with *Botryllus*. Is there any regularity in the number of a group connected with a common cloacal cavity?

2 With a sharp knife, cut a section vertical to the surface of the mass, and 2 or 3 mm. thick, and study it with a low power of the microscope. Other pieces should be squeezed in a fingerbowl half full of sea water, the expressed material (adult animals, fragments, embryos, etc.) allowed to settle, and then rinsed with clean sea water. A few entire adults may be picked out with a pipette.

In the adult animal you may find:

(a) *Oral* and *atrial openings*.

(b) *Pharynx*, with the *peripharyngeal bands* and *endostyle*, *esophagus*, the orange-brown corrugated *stomach*, and *intestine*.

(c) The *cerebral ganglia*.

(d) The long *postabdomen*, with its hollow *epicardium* connected with the pharynx. (The postabdomen is really a stolon. Recall *Perophora*.) If complete, the red-pigmented tip will be seen.

(e) The slowly pulsating U-shaped *heart*, situated very near the tip of the postabdomen.

3. In the atrium, which serves as a brood pouch, *embryos* in all stages may be found. How do the eggs compare in size with those of *Molgula*?

4. Look for *buds* formed by segmentation of the post-abdomen (stolon). The "inner vesicle" of these buds, which gives rise to the alimentary canal and atrial sacs, comes from the endodermic epicardium, as in *Perophora*. Compare this with *Botryllus*.

5. If the material squeezed in the fingerbowl was quite fresh, living embryos in all stages of development can be found. Fresh specimens kept in a large jar of sea water during the summer will discharge larvae. These swim rapidly, and usually swim away from the light. Does this correspond with *Botryllus*? Is this negative phototropism adaptive?

The tailed larvae may be picked up with a pipette while swimming, dropped into fixing fluid, and finally stained and mounted. Others may be transferred to watch glasses and studied. If the larvae are kept in watch glasses of sea water for some hours some will attach. The dishes may be kept in a cage under a wharf submerged in sea water, or in a dish where *pure* sea water can be conducted to it. Under these conditions they will develop readily, but they must be kept clean from sediment by washing them with a gentle current at least twice a day.

In larvae that have been previously stained and mounted observe:

(a) The shape of the animal and its division into body and tail.

(b) The thick test, and the *oral* and *atrial* openings.

- (c) The *adhesive organs*. How many are there?
- (d) The *notochord*. How far does it extend?
- (e) The tail *muscles*.
- (f) The *pharynx*, with as yet few gill clefts, the *endostyle*, *esophagus*, *stomach*, *intestine*, and *yolk mass*.
- (g) The *cerebral vesicle* with the *eye spot* and *otolith*.

If young individuals that have been attached but a short time, but have lost their tails, are stained and mounted, they will be found very instructive when compared with the larva. The complete degeneration of the tail and the final rotation into the position of the adult can be traced in a series of individuals.

Drawings of an adult individual, of a larva, and of a young individual are desirable.

van Name: Compound Ascidians of the Coasts of New England and Neighboring Provinces. Proc. Bost. Soc. Nat. Hist., 34, 1910.

SALPA CORDIFORMIS

Examine a specimen in a bowl of water without dissecting. Use a hand lens.

Sexual form (occurring in chains):

1. Note the transverse *muscle bands*. How many bands are there? Are they complete or interrupted? Do you know what they are for?
2. The *oral aperture* is dorsal and far forward. Are there any muscles for opening and closing it?
3. What is the form and position of the *cloacal aperture*? Is it provided with muscles?
4. Observe the processes of the tunic, one anterior, one midventral and two posterior. These processes (except the dorsal posterior) serve to unite the individuals of the chain.
5. Does the animal show perfect bilateral symmetry?
6. Posterior to the mouth, the *ganglion* and the pigmented *eye spot* may be found. Immediately anterior to these is the elongate *hypophysis*.

7. Note the *endostyle* in the floor of the pharynx, and the *dorsal lamina* between the pharynx and *atrial cavity*. From the anterior end of the dorsal lamina the *peripharyngeal bands* extend to the anterior end of the endostyle.

8. The pharynx communicates laterally with the atrium by means of two very large *stigmata*. These are probably homologous with the numerous stigmata of *Molgula*.

9. The "*nucleus*," the large mass in the posterior end of the body, contains the *stomach* and *intestine*.

The ova are fertilized by spermatozoa from individuals of another chain, since in the same chain the spermatozoa mature much later than the ova. The fertilized ova migrate to a spot in the right wall of the atrium, where they develop into the solitary, nonsexual *Salpa*.

In this species as many as three or four embryos may be seen attached by "*placentae*" to the cloacal wall on the right side. The placental connection finally separates, and the embryo passes out through the cloacal aperture.

Make an enlarged drawing (a laterodorsal view is best).

Brooks: The Genus *Salpa*. Mem. Biol. Lab. Johns Hopkins Univ., 2, 1893.

Groben: *Doliolum* und sein Generationswechsel. Arb. Zool. Inst. Wien, 4, 1882.

Metcalf: The *Salpidae*: A Taxonomic Study. Bull. U. S. Nat. Mus., 100, 1918.

CEPHALOCHORDA

AMPHIOXUS LANCEOLATUS

While living material is not easily provided for laboratory work, it should be understood that this form spends most of its time in the sand in rather shallow water and that it burrows with great ease by movements of the body.

1. In an alcoholic specimen note the dorsal, ventral, and caudal regions, and also the *median fin*, *metapleural folds*, *muscle plates* or *myotomes*, *buccal cavity fringed with cirri*, *atriopore*, and *anus*.

2. Using a specimen that has been macerated in 20 per cent nitric acid, remove the skin and myotomes from the

right side very carefully, by means of needles, exposing the *notochord*, *nerve cord*, *gonads*, and the entire *alimentary canal* (pharynx, intestine, and digestive diverticulum or "liver," which lies along the right side of the pharynx).

3. Examine microscopically and notice:

(a) The *nerve cord*, *cerebral vesicle*, *cerebral nerves*, *eye spot*, and *pigment cells*. Note also the alternate metamerism of the spinal nerves.

(b) The *buccal skeleton*.

(c) A large piece of the *pharyngeal wall*.

4. Examine an *Amphioxus* 1 cm. in length, stained and mounted.

Identify as many as possible of the structures mentioned above, and in addition note: *the olfactory pit*, *oral velum* with *velar tentacles*, and "*taste organ*" in the buccal cavity.

A drawing showing the general structure is desirable.

5. Make thick free-hand sections of various regions and study with a low power in a watch glass, to supplement the study of stained sections.

6. Prepared sections should be studied that show the following five regions: (a) buccal cavity; (b) anterior part of pharynx; (c) posterior part of pharynx with gonads and liver; (d) atriopore; (e) anus.

The five sections should be studied with a low power and drawn. In (b) (anterior part of pharynx), note especially the limits of the *coelom* and *atrium*, the *lymph spaces* in the metapleural folds, the two *dorsal aortae*, the *ventral aorta*, the *epibranchial groove*, the *endostyle*, the *subendostylar coelom*, and the two kinds of *gill bars*, *primary* and *tongue bars*.

With a high power study the nerve cord (best shown in region a) and the gill bars and endostyle (best shown in region b).

Drawings of these regions are desirable.

NOTES FOR GUIDANCE IN MAKING PERMANENT PREPARATIONS

Only very simple directions are here given, such as will serve to aid students who have had no experience in preparing objects for microscopic examination to make preparations when this is desirable for proper laboratory study. The best simple directions for technique of this type may be found in Guyer's Animal Micrology. Another excellent guide is Galigher's Practical Microtechnique.

For more elaborate work McClung's Microscopical Technique or Lee's Microtomist's Vade Mecum are valuable.

The steps taken in preparing total mounts include:

1. Narcotizing or anesthetizing.
2. Fixing or killing.
3. Washing.
4. Dehydrating and staining.
5. Clearing.
6. Mounting.

Narcotizing.—A great many animals or animal parts may be more easily fixed if first anesthetized. Many invertebrates can be anesthetized by placing in a dish with an ample supply of water to which crystals of magnesium sulphate are then added. As the crystals dissolve add more until anesthetization is complete. This method is especially good for delicate hydroids. Other narcotizing agents which may be used include the following: (1) chloretone, which may be used similarly to the method given for magnesium sulphate. Many small invertebrates may be transferred directly to dilute solutions of chloretone, *e. g.*, annelids (one part saturated aqueous solution of chloretone to four to nine parts of water). For marine animals this solution should be made up in sea water.

(2) Ethyl alcohol. Animals may be transferred gradually or directly to concentrations of alcohol varying from 1 to 8 per cent.

For finer cytological work it is well to avoid any use of anesthetics.

Fixing.—This is necessary in order to keep the cells and tissues as nearly as possible in their natural position, shape, and structure, and in order that the protoplasm composing them may be kept in condition to stain satisfactorily.

In selecting a fixing agent remember:

1. If the material is highly irritable and contractile, it will have to be killed practically instantly with hot solutions, or be previously narcotized.

2. If there is much lime, an agent that contains much acid should not be used, as the lime will be dissolved and the bubbles of gas are likely to tear or distort tissues.

3. Where rapid fixation is desirable, as in expanded hydroids and the like, hot Bouin's picroformol or hot sublimate-acetic is preferable. Where the tissue, or the animal, is not especially muscular, or liable to contraction, any of the fluids may be used. The time that objects should be left in the killing solution varies, approximately, directly as their size. Three minutes will suffice for killing hydroids in Bouin's picroformol or sublimate-acetic.

Washing.—All objects must be thoroughly washed, after using most killing agents. With most small objects alcohol is preferable, but if the object is large this is too expensive. In general the material used for washing will depend on the fixative employed. See Guyer.

Dehydrating and Staining.—From water, all objects should be placed successively in 35 per cent, 50 per cent, and 70 per cent alcohol, five to fifteen minutes in each for small objects, such as protozoa or individual hydroids. In subsequent changes from one grade to another allow about the same time. All tissues killed in a corrosive sublimate mixture should now be treated with a weak solution of iodine,

to dissolve the corrosive sublimate that still remains, and thus prevent the later formation of crystals of that substance. Such crystals would not appear immediately, but ever increasingly, as the preparation is kept. Put a few drops of iodine into the 70 per cent alcohol containing the object, leave a few minutes, and, if the yellow color caused by the iodine has disappeared, pour off the alcohol and use more 70 per cent alcohol with iodine, as before. The bleaching indicates that some corrosive sublimate remains. Repeat until the yellow color does not fade. Then transfer to clear 70 per cent alcohol. At this point either staining, or preparation for so doing, begins.

In case the stain you wish to use is a 70 per cent alcoholic solution, it may be used immediately. Otherwise, the object must be run through the grades of alcohol, up or down as the case may be, to that medium in which the stain to be used is dissolved. If an aqueous stain such as alum carmine is to be used, pass through 50 per cent and 35 per cent alcohol to water. If a 95 per cent alcoholic stain is to be used, pass through 80 per cent and 95 per cent alcohol.

The time an object should be treated with stain varies with the stain and the size of the object. Alum carmine should be used from six to twenty hours, according to circumstances. Borax carmine should be used for from five minutes to half an hour. Aceto-carmine, used for killing and staining, acts very rapidly. Delafield's hematoxylin (a dark wine-colored solution in water) requires ten minutes to half an hour. In all these cases, examination of the objects themselves is the only means of deciding when staining is sufficient. It is usually best to overstain slightly and then to bleach out, as certain parts of the protoplasmic structure will retain the stain better than others, and thus better differentiation will be secured. After staining, bring the tissues gradually into 70 per cent alcohol, and then treat with acidulated alcohol to remove excess of stain. After this, every trace of the acid must be removed by washing in clean al-

cohol, or the tissues will continue to bleach after they are mounted. The specimen is now ready for final dehydration. In damp climates, as at the seashore, your stronger alcohols must be kept closely covered *all of the time* or they will take water from the atmosphere and be unfit for the purpose. Absolute alcohol may be kept from excessive dilution under these conditions by placing in it a fine-mesh cloth bag containing anhydrous copper sulphate which must be renewed from time to time. Run through 80 per cent, 95 per cent, and 100 per cent alcohol, thus completing dehydration. *Every trace of water must be removed and then kept out.*

Clearing and Mounting.¹—From absolute alcohol, place objects in some clearing fluid (clove oil, cedar oil, or xylol) and leave till they have a clear, translucent appearance, after which place on a *clean* slide, with some Canada balsam or damar, and cover with a cover glass.

If the object turns cloudy or milky when placed in the cleaning fluid, it is evidence that all of the water has not been removed, and it should be returned to absolute alcohol for complete dehydration. Tissues left in the clove oil or xylol for any great length of time will become hard and brittle. In case tissues in the process of preparation must necessarily be left untreated for several days, they should be left in a 70 per cent or 80 per cent alcoholic medium.

Sectioned Material.—In a few cases sectioned material, previously stained *in toto*, may be distributed to the class. Be sure that the slide on which you intend mounting the sections is *thoroughly clean*. Remove any greasy substance with 95 per cent alcohol. On a cleaned slide, smear a *very little* albumen fixative with your fingertip and remove all except the thinnest film. On water placed over this film of albumen float the sections. Very gently heat the water until the sec-

¹Specimens may be successfully mounted in euparal from 95 per cent alcohol. This avoids labor of dehydration and clearing and gives permanent mounts. For total mounts of parapodia, etc., methyl salicylate (synthetic oil of wintergreen) may be used to clear directly from 95 per cent alcohol.

tions stretch out flat, but *do not melt* the paraffin. Carefully drain off excess water and set slides aside to dry. If the air is dry, the slides should be ready for further treatment in about twelve hours. The value of this method is that it gives perfectly flat sections. At the end of this time the slides may be placed in xylol to dissolve the paraffin. When the paraffin is completely dissolved (this will take a few minutes), drain off the xylol, apply a drop of balsam, and cover as in total mounts. The preparation is now ready for use, and is permanent, but must be handled carefully while fresh.

Application of Above Directions in the Case of a Hydroid:

Hot Bouin's picroformol, fifteen seconds.

Cold Bouin's picroformol, five to fifteen minutes.

Fifty per cent alcohol, four changes, three or four minutes each.

Seventy per cent alcohol, five minutes.

One half of your material may now be placed in borax carmine. Leave the material in this till objects have taken on a good color. (Ask an instructor about this.) When sufficiently stained, put into acidulated alcohol till the color assumes a brilliant appearance, but do not allow it to fade too far. Wash in 70 per cent and then run through 80 per cent and 95 per cent alcohol, five minutes each, and mount directly in euparal.

If balsam is to be used, continue from the 95 per cent alcohol to 100 per cent alcohol, five minutes, thence into clove oil, or cedar oil, keeping all reagents carefully covered, and leave till the object is thoroughly penetrated. This latter process may take five to ten minutes.

If, on putting your objects into the clearing medium, the latter exhibits a milky-white appearance, the material is not sufficiently dehydrated, and must be returned to 100 per cent alcohol.

After clearing is completed, put the object on a clean slide with a little balsam and cover.

The material not treated with borax carmine may be run

back through 50 per cent and 35 per cent alcohol to water, to which a few drops of hematoxylin has been added, or put from water into alum carmine. The former stain, if dense, should not require over twenty to thirty minutes, but objects must be left in alum carmine ten to twenty hours. When a good color is obtained, run the material through the grades of alcohol, from the lowest to the highest (five minutes in each), and mount as in the case of the borax carmine objects.

Objects stained in alum carmine will probably not over-stain; but excess of hematoxylin should be extracted with acidulated alcohol when the 70 per cent grade is reached, after which it is very essential that *all of the acid* be removed by repeated changes of 70 per cent alcohol. Otherwise the objects will fade.



GLOSSARY

NOTE: The definitions given below are, of course, intended primarily to apply to the invertebrates. With a few exceptions no attempt has been made to supply the meanings which apply to the vertebrates.

- Abdomen.** In invertebrates, the posterior division of the body.
- Aboral surface.** The surface of the body opposite the oral or mouth surface.
- Aciculum.** A supporting rod in an annelid parapodium.
- Acinous.** Saccular or granular.
- Acontium.** In sea anemones, a threadlike organ containing nettle cells.
- Acraspedote medusa.** A medusa without velum. Typical of Scyphozoa.
- Acrocyst.** An extracapsular brood chamber attached to distal end of gonosome in certain calyptoblastic hydroids.
- Actinopharynx.** Tube leading from mouth to coelenteron in sea anemones.
- Actinule or actinula.** A specialized larval form, having aboral and oral tentacles and developed in the medusa of tubularian hydroids. Ultimately gives rise to a new colony.
- Adductor muscle.** A closing or withdrawing muscle.
- Adhesive organ.** A sucker or sticky pad that will adhere.
- Adnate.** Said of hydranths growing with one side adherent to a stem.
- Adradial canal.** In a medusa, a canal lying between adjacent per- and inter-radial canals.
- Afferent.** Carrying toward, as a vessel which leads to an organ.
- Alga.** A simple chlorophyll-bearing plant.
- Alimentary canal.** Digestive tube.
- Alternation of generations.** Alternation of sexual and asexual generations in the life cycle of an organism.
- Alveolus.** A little sac or cavity; also one of the plates that bears the teeth in an echinoid.
- Ambulacral area.** The region bearing the tube feet of an echinoderm.
- Ambulacral foot.** A tube foot of an echinoderm.
- Ambulacral groove.** One of the depressions in which the tube feet of a starfish are placed.
- Ambulacral plate.** One of the plates of an ambulacral area.

- Ambulacral pore.** The opening through which a tube foot projects.
- Ambulacral ridge.** The elevation in the coleom of starfish arm, caused by the ambulacral plates.
- Ambulacral sucker.** The sucker at the end of a tube foot.
- Amphiblastula.** A characteristic embryonic stage of a sponge.
- Ampulla.** A reservoir connected with the tube foot of an echinoderm.
- Anal plate.** In the periproct of an echinoid, the plate in which the anus lies.
- Analogous.** Similar in function.
- Anastomosis.** In the simplest form, a cross connection between two adjacent blood vessels or nerves. Frequently many such cross connections among several related vessels or nerves result in the formation of a network.
- Annulation.** A ringlike part or annulus as in the ringed stem of certain hydroids.
- Annulus.** A ring or ringlike part or structure.
- Antenna.** A sensory head appendage of an arthropod.
- Antennule.** A sensory paired head appendage of an arthropod, placed just anterior to the antenna when present. Usually designated as first antenna.
- Anterior.** Front or head end.
- Anteroposterior.** Lengthwise of the body.
- Anus.** The opening (usually posterior) of the alimentary canal through which the feces are discharged.
- Apical plate.** An ectodermal thickening at the anterior end of trochophore larva.
- Apical system.** A group of plates surrounding the periproct of an echinoid.
- Apical tuft.** A group of large cilia on the apical plate.
- Apopyle.** The opening of a radial canal of a sponge into the gastric cavity or cloaca.
- Arthrobranch.** A gill of a crustacean, borne by the articular membrane at the base of an appendage. A joint gill.
- Asexual reproduction.** Reproduction without sexual phenomena, *e. g.*, binary fission, multiple fission (sporulation), budding, etc.
- Atriopore.** External opening of the atrium.
- Atrium.** In tunicates and Amphioxus, a chamber partly enclosing the pharynx and receiving water from the latter through the pharyngeal slits. Genital products and, in adult tunicates, feces also pass into the atrium.
- Auricle.** A receiving chamber of the heart.
- Avicularium.** A structure shaped like a bird's head, present in some Bryozoa.

- Axial organ.** A structure near the stone canal of echinoderms which is apparently connected with genital organs.
- Basipod.** Second segment from proximal end of protopod in a crustacean appendage.
- Beak.** Horny mouth parts; the point from which growth has proceeded in a clam shell. Cf. Umbo.
- Bilateral symmetry.** Body plan such that the organism may be cut in but one plane to produce equivalent or mirror-image halves (right and left).
- Biramous.** Composed of two branches, as a typical crustacean appendage.
- Bivalve.** Having two valves or pieces, as a clam shell.
- Bivium.** The two rays of a starfish which are nearest the madreporic plate.
- Blastostyle.** The reproductive zooid (probably a degenerate hydranth) in certain hydroids. The gonophores (sporosacs and medusae) are developed on the blastostyle.
- Body cavity.** See Coelom.
- Body wall.** The outer wall of the body.
- Brain.** In invertebrates, frequently applied to the cerebral ganglia.
- Branchiae.** Gills; organs adapted for aquatic respiration.
- Branchial heart.** An accessory heart placed at the base of a gill, as in the squid.
- Brood sac.** A cavity or pouch in which developing embryos are carried.
- Bud.** An outgrowth or ingrowth which will become a new individual.
- Byssal gland.** A gland in the reduced foot of certain pelecypods, *e. g.*, *Mytilus*. It produces the byssal threads, collectively known as the byssus.
- Byssal thread.** One of the threads by which certain pelecypods attach themselves.
- Caecum.** A blind saclike outgrowth of the alimentary canal.
- Calciferous glands.** Esophageal glands of some annelids.
- Calyptoblastic.** Possessing hydrothecae and gonothecae.
- Capitate tentacle.** One which is enlarged or globose at its distal end.
- Carapace.** The covering of the head and thorax in some crustaceans.
- Cardiac stomach.** Anterior or first division of the stomach.
- Caudal cirrus.** A cirrus found on the caudal end, especially of nemertines and annelids.
- Carpopod.** Third segment from proximal end of endopod in crustacea.
- Cellulose.** The most important material in the walls of plant cells. A cellulose-like substance (tunicin) is found in the tunic of many Urochorda.

- Cephalont.** Attached stage in the life history of Gregarina.
- Cephalothorax.** Fused head and thorax in many crustaceans.
- Cervical groove.** A groove, in the carapace, which marks the boundary between the head and the thorax.
- Chela.** Large claw of many crustaceans; also applied to pincer-like claws on other appendages.
- Chelate.** Bearing pincer-like claws.
- Chelicera.** One of the anterior pair of mouth appendages of Arachnida.
- Chitin.** The material which forms the outer covering of insects and many other invertebrates.
- Chlorogogue.** Modified cells of the peritoneal covering of the intestine in annelids.
- Chlorophyl.** The green coloring matter of plants.
- Chloroplastid.** One of the chlorophyl-containing bodies within certain cells of green plants.
- Choanocyte.** A "collar cell." *e. g.*, Choanoflagellates and the gastric layer in sponges.
- Chromatophore.** In zoology, a pigment-bearing tissue element or cell; in botany, a colored body or plastid found commonly in plant cells, *e. g.*, a chloroplastid.
- Cilia** (sing., Cilium). Minute, hairlike, motile, protoplasmic processes of cells, *e. g.*, ciliate protozoa. Also widespread throughout the animal kingdom.
- Cinclides** (sing., Cinclis). Minute openings in the body wall of sea anemones.
- Circular canal.** Marginal canal of a medusa; also applied to the water canal which surrounds the mouth of an echinoderm.
- Circumferential canal.** Circular canal of a medusa.
- Cirrus.** A soft, elongate outgrowth or appendage; in protozoa, an organelle composed of a cluster of fused cilia.
- Cleavage.** Rapid cell division not accompanied by growth.
- Clitellum.** The thickened, glandular region which secretes the cocoon of an earthworm.
- Cloaca.** The modified posterior part of the alimentary canal which receives products from the excretory organs and frequently from other organs.
- Cnidoblast.** Stinging cell in coelenterates. Contains the nematocyst.
- Cnidocil.** Slender process of a cnidoblast, stimulation of which may cause the ejection of the nematocyst.
- Coelenteron.** The digestive cavity of a coelenterate.
- Coelom or true body cavity.** The cavity between the alimentary canal and the body wall. Lined with mesoderm.
- Coenosarc.** The living part of the stalk of a coelenterate. It is continuous with the polyps.

- Collar cell.** A cell provided with a protoplasmic collar; choanocyte.
- Colon.** Variously applied to a portion of last part of digestive tract.
- Columella.** Axis around which the spire of a gastropod shell is wound.
- Commensal.** Organisms living together and usually partaking of the same food.
- Commissure.** In invertebrates, a nerve connecting two ganglia of a pair.
- Compound eye.** An arthropod eye which is composed of many similar units, called ommatidia.
- Connecting canal.** The canal which joins the tube foot to the radial canal of an echinoderm.
- Connective.** In invertebrates, a nerve connecting two ganglia not of a pair.
- Contractile vacuole.** Pulsating ectoplasmic organelle present in fresh-water protozoa and commonly absent in marine forms. It discharges water and possibly excretory material.
- Copulation.** Union for the purpose of transferring spermatozoa from male to female.
- Cormidium.** An assemblage of structures of a siphonophoran colony consisting of a hydrophyllium, a gastrozoid, a dactylozoid and a gonozoid.
- Coxa.** Basal segment of the leg of an insect.
- Coxopod.** Basal segment of the protopod in crustacea.
- Craspedote medusa.** A medusa possessing a velum.
- Crop.** An enlargement of the alimentary canal for storage of food.
- Crystalline style.** A transparent rod found in the alimentary canal of many polycypods.
- Ctenophoral row.** A row of swimming plates on a ctenophore.
- Cuticle.** Nonliving external layer or protective covering.
- Cyst.** A sac or pouch, *e. g.*, as in the larval stage of tapeworms or resting condition in protozoa.
- Cysticercus.** A stage in the development of many tapeworms.
- Dactylopod.** Terminal segment of endopod in crustacea.
- Dactylozoid.** Elongated tentacle-like zooid of a coelenterate, *e. g.*, a siphonophore.
- Denticle.** Small, toothlike protuberance, as in the buccal cavity of some annelids.
- Dermal branchiae.** Epithelial projections from the surface of the body which are used for respiration in echinoderms.
- Development, embryonic.** The series of changes which lead from the fertilized egg to the mature animal.
- Digestive gland.** Any gland which secretes a digestive fluid.
- Dimorphism.** Two distinct forms of individuals in the colony or species.

- Dioecious.** Said of species in which the male and female gonads are in separate individuals.
- Directive septa.** Those placed opposite the siphonoglyphs of an actinozoan.
- Disk.** In a starfish, the central portion from which the arms radiate.
- Dissepiment.** Septum.
- Distal.** Remote from the point of origin or attachment. Converse of proximal.
- Diverticulum.** An outpocketing from a tube, a caecum.
- Dorsal.** Pertaining to the back.
- Dorsal lamina.** A ciliated ridge on the dorsal side of the pharynx of an ascidian.
- Dorsoventrally.** From the dorsal to the ventral position.
- Ectoderm.** The outer embryonic or germ layer.
- Ectoparasite.** A parasite on the outside of the body.
- Ectoplasm.** Outer protoplasmic layer of a protozoan.
- Efferent.** Carrying away, as a vessel which leads away from an organ.
- Elytra.** The modified forewings of a beetle; the scales of a scale-worm.
- Embryo.** An immature organism.
- Encyst.** To enclose in a cyst.
- Endoderm.** The inner embryonic or germ layer.
- Endoparasite.** A parasite inside the body.
- Endophragmal skeleton.** Chitinous plates which cover the nerve chain and ventral blood sinus in the thorax of certain crustaceans.
- Endoplasm.** Inner protoplasmic portion of a protozoan.
- Endopod.** In a biramous appendage of an arthropod, the branch which is nearer the midline of the body.
- Endoskeleton.** An internal skeleton.
- Endostyle.** A ciliated groove in the ventral wall of the pharynx of certain primitive chordates.
- Ephyra.** The stage which follows the scyphistoma in the development of a scyphomedusan. It consists of a disklike body with 8 marginal notched lobes.
- Epicardium.** A hollow process from the pharynx of some ascidians.
- Epipharynx.** In some insects, a projection from the roof of the mouth.
- Epiphysis.** A plate joined to the base of the alveolus in the mouth parts of an echinoid.
- Epipod.** A membranous projection from the basipod of certain crustacean appendages. It extends into the gill chamber.
- Episternum.** Among invertebrates, a lateral piece next to the sternum, as in arthropods.

- Epistome.** A projection above the mouth as in *Pectinatella*.
- Esophagus.** The portion of the alimentary canal which leads back from the buccal cavity or pharynx.
- Euglenoid.** Similar to *Euglena*, especially in movements.
- Exopod.** The branch of the biramous appendage of an arthropod which is away from the midline of the body.
- Exoskeleton.** An outer covering, as a shell.
- Exumbrella.** The convex or aboral side of a medusa.
- Eye spot.** A pigment spot generally supposed to be photoreceptive.
- Femur.** In an insect appendage, the third segment from the body of the animal.
- Fission.** Reproduction by division.
- Flagellum.** An elongated, protoplasmic, motile process of a cell.
- Flame cell.** An excretory cell characteristic of certain invertebrates, *e. g.*, platyhelminthes, nemertines, rotifers and certain polychaetes. It possesses a cavity continuous with the lumen of the excretory duct. This cavity may contain a bundle of cilia or a flagellum.
- Food vacuole.** In Protozoa, a temporary space in which food is digested.
- Foot.** Among invertebrates, a locomotor organ characteristic of molluscs.
- Funiculus.** A strand of tissue which connects the stomach with the body wall in Bryozoa.
- Funnel.** The tube through which water is expelled from the mantle chamber by cephalopods.
- Ganglion.** A group of nerve cells.
- Gastric filament.** One of the filaments in the digestive cavity of Scyphozoa.
- Gastrovascular.** Digestive and circulatory in function, as the gastrovascular cavity or coelenteron of coelenterates.
- Gastrozoid.** Feeding individual in a hydrozoan colony.
- Genital atrium.** A cavity receiving the genital ducts, as in *Bdelloura*.
- Genital gland.** A gonad.
- Genital plate.** One of the plates bearing the external openings of the genital ducts in echinoderms.
- Genital pore.** The external opening of a genital duct as in the genital plate.
- Gill.** Aquatic respiratory organ.
- Gizzard.** A heavy, muscular, grinding division of the alimentary canal.
- Gonad.** A gland which produces sex cells.
- Gonangium.** See *Gonotheca*.

- Gonophore.** In coelenterates, the specialized form which produces the sex cells; whether a free-swimming medusa or any of the various intermediate stages between it and the sporosac.
- Gonosome.** The assemblage of structures directly connected with sexual reproduction in hydroids. The comprehensive term which includes gonophores, blastostyles, ovaries, gonangia, etc.
- Gonotheca.** The chitinous covering of a gonozooid in calyptoblastic hydroids.
- Gonozooid.** The reproductive zooid in coelenterates.
- Green gland.** In certain crustaceans, a paired excretory gland, the duct of which opens to the outside at the base of the second antenna.
- Gullet.** Esophagus.
- Gut.** Digestive tube.
- Gymnoblasic.** Lacking hydrothecae and gonothecae.
- Head.** The more or less differentiated anterior end in bilaterally symmetrical animals.
- Hepatic caeca.** Digestive glands opening in the stomach in certain echinoderms.
- Hermaphrodite.** An individual in which both male and female gonads are present.
- Holophytic.** The nutrition characteristic of green plants.
- Holozoic.** The nutrition characteristic of animals.
- Homologous.** Of similar structure or origin.
- Host.** The organism which harbors a parasite.
- Hyaline.** Transparent, glassy.
- Hydranth.** An individual of a hydroid colony.
- Hydrocaulus.** The stem of a hydroid colony.
- Hydrophyllium.** A disk-shaped protective structure covering other parts of the cormidium in a siphonophore.
- Hydrorhiza.** The rootlike attachment of a hydroid colony.
- Hydrotheca.** The chitinous covering of a vegetative hydranth in calyptoblastic hydroids.
- Hypodermis.** The single-layered integument in invertebrates possessing a cuticle.
- Hypopharynx.** A projection borne on the lower side of the pharynx of some insects.
- Hypostome.** In a hydranth, the projection which bears the mouth.
- Incurrent canal.** A canal that admits water to a sponge.
- Integument.** Outer covering of an animal.
- Interambulacral area.** One of the areas of an echinoderm which lies between adjacent ambulacral areas.

- Interfilamentar junction.** A connection between adjacent filaments in a pelecypod gill.
- Interlamellar junction.** A connection between adjacent lamellae in a pelecypod gill.
- Internode.** In hydroids, that portion of a stem or branch between two branches.
- Inter-radial canals.** In a medusa, the canals which run from the stomach to the circular canal and which lie midway between the per-radial canals.
- Introvert.** A portion which may be drawn in, as the anterior end of *Phascolosoma*.
- Ischiopod.** The first segment of a crustacean endopod.
- Kidney.** Frequently but improperly applied to the excretory organ of an invertebrate.
- Labrum.** The fused pair of appendages which forms the lower lip of insects and some other arthropods.
- Lamella.** One of the two sides which form a pelecypod gill; a flat structure.
- Lamelliform.** Like a lamella; thin and flat.
- Lamina.** A thin plate or a scale.
- Lancet.** A sharp structure; a portion of the sting of a bee.
- Larva.** An embryo; a stage in the development of an animal.
- Lateral.** At or toward the side.
- Ligament.** In pelecypods, the elastic structure which unites the valves.
- Lithite.** One of the concretions in a tentaculocyst of a medusa.
- Liver.** Frequently but improperly applied to the largest digestive gland of many invertebrates.
- Lophophore.** The disk which surrounds the mouth and bears the tentacles in the Molluscoidea.
- Lorica.** The transparent covering of a rotifer.
- Macronucleus.** In general, the larger of the two nuclei of ciliate Protozoa.
- Madreporic plate.** The perforated plate through which the water-vascular system of an echinoderm communicates with the outside.
- Mandible.** One of a pair of mouth appendages in arthropods.
- Mandibulate.** Possessing mandibles.
- Mantle.** The outer fold of the body of many molluscs; the entire body wall in tunicates. In both groups it secretes a protective covering.
- Manubrium.** The hollow outgrowth supporting the mouth of a medusa.

- Marginal lappets.** Small flaps of tissue near the sense organs of Discomedusae.
- Mastax.** In rotifers, the very active muscular grinding part of the digestive tract.
- Maxilla.** One of the mouth appendages of arthropods.
- Medusa.** Jelly fish; the sexual stage of certain coelenterates.
- Membranelles.** Organelles formed of a double row of fused cilia found in some ciliates.
- Meropod.** The second segment from the base of the endopod of a crustacean.
- Mesenteric filament.** In Actinozoa, the thickened free edge of certain mesenteries. Gland cells and nettle cells are present here.
- Mesentery.** A membrane which supports the intestine. In Actinozoa, a membranous lamella or sheetlike portion having mesoglea for its middle layer and covered on either face by endoderm.
- Mesoglea.** The jelly-like substance which separates the ectoderm and endoderm of a coelenterate.
- Metagenesis.** In animals, alternation of generations.
- Metamere.** One of the serial body segments of an animal, as in annelids.
- Metamorphosis.** In animals, a change in structure, as from larval to adult stage.
- Metapleural fold.** One of a pair of folds on the sides of Amphioxus.
- Micronucleus.** In general, the smaller of the two nuclei in ciliates.
- Moniliform.** Resembling a string of beads.
- Monoecious.** Said of species in which the male and female gonads are present in the same individual.
- Mouth.** The opening through which food is taken.
- Myoneme.** A contractile fiber, as in Vorticella.
- Nacre.** The innermost layer of a mollusc shell.
- Nematocyst.** The stinging organ contained in the cnidoblast.
- Nephridiopore.** The external opening of a nephridium.
- Nephridium.** Excretory tubule.
- Nephrostome.** The opening from the coelom into a nephridium.
- Nerve connective.** In invertebrates, a nerve connecting two ganglia not of a pair.
- Nettle cell.** Cnidoblast.
- Neuropodium.** The ventral division of a parapodium of an annelid.
- Nidamental gland.** In certain molluscs, an accessory reproductive gland possessed by females.
- Notochord.** A dorsal, living, internal, supporting structure characteristic of Chordata.
- Notopodium.** The dorsal division of a parapodium of an annelid.

- Odontophore.** A special structure in the mouth of most mollusca except pelecypods. The name is applied to the whole structure, cartilage, radula and muscles. (It is used by some authors as the equivalent of radula.)
- Ocellus.** A simple eye of an arthropod.
- Ocular plate.** In echinoderms, a plate at the end of an ambulacral area.
- Olfactory organ.** An organ to distinguish odors.
- Ooecium.** A structure in Bryozoa in which the embryo develops.
- Ootype.** The region in flat worms where the eggs are supplied with shells.
- Operculum.** The horny lid which fits into the aperture of the shell of some gastropods. A chitinous protective structure on the hydrotheca of certain hydroids which closes the hydrothecal aperture when the hydranth is retracted.
- Oral.** Pertaining to the mouth.
- Organelle.** A specialized part of a cell, as a cirrus, performing functions analogous to those of the organs of metazoa.
- Osculum.** The opening of a sponge through which water escapes.
- Osphradium.** A structure in the gill region of aquatic molluscs and supposedly of sensory function.
- Ossicle.** A small hard plate.
- Ostium.** A small pore; in pelecypod gills, one of the pores through which water is passed. In sponges, the opening through which water enters an incurrent canal.
- Otocyst.** A statocyst.
- Otolith.** Statolith.
- Ovary.** A gonad which produces eggs.
- Oviducal gland.** A glandular portion of an oviduct, as in the squid.
- Oviduct.** In a female, a duct which carries eggs to the uterus or the exterior.
- Ovipositor.** In some insects, an elongate structure used in depositing eggs.
- Ovum.** Egg.
- Pallial line.** The depression in the shell of a pelecypod at the point of attachment of pallial muscles.
- Pallial sinus.** The indentation in the pallial line of some pelecypods at the point of insertion of the retractor muscles of the siphons.
- Palp.** A process near the mouth. It occurs in several invertebrate groups: *e. g.*, in polychaete annelids, one of a pair of processes on the ventral side of the prostomium; in pelecypods, one of four fleshy processes around the mouth.

- Papilla.** A small projection.
- Paragnatha.** Lamellae behind the mandibles of some Crustacea.
- Parapodium.** Typical annelid appendage. Usually one pair per segment.
- Parenchyma.** Any soft tissue; specifically, the tissue occupying much of the space between the body wall and digestive tract in a flat worm.
- Pectine.** One of a pair of appendages of scorpions.
- Pedicel.** The stalk supporting a hydranth or gonozooid.
- Pedicularia.** A minute pincer-like organ which is present on Assterioidea and Echinoidea.
- Pedipalpi.** The second pair of appendages in arachnids. They lie on each side of the mouth.
- Peduncle.** A short stalk.
- Pelagic.** Said of organisms which live at or near the surface of the water.
- Pen.** Vestigial internal shell of a cephalopod, as in the squid.
- Penis.** Male intromittent organ.
- Pericardium.** The membrane surrounding the heart.
- Pereiopod.** The walking leg of a crustacean.
- Peripharyngeal bands.** The ciliated bands situated in the anterior region of the pharynx and connecting the endostyle with the dorsal lamina in many protochordates.
- Periproct.** The region around the anus (especially applied to the echinoderms).
- Perisarc.** The chitinous covering of the coenosarc in hydroids.
- Peristalsis.** The motion caused by the relaxation and contraction of the muscle fibers in the walls of a tube.
- Peristome.** The region around the mouth (especially applied to echinoderms).
- Peristomium.** The first somite of an annelid. In it the mouth lies.
- Peritoneum.** The membrane which lines the coelom.
- Per-radial canals.** In a medusa, the canals which run from the stomach to the circular canal and which lie opposite the corners of the mouth.
- Pharynx.** An anterior division of the alimentary canal.
- Planula.** A young ciliated, free-swimming coelenterate embryo consisting of two cell layers.
- Pleopod.** Any abdominal appendage of a crustacean.
- Pleurobranch.** A crustacean gill which is borne on the body wall.
- Pleuron.** One of the lateral pieces or processes of a somite of an arthropod.
- Podobranch.** A crustacean gill which is borne on the basal joint of an appendage.

- Polymorphism.** Many distinct forms of individuals within a single species.
- Polyp.** An individual of a hydroid stage of a coelenterate.
- Postcava.** In some invertebrates, a blood vessel which leads to the heart from the posterior portion of the body, as in the squid.
- Posterior.** Hinder; anal end.
- Precava.** In some invertebrates, a blood vessel which leads to the heart from the anterior end of the body.
- Primary mesentery.** A mesentery which extends from the body wall to the actinopharynx in Actinozoa.
- Proboscis.** Applied to various tubelike organs around the head sometimes capable of being everted or protruded.
- Proglottid.** One of the numerous "segments" which are formed by the scolex and which, together with the scolex, make up a tapeworm.
- Propod.** The next to the last segment, fourth, of a typical crustacean endopod.
- Prosopyle.** One of the pores through which water passes from an incurrent to a radial canal in most sponges.
- Prostomium.** The anterior process which overhangs the mouth of an annelid.
- Prothorax.** Anterior division of the thorax of an insect.
- Protomerite.** The anterior part of a eugregarine, *e. g.*, Gregarina.
- Protopod.** The basal portion of a crustacean appendage. It consists of two segments, a basal coxopod and a distal basipod. The latter frequently bears both an exopod and an endopod. In some cases it also bears an epipod or bract and a gill.
- Proximal.** Nearest to the point of origin or attachment. Converse of distal.
- Pseudopodium.** A temporary protrusion of the protoplasm of a cell. Found in all Sarcodina and in certain tissue cells of higher animals.
- Pyloric stomach.** Posterior or second division of the stomach.
- Radially symmetrical.** The condition of having similar parts radially arranged about a central axis; as in a jelly fish.
- Radius.** One of the parts of the jaw apparatus of an echinoid; from center to periphery.
- Radula.** The flexible, tooth-bearing, ribbon-like membrane of an odontophore.
- Ray.** One of the arms of a starfish or a brittle star.
- Rectum.** The posterior division of the alimentary canal.
- Renal organ.** An organ which excretes nitrogenous wastes and other materials.
- Reservoir.** The place where anything is stored; the poison sac of a bee.

- Respiratory tree.** The respiratory mechanism of some holothurians, opening into the cloaca.
- Retractor muscle.** A muscle that withdraws an organ or part of an animal.
- Root stalk.** A creeping stem from which the hydrocauli originate.
- Rostrum.** The anterior spine of a lobster and of other crustaceans.
- Rotula.** One of the calcareous pieces of the jaw of an echinoid.
- Rudimentary.** When applied to adult animals, means permanently undeveloped; vestigial.
- Sagittal plane.** The median plane, *i. e.*, that which divides a bilaterally symmetrical animal into two equivalent halves.
- Salivary gland.** In invertebrates, any gland which opens into the mouth cavity.
- Scaphognathite.** The flattened structure composed of the fused endopod and epipod of the second maxilla of certain crustacea. It is used to expel water from the branchial cavity.
- Schizogony.** Spore formation of the type characteristic of schizonts.
- Schizonts.** In Sporozoa a cell, formed by the growth of a sporozoite (merozoite) in a cell or corpuscle of the host, which forms merozoites by sporulation (multiple fission).
- Schizopod.** A biramous arthropod appendage.
- Scolex.** Anterior or attaching portion of the tapeworm from the posterior end of which the proglottids are formed.
- Scyphistoma.** The attached, hydra-like larval stage of many Scyphozoa.
- Segment.** One of a series of divisions of an animal's body or appendage. In the former case it is synonymous with somite.
- Segmentation.** (a) Cleavage; (b) division of body into segments.
- Seminal receptacle.** A sac in which spermatozoa are stored.
- Seminal vesicles.** The sacs which inclose the testes of an earthworm.
- Septum.** A plate which divides two spaces. In annelids the partition which separates the coelomic cavities of adjacent segments.
- Sessile.** Attached; without the power of locomotion. Also applied to hydranths without a stalk.
- Seta.** A stiff, slender, bristle-like structure. Most commonly applied to one of the chitinous bristles imbedded in the body wall or parapodium of an annelid.
- Setigerous gland.** An integumentary gland which forms setae. Typically two pairs per segment.
- Shell gland.** A gland which secretes the egg shell; sometimes applied to the excretory organs of Entomostraca.

- Siphon.** One of the two tubes concerned with the passage of water through a mollusc or a tunicate. Water enters through the in-current siphon and flows out through the excurrent siphon.
- Siphonoglyph.** The ciliated groove leading into the actinopharynx from a corner of the mouth.
- Somite.** Metamere; one of the serial body segments of an animal.
- Sperm.** Spermatozoon; male reproductive cell.
- Spermary.** A temporary sperm-producing organ; a testis.
- Spermatheca.** A seminal receptacle, used for storing spermatozoa in the female.
- Spermatophore.** A specially formed packet of spermatozoa.
- Spermatozoon.** Male reproductive cell.
- Sperm sphere.** A mass of spermatozoa in the earthworm.
- Spicules.** Minute skeletal bodies characteristic of sponges.
- Spinneret.** One of the organs by means of which a spider spins its thread.
- Spiracle.** Breathing pore; external opening of the tracheal system.
- Spiral valve.** The spirally wound internal fold of the wall of the intestine in elasmobranchs and certain other fishes.
- Spongin.** The specialized spicule material of which the fibers of the commercial sponges are composed.
- Spore.** In animals, especially Protozoa, a cell, the product of multiple fission, which is capable of developing into a new organism.
- Sporoblasts.** In sporozoa, a cell which divides into sporozoites.
- Sporogony.** Reproduction by multiple fission (sporulation). Specifically, in Sporozoa, spore formation by a zygote after encystment.
- Sporont.** The detached stage of Gregarina.
- Sporosac.** In coelenterates, a sac which contains the generative cells—an undeveloped, possibly degenerate medusa.
- Sporozoite.** In Sporozoa, a small, usually elongate, sickle-shaped or ameboid spore produced after zygote formation.
- Sporulation.** The act of forming spores; multiple fission.
- Stalk.** A stem or a peduncle.
- Statoblast.** Asexual reproductive body of certain Bryozoa.
- Statocyst.** An organ of equilibrium present in many invertebrates.
- Statolith.** The body or bodies present within the cavity of a statocyst.
- Sternum.** The ventral covering of a segment of an arthropod.
- Stigma.** In insects, one of the external openings of the trachea; one of the apertures in the pharynx of an ascidian.
- Stolon.** An extension of the body wall from which buds are developed.
- Stomach.** In invertebrates, the storage (food) division of the alimentary canal.
- Stomach-intestine.** A division of the alimentary canal which functions as both stomach and intestine, as in the earthworm.

- Stomodaeum.** The anterior portion of the alimentary canal which is ectodermal in origin.
- Stone canal.** The tube which leads from the madreporic plate to the circular water canal in echinoderms.
- Stylet.** A small, sharp instrument.
- Subgenital Pits.** The pouches adjacent to the gonads of the Discomedusae, on the subumbrellar side.
- Subumbrella.** The concave or oral side of a medusa.
- Sulcus.** A furrow or groove.
- Suture.** An immovable union between plates or ossicles.
- Swimmeret.** Pleopod; any abdominal appendage of a crustacean.
- Swimming plate.** One of the ciliated swimming organs of a ctenophore.
- Synchronous.** Happening at the same time.
- Tactile.** Capable of feeling.
- Tarsus.** The segmented foot of an insect.
- Telson.** Hinder division of a crustacean, usually not considered a segment.
- Tentacle.** An elongated, tactile organ, usually unsegmented. In polychaetes, a prostomial structure usually arising from the anterior end and more dorsal than the palps.
- Tentaculocyst.** A marginal sense organ in certain medusae.
- Tergum.** The dorsal covering of a segment of an arthropod.
- Test.** Hard or firm outer covering as the shells of many protozoa, echinoderms, molluscs, etc., and the tunics of Urochorda.
- Testis.** Sperm-producing organ.
- Thorax.** The body division of arthropods between the head and abdomen.
- Tibia.** In an insect leg, the segment lying between the femur and the tarsus.
- Trachea.** One of the respiratory tubes of certain arthropods.
- Trichocyst.** An ectoplasmic organelle, often considered as offensive or defensive in function, formed in many holotrichous ciliates.
- Trivium.** The three rays of a starfish which are farthest from the madreporic plate.
- Trochal disk.** The ciliated disk of a rotifer.
- Trochanter.** In an insect leg, the second segment from the body of the animal.
- Trochophore.** The free-swimming larva, with bands of cilia, especially typical of the annelids and Mollusca.
- Trochosphere.** Trochophore.
- Tubercle.** A small knoblike prominence.
- Tunic.** The outer covering of an ascidian.

- Tunicin.** A cellulose-like substance found in the tunics of certain Urochorda.
- Typhlosole.** A longitudinal internal fold in the wall of the intestines of some invertebrates, *e. g.*, the earthworm.
- Umbo.** In the valve of a clam shell, the raised portion which ends in the beak. It is the first part of the shell to be formed.
- Umbrella.** The umbrella-shaped major portion of the body of a jelly fish.
- Uriniferous tube.** One of the tubes of an excretory organ.
- Uropod.** One of the pair of broad, leaflike appendages of the last abdominal segment of a crustacean.
- Uterus.** A female organ in which young develop.
- Vagina.** In invertebrates, the terminal division of the female reproductive duct.
- Vas deferens.** The duct which leads away from the testis.
- Velum.** The ledgelike, delicate, muscular membrane extending inward from the subumbrellar margin of certain jelly fishes.
- Ventral.** Under surface; belly.
- Ventricle.** A division of the heart which forces blood to the body.
- Ventriculus.** In insects, the division of the alimentary canal which leads into the stomach.
- Vestibule.** A depression near the mouth in certain Protozoa as in Vorticella.
- Vestigial.** An organ which remains undeveloped and has no function; rudimentary as applied in anatomy.
- Viscera (sing., Viscus).** Internal organs taken collectively.
- Visceral mass.** Applied to the portion of a mollusc which contains stomach, intestine, liver, gonads, etc.
- Vitellarium.** A female reproductive gland which supplies cells to be used as food for developing embryos, as in Bdelloura.
- Vitelline glands.** Same as vitellarium.
- Water tube.** One of the tubes between the lamellae of a pelecypod gill.
- Whorl.** One of the turns of a gastropod shell.
- Yolk mass.** A mass of food material for the nourishment of an embryo.
- Zoophyte.** An animal which is somewhat plantlike in appearance.
- Zoid.** One of the more or less independent individuals which go to make up an animal colony as in Obelia and Bugula. Zooids may be nutritive, reproductive, defensive, or sensory.

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