British Museum (Natural History).

Instructions for Collectors:

No. 12.—WORMS.

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Instructions for Collecting Worms.

INTRODUCTORY REMARKS.

On Collecting.

The term "Worms," as here used, embraces a great many animals which bear very little resemblance to the popular conception of a worm. Some little idea of their variety and of the situations in which they are most likely to be found may be gathered from the lists given below.

It will be seen that the parasitic groups are A 2 and 3, C, D, E, and those mentioned on pp. 10–11.

The majority are internal parasites of vertebrate animals; but their position in their hosts varies very greatly. Many live in the alimentary canal, but many occur in other cavities and organs of the body, *e.g.* the heart, liver, lungs, kidneys, bladder, body-cavity, nasal cavities, or even in the blood-vessels, bronchial tubes, and among the muscles and connective tissue. Some of the Trematodes and Leeches are external parasites of fishes and other aquatic or amphibious animals, and may be found attached to their skin, especially on the fins, or on the gills and in the cavity of the mouth. Hence one must not be content merely to open the intestines of an animal and make a hasty examination there, but all parts of the animal ought to be inspected. Even the connective tissue between the skin and flesh often contains parasites. Filariæ (Nematodes), for instance, often choose this situation, where they lie coiled up in capsules or "cysts"; and the larval forms of various other parasites may be discovered in similar positions. When found in the connective tissue or in the peritoneum covering the internal organs and body-cavity, or in the walls of the stomach, etc., it is advisable to cut out and preserve the cyst with a small portion of the surrounding tissue.

It is worth while to collect all parasites seen, since it is often only the most careful microscopic investigation that can determine a species.

Some small Nematodes parasitic in the intestines, more especially of birds, are so fine that they are difficult to see unless some of the contents of the intestine or cæca are shaken up in salt solution or water, when the little hair-like creatures can be picked up with a needle, to which they adhere readily. For many

Nematodes it is a good plan to shake or stir up the intestinal matter in a vessel of weak salt solution, and allow it to stand, when the worms will sink to the bottom, and the dirty fluid can be poured off. Clean fluid may then be added, and the process repeated until the worms are clean and free from *débris*.

In collecting Tape-worms the intestine of the host should be slit up with a pair of scissors (preferably blunt-ended) and the contents scraped out gently with the back of a knife or scalpel. The scraping ought to be so managed as to secure the heads of the worms, which may be attached more or less firmly to the lining of the intestine. If any are very firmly attached a small portion of the intestine may be cut out round them, and placed with the

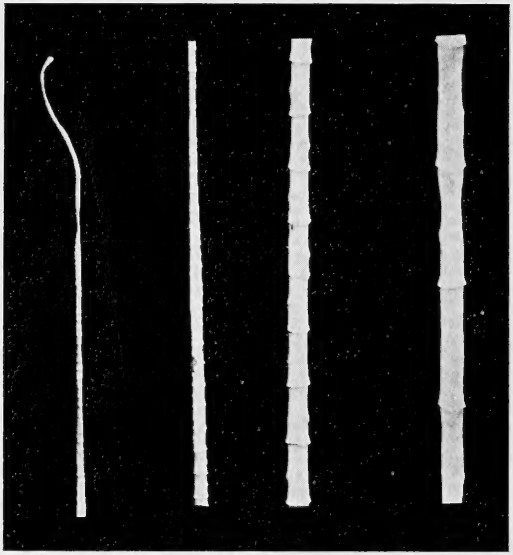

FIGURE 1.

Portions of an adult Tapeworm, *Taenia serrata*, natural size. *a*, the head (scolex) and some of the young segments; *d*, the most fully developed (gravid) segments, near the hinder end of the worm: *b*, *c*, portions from intermediate regions. [CESTODA.]

worms in a dish of weak salt solution, when by gentle persuasion, by means of scalpel or needles, the worms may be made to loosen their hold. In general, however, it is best to place the mass of worms and ingested matter in a tall vessel of salt solution, any floating lumps being broken up, and the worms then washed by gentle shaking, as directed on p. 22.

On Labelling.

It is extremely important to label all specimens fully.

The label bearing data as to the host in which a parasite occurred, its position in the host, the locality, date, and any other observations made at the time of collecting, is almost as valuable as the specimen itself; a specimen without its label is practically useless. These remarks, of course, apply to free-living forms as well as to parasites—notes as to the position in which they were found should always be given. The method of preservation used should also be stated. In addition to the labels giving such information, it is well to number the specimens, and to keep a note-book with full notes of each specimen or set of specimens opposite its number.

In the case of parasites, the species of the host (if known) should always be given. If unknown, an accurate description of it, and if possible a drawing, will help greatly in the labour of determination. In some cases (*e.g.* small Fishes, Reptiles, Mammals, &c.) it would be well to preserve the host as well as the parasite, giving it a corresponding label and number.*

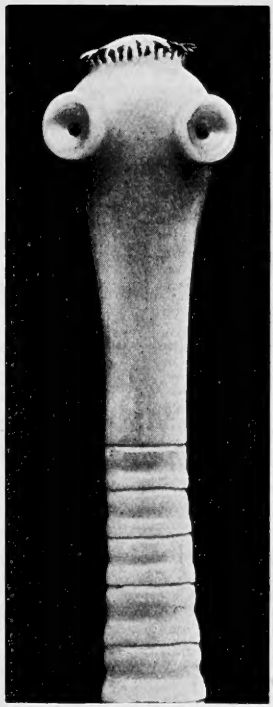

FIGURE 2.
The head (scolex) of a Tape-worm, greatly enlarged, showing the hooks and two of the suckers. From a wax model of *Taenia solium*. [CESTODA.]

Labels should be written clearly with a soft lead pencil on unsized paper, and placed in the preserving fluid with the specimens. Ordinary *ink* will not do for this purpose, as it will wash out. Certain kinds of waterproof Indian ink, however, are good, if allowed to dry thoroughly before being placed in the liquid. In some cases an additional label in ink may be placed outside the bottle.

* Fishes, Amphibians, Reptiles, &c., are best preserved in spirit; in the case of Birds and Mammals the skins must be preserved, and the skulls of Mammals should be dried and labelled to correspond with the skins. (See "Instructions for Collectors," Nos. 3, 2, 1.)

SCIENTIFIC AND POPULAR NAMES OF THE CHIEF GROUPS OF "WORMS."

A. PLATYHELMINTHES (Flat-worms). Including:—
   1. TURBELLARIA (Planarians).
   2. TREMATODA (Flukes).
   3. CESTODA (Tape-worms).

B. NEMERTINEA (Long unsegmented worms, mostly marine).

C. NEMATODA (Thread-worms or Round-worms).

FIGURE 3.
Fasciola hepatica, the Liver-fluke of the Sheep and Ox, seen from below and enlarged about 2 diameters. The ventral sucker is visible at a short distance behind the mouth. In some related species it is situated further back. [TREMATODA.]

D. NEMATOMORPHA or GORDIACEA (Hair-worms).

E. ACANTHOCEPHALA (Including *Echinorhynchus*).

F. CHÆTOPODA (Bristle-worms). Including:—
   1. OLIGOCHÆTA (Earth-worms and their allies).
   2. POLYCHÆTA (Marine bristle-worms).

G. GEPHYREA.

H. HIRUDINEA (Leeches).

I. PHORONIDEA (Phoronis).

J. ENTEROPNEUSTA (Balanoglossus).

Some of the distinguishing features of the groups mentioned above are outlined in the following

BRIEF SYNOPSIS OF THE CHIEF GROUPS OF WORMS.

A. PLATYHELMINTHES. Animals of flattened shape, their viscera not contained in a "body-cavity." The alimentary canal, which is either simple or branched, has no anus or vent. Mostly aquatic or parasitic; exceptionally living on land in moist situations. These include:—

(1) TURBELLARIA (see Fig. 14). Usually with soft, oval or elongate, unsegmented body, covered with minute cilia, or vibratile "hairs." Mouth often in centre of ventral surface, or even further

back, with protrusible tubular pharynx. Alimentary canal simple or much branched. Chiefly aquatic (marine and fresh-water); some terrestrial, living in damp places, generally under logs or stones.

(2) TREMATODA (*e.g. Fasciola hepatica*, the well-known Liver-fluke of the Sheep.—See Fig. 3). Internal or external parasites; usually of oval shape. Unsegmented. With suckers and sometimes horny hooks for attachment to host. Alimentary canal bifurcate, sometimes branched.

(3) CESTODA (*e.g. Tænia* of various species, parasitic in man, dog, etc.—See Figs. 1 and 2).

Elongate parasitic creatures, of ribbon-like form, with a rounded "head" bearing suckers or hooks and suckers, followed by a series of segments increasing in size posteriorly. The hinder segments are generally full of eggs. The larval or young forms (see Fig. 4) of these worms are found as bladder-like "cysts" of various sizes embedded in the organs (*e.g.* the liver) of some animal which is liable to be eaten by their final host. Alimentary canal absent in all stages.

FIGURE 4.
A "Bladder-worm," *Cysticercus tenuicollis*, enlarged about 1½ diameters. This is the larval stage of one of the common Tapeworms of the Dog, and is found in the lining of the body-cavities of Sheep, Cattle, etc. [CESTODA.]

[*Note.*—In collecting Tapeworms great care must be taken to find the head (Figs. 1 and 2), which is very minute and easily broken off, as it may be attached firmly to the host's tissues. (See p. 2.)]

B. NEMERTINEA (see Fig. 15). (Such as *Lineus* of the sea-shore.) Generally very elongate, unsegmented, more or less flattened worms, capable of great extension and contraction. Sometimes several yards in length when extended. Possessing a remarkable protrusible proboscis, in a sheath lying above the alimentary canal. Mostly marine, rarely on land or in fresh water, living under stones, etc.

C. NEMATODA (see Fig. 5). (Such as *Ascaris lumbricoides*, the largest round-worm found in man.)

Mostly parasitic worms of elongated form, with flexible but not extensible cylindrical, unsegmented body. (There are many free-living Nematodes, but these are mostly very minute, living in

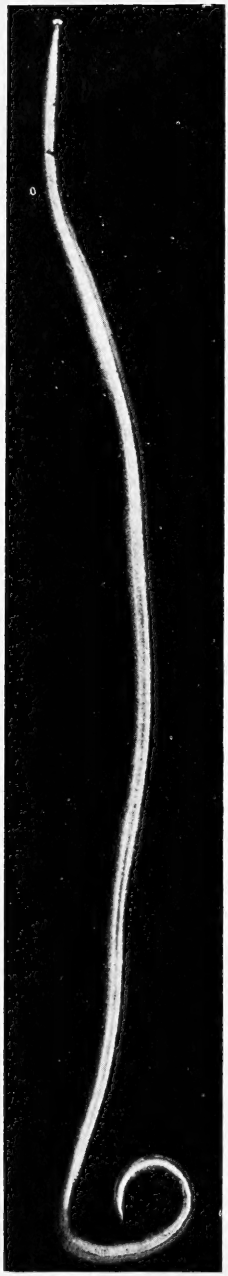

FIGURE 5.

The large Nematode, *Ascaris megalocephala*, natural size, of the intestines of the Horse. This specimen shows the curvature of the tail, characteristic of the male sex of many Nematodes. [NEMATODA.]

water or in the soil; some are also parasites of plants.) Tail of *male* nearly always curved downwards, and provided either with a membranous expansion, or with curved horny spicules, or both.

D. NEMATOMORPHA (Gordiacea) (see Fig. 13). Extremely long, thin, wiry-looking worms, traditionally compared to "animated horse-hairs." Parasitic at first in the body-cavity of Insects; afterwards emerging from them to lead a free life, usually in fresh water.

E. ACANTHOCEPHALA (*Echinorhynchus*, etc.—See Fig. 6). Unsegmented, cylindrical worms, more or less resembling Nematodes, but having a proboscis armed with many hooks (see Fig. 7), with which they attach themselves to the tissues of the host—usually to the lining of the stomach or intestines. Larvæ in cysts, usually in aquatic insects or crustacea, sometimes in fishes, preyed upon by the final host. Alimentary canal absent.

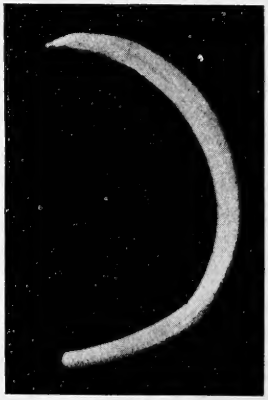

FIGURE 6.
Gigantorhynchus gigas, natural size, of the Pig. This is a male specimen, the female being considerably larger. [ACANTHOCEPHALA.]

F. CHÆTOPODA. Body divided into a number of "rings" or segments, each of which is provided with certain groups or rows of bristles.

(1) OLIGOCHÆTA (Earthworms, &c.).

Chiefly living below the surface of the soil (often crawling on the surface after rain, &c.), or among rotting timber or other vegetation. Others, of small size, in fresh water, or in mud at the bottom of ponds and streams.

(2) Polychæta (see Figs. 8, 9 and 16). Each segment bears generally a fin-like expansion on each side, from which bristles project fanwise. These expansions may be wanting, or confined to the front end of the body, in forms which inhabit tubes.

Marine. Some live under stones, or buried in sand at low-water mark; many in tubes of their own construction, attached to solid objects under water. The tubes are of very various appearance, some being hard and chalky, others covered with sand-grains, bits of shells, seaweed, &c., others quite transparent.

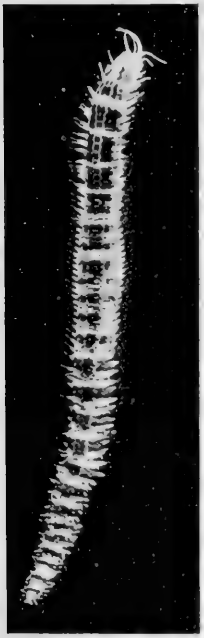

Figure 8.
An example of a free-living Polychæte worm, *Lepidasthenia elegans*, slightly enlarged, showing the characteristic paired appendages. [Chætopoda.]

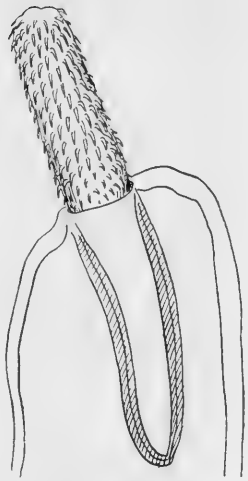

Figure 7.
The "head" end of a typical *Echinorhynchus*, greatly magnified, showing the cylindrical proboscis covered with hooks. [Acanthocephala.]

Many of the tube-living worms (see Fig. 9) have a flower-like crown of feathery "gills" on the head, which can be protruded from the opening of the tube.

A few Polychætes lead an entirely free-swimming life at the surface.

Certain forms, rather unlike the ordinary conception of a "worm," are included in this group (Polychæta)—such as the Polynoids, some of which are of woodlouse-like shape and appearance; and *Aphrodite* ("Sea-mouse") and its relatives, which are thick-set animals covered with strong bristles and beautifully iridescent hairs (see Fig. 16).

G. GEPHYREA (see Fig. 10). Marine forms, usually with a simple distended sac-like body, which shows no external traces of segmentation. Sometimes a crown of short tentacles may be seen expanded at one end. The viscera are enclosed in a spacious body-cavity.

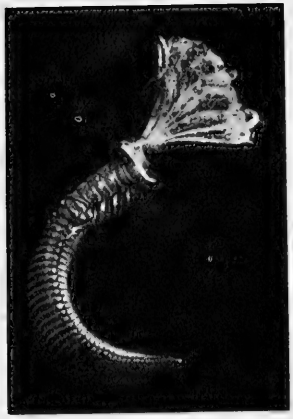

FIGURE 9.
One of the tube-forming Polychætes, *Sabella bombyx*, slightly enlarged. The specimen has been removed from its tube, and shows the crown of tentacles. [CHÆTOPODA.]

H. HIRUDINEA. (Leeches.) Worm-like animals, narrower at the head end than at the tail, and having a rounded sucker at each end. External, temporary parasites mostly found on vertebrates, sometimes entering the mouth or nose; living in water (either fresh or salt) or on land in moist places, sometimes climbing on to low bushes, &c.

I. PHORONIDEA (see Fig. 11). Unsegmented worms living in tubes, commonly found associated in large numbers. Tube membranous, sometimes covered with mud and other foreign substances. The mouth is in the middle of a terminal crown of tentacles, arranged on a more or less obvious horseshoe-like plan. Alimentary canal U-shaped, the intestine running up by the side of the œsophagus and opening near the mouth. The blood is red, with conspicuous red corpuscles.

Marine. May be found attached to solid objects in shallow water, or inhabiting tunnels bored in calcareous rocks, shells, &c.

J. ENTEROPNEUSTA (see Fig. 17). Soft-bodied worms which may have a strong odour of iodoform. The front end has the form of a conical or cylindrical "proboscis," which is followed by a short region termed the "collar," encircling the mouth. The rest of the animal, unsegmented and often of considerable length, is composed of several distinct regions, the first of which is often produced into large lateral flaps. A double series of pores occurs on the back or at the sides of this "branchial" region.

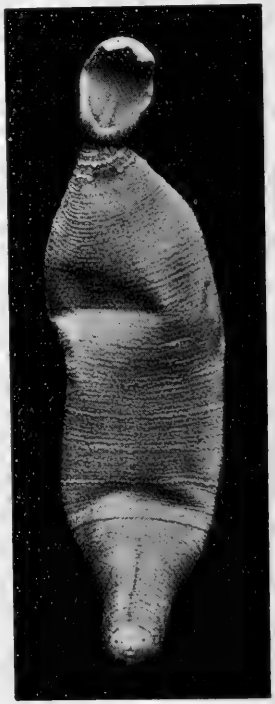

FIGURE 10.
Echiurus vulgaris, the "Spoon-worm," slightly enlarged. [GEPHYREA.]

Marine; to be looked for on sandy shores at extreme low tide-mark.

[*Note.*—The body is so easily injured that great care is required to obtain complete specimens; but they are worth preserving if the front half can be obtained in good condition.]

FIGURE 11.

A portion of a crowded colony of *Phoronis*, seen from above and slightly enlarged. The tentacles of the individual worms are not fully expanded. [PHORONIDEA.]

OTHER PARASITES WHICH ARE LIABLE TO BE CONFUSED WITH WORMS.

When searching, by dissection or otherwise, for parasitic worms, it is not improbable that various other parasites of more or less worm-like appearance will be found.

The importance of collecting *everything* of this kind, especially if there is any question as to its identity, cannot be too strongly emphasised. Hence a brief reference is made here to some of the parasitic creatures which bear some resemblance to worms.

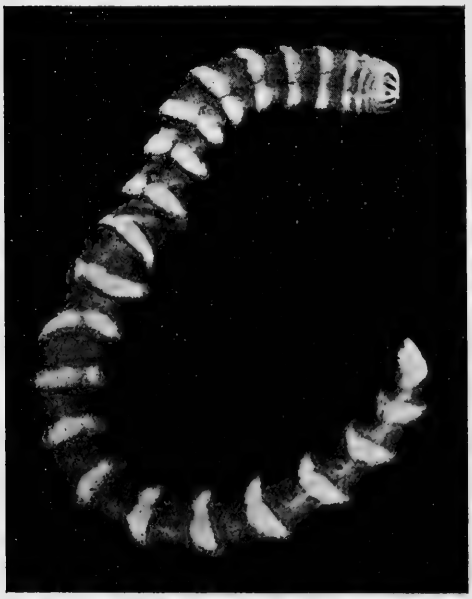

FIGURE 12.
A large Pentastomid, *Pentastoma polyzonum*, found in the Puff-adder; natural size. [PENTASTOMIDA.]

Certain parasitic Arthropods, in which the limbs are degraded or entirely lost, resemble worms in general appearance and are commonly confused with them. Among these may be mentioned the insect larvæ or MAGGOTS, which make their way into the mouth, nostrils or stomach, or under the skin of Mammals, including Man. These are the larval stages of certain Flies, and most of them attack Ungulates—(Horses, Cattle, &c.). Some, like the "Warble" flies, raise tumours of considerable size under the skin, in which the larvæ live and feed, and from which they eventually emerge. The so-called "Screw-worm" similarly attacks Man. In other cases, like the *Gastrophilus* of the Horse, the eggs swallowed by the animal hatch in its stomach, and the larvæ thereupon attach themselves as true internal parasites to the stomach-wall. They are true maggots and have the usual shape of such creatures, tapering in front and broadened behind, and armed with various rings of spines encircling the body.

The PENTASTOMIDA (*Pentastoma* or *Linguatula*, &c.) are curious worm-like creatures (see Fig. 12), of doubtful affinities, usually considered to belong to the Arachnid group, *i.e.* to be allied to Spiders and Mites. But in general appearance they resemble worms, having a comparatively soft elongated body, without limbs, and the head being armed with two pairs of sharp, horny hooks. In their immature stages they may occur enclosed in cysts among the tissues of almost any part of the body, and in almost any Mammal or Bird, or living free in its various cavities, more especially the general body-cavity. The adult forms usually occur in Snakes or other carnivorous Reptiles, and mostly take up a position in the lungs of their host.

Parasitic CRUSTACEA of various sorts may also occur. They are found mostly on Fishes and Whales, or on other Crustacea, on Mollusca and other aquatic invertebrates; they may assume a very different appearance from a typical Crustacean. The body may be reduced to a mere soft sac, without appendages, or with only vestiges of them. Sometimes there is a pair of elongated bags of eggs attached to the body, and looking almost like a pair of legs, or like a pair of long strings. These parasites are very commonly found, for example, clinging to the gills of Fishes.

Any of the above-mentioned creatures may be safely treated by ordinary methods of killing, such as corrosive sublimate, and preserved in alcohol.

Insect-larvæ may be killed by immersion for a *few seconds* only in boiling water, and then placed in weak spirit, in which they are to remain till hardened (two or three weeks). They can finally be transferred to stronger spirit.

HABITAT OF WORMS.

The following is intended as a rough indication of what may be looked for in a particular kind of locality, though it is impossible to give a complete guide:—

(i) *Inland localities.*

(*a*) Parasitic worms.—Any kind of mammal, bird, reptile, amphibian, or fresh water fish may be found to harbour parasites of many kinds. NEMATODES, TREMATODES, CESTODES, and ACANTHOCEPHALA occur in all these groups of animals, either in their larval or adult stages. As a rule, dissection is the only method of obtaining them, a special search being made in the alimentary canal. But some of the TREMATODES attach themselves to the skin of Fishes. LEECHES are sometimes found adhering to animals (*e.g.* Frogs) which have recently been in or near water. Reptiles, especially Snakes, are sometimes found to harbour *Pentastoma* (or *Porocephalus*, see

Fig. 12), a wormlike creature of doubtful affinities, which usually takes up a position in the lungs of its host.

Molluscs frequently contain the immature stages of TREMATODES.

Insects, such as beetles, grasshoppers and flies, often harbour the immature stages of "HAIR-WORMS" (*Gordius* and a Nematode worm of somewhat similar appearance, *Mermis*). These live coiled up in the body-cavity of the host. It is to be desired that the adult stages of these worms should be obtained, and if possible some knowledge acquired as to their eggs and larvæ, and the mode of infection of new hosts by the larvæ. It is recommended, therefore, that when these worms are seen emerging from insects, or found crawling about, an attempt should be made to keep them alive for a time in damp earth, water, etc., in the hope of learning more of their life-history, as the mature stage is usually only reached after leaving the insect host. Some small NEMATODES are also parasitic in insects, but usually require the microscope for their detection.

(*b*) Fresh-water worms.

TURBELLARIA are commonly found in any standing water, crawling on water-weeds or on the bottom, or even upside down on the surface-film. Since many of them are carnivorous, and have a liking for decaying matter, they may sometimes be conveniently caught by sinking a pot of dead earthworms in a pond or ditch, drawing it up after a day or two and collecting the Planarians that have been attracted by it.

Fresh-water fishes,* amphibians, etc., as mentioned above, should be examined for parasites. Some of the Insects and Crustacea also harbour NEMATODES and NEMATOMORPHA.

NEMERTINEA are rare in fresh water, but may be found occasionally.

Many NEMATODA occur in fresh water, in the mud at the bottom, or among plants, but these are chiefly of microscopic size.

NEMATOMORPHA habitually pass their adult stage of existence in fresh water, sometimes in standing water, sometimes in quite rapid streams. They may often be

* Fishes frequently harbour parasitic Crustacea, especially on their gills. These parasites should, if possible, be preserved, with a note of the position in which they were found, and information about the "host."

found tangled together (see Fig. 13) in great numbers, and twisted round weeds, etc., on which their eggs are laid.

The fresh-water CHÆTOPODS are practically all OLIGOCHÆTA, chiefly small forms living in the mud round the roots of aquatic plants, or plants growing near the edge of the water. Samples of mud scraped up from the bottom of any pond, ditch or stream may contain many of these worms. POLYCHÆTA are exceptionally found in fresh water, and these are most likely to be obtained by dredging in large lakes. In brackish water, on the other hand, POLYCHÆTES are numerous, living in the mud, under stones, etc., as do those of the sea-shore.

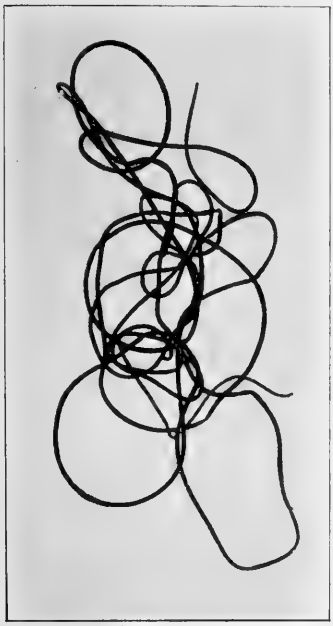

FIGURE 13.
A tangled mass of *Gordius aquaticus*, natural size. [NEMATOMORPHA.]

LEECHES are often abundant in ponds, ditches and streams, where they swim with an undulating movement, or attach themselves to solid objects, and walk like "looper" caterpillars.

(*c*) Land forms.

A number of PLANARIANS (see Fig. 14) crawl about in moist places, or may be found among wet vegetation, such as mosses, or under stones and decaying logs. Some of them suggest slugs, others are more elongated, but they are to be recognised by their extremely soft and slimy bodies, having no tough skin like molluscs or other worms. Land Planarians are chiefly, but not exclusively, tropical.

NEMERTINES are rare on land, but one or two have been recorded. They are likely to be found in wet places, under stones, &c.

NEMATODES of minute size are abundant in soil and among roots of grasses and other plants. Some are parasitic on plants, and form various kinds of galls and other diseased growths on grasses, &c. *Mermis*, as already mentioned, is at first parasitic in Insects, but after emergence from them is often found crawling about on the soil or on plants, sometimes in large numbers, especially after rain. It resembles *Gordius* in its very wiry appearance.

Gordius and its relatives are also sometimes found crawling on the ground, among dead leaves, &c., especially in wet forest districts.

Figure 14.
A land Planarian, *Geoplana fryi*, natural size. [Turbellaria.]

Oligochæta (Earthworms and their allies) are abundant everywhere in damp soil, under stones, or among rotting vegetation, sometimes even in the accumulated water and vegetable *débris* in hollows in the branches of trees. Many species are very small and inconspicuous. The earthworms may be obtained by digging, but many of them emerge from their burrows after rain, and may then be collected, while some species habitually lie with part of their bodies out of their holes at night, except in frosty weather or bright moonlight. They may be caught with forceps when found in this position, but are very sensitive to light and vibration, and dart back into their burrows with astonishing speed if disturbed.

Land-leeches occur chiefly in the tropics, lying in wait for their vertebrate prey, either on the ground or on forest undergrowth, always provided that the situation is moist. They often attack man and domestic animals passing through such places.

(ii) *Marine localities.**

(*a*) Parasites. As on land and in fresh water, all kinds of mammals, birds, reptiles, and fishes may be examined for Platyhelminthes, Nematoda, Acanthocephala, and Hirudinea (see Synopsis). The last-named are not always attached to their hosts (usually fishes), but may be found free in the water, or on stones and weeds.

* Much valuable information about the collection and preservation of Marine organisms will be found in a most useful book, "Science of the Sea," issued by the Challenger Society. London: John Murray. 6*s*.

It is also worth while to examine the gills and body-cavities of crustacea (crabs, &c.), for larval NEMATODES and TREMATODES, and the various cavities of shell-fish for larval TREMATODES, and their empty shells for NEMERTINES, &c.

(*b*) Free-living forms in the water or on shore.

PLANARIANS and NEMERTINES (see Fig. 15) are numerous, being generally found under stones between tide-marks or dredged in shallow water. The NEMERTINES are often in tangled masses. Empty shells should be examined for them, and also sea-weeds. Some Nemertines make gelatinous tubes, or even burrows in the mud. Pelagic Nemertines, swimming freely at the surface, are very rare.

Free-living NEMATODES are known in the sea, but many more no doubt remain to be discovered.

A single NEMATOMORPH, *Nectonema*, has sometimes been caught by the tow-net swimming at the surface.

POLYCHÆTA (see Figs. 8, 9, and 16, and p. 7) are very abundant. Unless dredging operations can be carried out, they are best obtained by shore-collecting at low tide. The burrowing kinds often betray their presence by leaving "casts" of sand which has passed through the alimentary canal on the surface of the ground, and may be obtained by digging in such spots.

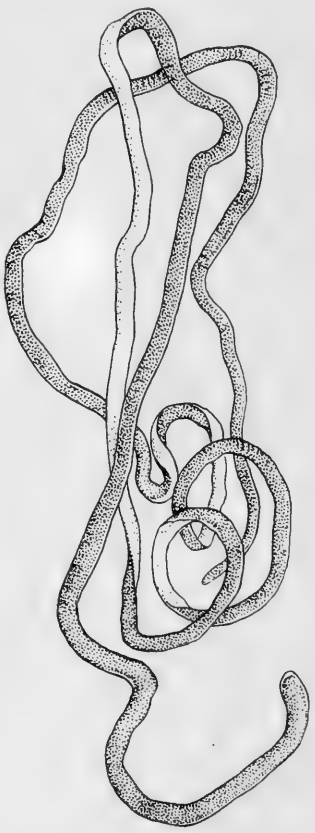

FIGURE 15.
A Nemertine, *Eunemertes gracilis*, enlarged, in a characteristically twisted position. [NEMERTINEA.]

GEPHYREA (see Fig. 10) will, as a rule, only be obtained by dredging, but a few may be found between tide-marks, burying themselves in mud, or forming tubes, or tubular excavations in soft rock. Some live as "commensals" with corals or sea-urchins, or in empty shells.

Oligochæta are rare on the shore, but a few forms have been found among seaweed above high-water mark, and some small species are partly marine.

For Leeches, *cf.* ii (a), p. 14.

For Phoronidea, see p. 8.

Enteropneusta (see Fig. 17) may be looked for on sandy shores at extreme low-tide mark. They

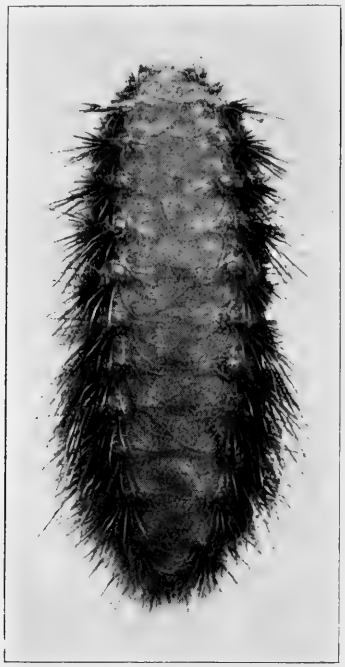

Figure 16.
Hermione hystrix, one of the spiny Polychætes, related to the "sea-mouse"; slightly enlarged. [Chætopoda.]

form large sand-casts, resembling those of Polychætes, and they may be found by digging in the neighbourhood of these casts. They must be handled with great care (see p. 9).

LIST OF USEFUL CHEMICAL REAGENTS.

Acetic Acid, glacial (see "Corrosive Sublimate"). Only a very small quantity of this will be required. Its use is to counteract the shrinkage of tissues, caused by solutions containing corrosive sublimate. A few drops added to the solutions are sufficient

Alcohol. The best is "pure spirit," which can often be obtained more easily abroad than in England, where the heavy duty makes its price almost prohibitive. For export only it may be obtained from English firms at about 2s. 6d. per gallon, or 10s. for a 10-gallon drum (returnable). The strength of the spirit as purchased should be about 90 per cent. For most purposes a strength of 70 per cent. is sufficient for use; this is obtained by adding 70 volumes of the 90 per cent. spirit to 20 volumes of water. Similarly, a 50 per cent. solution is obtained by diluting 50 volumes of 90 per cent. spirit with 40 volumes of water

Ordinary "methylated" spirit can be used for some animals, but cannot be diluted, as a white precipitate is formed on addition of water. For delicate marine animals it is quite useless. What is known as Industrial methylated spirit is, however, suitable for preserving specimens. In England it can only be obtained by special permit from the Customs authorities, and only in considerable quantities.

Sudden changes in the strength of this reagent should be avoided. Thus if it be desired to transfer specimens from corrosive sublimate to alcohol, they should be placed, after washing with water, in a weak solution of alcohol before being transferred to stronger spirit.

Figure 17.
Ptychodera minuta, enlarged about 1½ diameters. [Entero-pneusta.]

Chloral Hydrate (Crystals), used for narcotizing certain kinds of worms (*e.g.* Nemertines) before killing them, so as to cause them to die expanded. A few of the crystals may be added to the sea-water, or the animals may be placed in a 1 per cent. solution of the substance made up in sea water. Further remarks about narcotizing are made on page 21.

Corrosive Sublimate ($HgCl_2$). A saturated solution of this exceedingly poisonous substance in fresh water (or sea-water for marine animals) is a very useful killing and preserving fluid. Its action is extremely rapid, and is made even more so by heating. The solution is best made by putting more of the crystals in a vessel of water than will dissolve; but as solution takes place slowly the mixture should be made some hours before it is likely to be wanted. As the solution is used more water can be added at intervals. So long as some of the crystals remain undissolved the solution will remain "saturated." For delicate specimens which are not calcareous it is useful to add a few drops of glacial acetic acid to the solution. Steel instruments should not be brought into contact with solutions of corrosive sublimate.

Objects hardened with corrosive sublimate should be soaked for some hours in water or, better, in iodised alcohol (alcohol 70 per cent. 100 parts; tincture of iodine 2·5 parts) before being placed in alcohol for keeping. Otherwise the sublimate may crystallise out in the tissues, and spoil the specimens, when subsequently mounted for the microscope.

N.B.—The saturated solution of sublimate looks exactly like plain water, and the bottle containing it should be labelled "Poison."

Formalin. The commercial formalin as bought is a solution of the gaseous compound *formaldehyde* in water. Its strength is about 40 per cent. For use it may be diluted with several times its bulk of water; hence it is very convenient for certain purposes, owing to its portability. It is also much cheaper than spirit. The strength most convenient for preserving animals is a 10 per cent. solution of formalin (or, which is the same thing, a 4 per cent. solution of formaldehyde). This is made by diluting one volume of commercial formalin with 9 volumes of water. After having been preserved in this solution specimens may be kept in a weaker one—say 5 per cent. (5 parts commercial formalin to 95 of water).

Note.—As formalin does not give good results with many kinds of animals, some caution must be used in employing it. It is advisable not to preserve *all* the specimens of a kind in it, but to keep some in alcohol. Further, formalin contains a certain amount of acid, which is injurious to animals with calcareous structures, such as worms with calcareous tubes, or jaws in which the hardening matter is calcareous. Hence Polychætes should generally be preserved in alcohol rather than in formalin. For marine animals the formalin may be diluted with sea-water instead of with fresh water—an advantage which this reagent has over alcohol. N.B.—Formalin should not be used for preserving Nematodes, as it is liable to cause the specimens to burst.

Glycerine. This may be used, with special precautions, to preserve the natural colour of an animal. But as this reagent produces strong shrinkage in the tissues, it is essential to transfer the specimen into it gradually. This can be done by using successively stronger solutions of glycerine. The specimen may first be placed in a 10 per cent. solution of formalin containing a *small* quantity of glycerine. After a suitable interval (perhaps half an hour) the specimen, now hardened by the formalin, may be placed in a stronger solution of glycerine in water; and this process must be repeated each time with an increasing strength of glycerine. If the colour is not too fugitive, the specimen may be hardened in alcohol *for a short time*, transferred to a dilute solution of glycerine in alcohol, and then left in an open dish for the glycerine to concentrate itself by evaporation. In a hot, dry climate a formalin solution of glycerine might be allowed to concentrate itself in the same way.

Whatever method is adopted, the specimen should be brought into glycerine of full strength before the colour is lost.

Iodine, tincture of. A small quantity of this will be useful (see "Corrosive Sublimate").

Sodium Chloride (common salt). A weak solution of this substance should always be kept ready to hand for the preliminary washing of parasitic worms. 1 gramme of salt dissolved in 99 cubic centimetres of water will give the required strength (or about 1 oz. in 5 pints).

APPARATUS.

A few dissecting instruments will be required by those who intend to look for parasites. They need not be many or costly, but the following would be indispensable :—

Forceps.—Two or three pairs of various sizes, including one large and strong pair, and one with slender points.

Scissors.—A large strong pair for cutting open animals (sometimes better than a knife). One or two pairs of smaller dissecting scissors.

Scalpels.—Three or four of different sizes and shapes.

Needles.—A few of different sizes mounted in wooden handles.

Camel's hair brushes.—One or two.

Glass vessels.—Specimen tubes, with corks, of various sizes, will be required, and can be obtained cheaply from dealers in natural history requisites.

For larger specimens any kind of wide-mouthed bottles with sound corks or stoppers will do, but perhaps the best are those with screwed-on metal lids and rubber rings.

Tins, closed by soldering, may be used for specimens in spirit. They should under no circumstances be employed for specimens in formalin, which corrodes the metal.

Glass pipettes.—Fitted with rubber caps, are useful for picking up and transferring small specimens. "*Dipping tubes*," or straight pieces of glass tubing, with their cut edges rounded by placing for a few seconds in a flame, are useful for similar purposes, especially for capturing small creatures contained in deep vessels of water. (The method of using a dipping tube is as follows: close one end with a finger, and bring the other end down into the water until close to the specimen. Then remove the finger, when water will rush up the tube, carrying the specimen with it. Replace the finger over the upper end of the tube, and withdraw the tube from the vessel.)

Test-tubes.—A few of these will be useful in cleaning specimens, as described below.

Dishes.—Shallow glass or porcelain dishes may be used for containing the fluids in which specimens are to be killed or washed, but an ordinary saucer, plate, or other such vessel will serve most purposes equally well. Enamelled iron vessels are particularly useful when travelling—*e.g.* for heating the alcohol with which Nematodes are to be preserved. For this purpose they need only be wide enough to allow the specimens to be straightened out, and should not be too shallow, in order to lessen the possibility of the spirit being set on fire.

SPECIAL METHODS FOR THE TREATMENT OF SPECIMENS.

Cleaning. Having collected the specimens, the first thing to do is to clean them from foreign matter, which may be difficult to remove after they have been killed.

All parasitic worms may be washed by shaking in the 1 per cent. salt solution. Nematodes and Trematodes can be shaken up vigorously in a test-tube without damage, but care must be taken with Tapeworms, which are very fragile. In some cases, such as Chætopods, a soft camel's hair brush may be used to remove particles of sand, etc., which cling to their bodies, especially if they

are very bristly. Water alone may be used for the washing if salt solution cannot be had, but specimens, especially Nematodes, must not be left long in it.

Killing. After washing, the specimens must be killed with some reagent which leaves them in a suitable condition for subsequent examination—*i.e.* which "fixes" their tissues in a condition as nearly as possible approaching that of life.

The most generally useful reagent is *Corrosive Sublimate* (see list of reagents). The effect is more instantaneous if the solution be heated, and the animals then plunged into it; but it may also very well be used cold. An hour or two in this solution will be long enough for the largest specimens; for smaller ones a few minutes will suffice. For necessary treatment *after* corrosive sublimate, see this heading in the list of reagents.

Formalin (10 per cent. solution) is also good for most parasites, except for Nematodes, but it cannot be recommended for the majority of marine worms; specimens left in this reagent for 24 hours can then be transferred to the weaker solution, or to alcohol.

For killing parasitic worms, see also other methods given below.

Narcotizing. As it is desirable to get the animals to die in an expanded condition, it is useful in some cases to anæsthetize them before killing. This is especially the case with Nemertines, and worms which live in tubes. They may be placed for from 6 to 12 hours in a solution of chloral hydrate in sea water (1 per cent.), or a few of the crystals may be simply added to the sea water in which they lie. When fully extended and showing no sign of sensation, they may be preserved with formalin or alcohol. Cocaine may be used with advantage instead of chloral hydrate.

Another method is to place the worms in a shallow dish of sea water, and gently pour about $\frac{1}{20}$ part of strong alcohol on the surface. This will slowly mix with the water and anæsthetize the animals gradually. They can then be transferred to alcohol to harden, and preserved in it.

Some of the free-living Polychæta must be treated in this way, or they will break themselves into a number of little pieces when a killing reagent is added.

Note.—In tropical countries narcotizing is sometimes found difficult, owing to the fact that decomposition sets in before the anæsthetic has had time to take effect. In such cases the alcohol method will be found to give better results than chloral hydrate.

Special Methods for Parasitic Worms.

Nematodes.

After washing thoroughly as above directed,* kill by plunging each separately into a quantity of *hot* 70 per cent. alcohol. A temperature of 50°–60° C. is high enough.

(N.B.—Care must be used, as the vapour given off is highly inflammable.) The advantage of this method is that the worms should die in a straight position.

After being killed in this way the worms are to be stored in fresh 70% alcohol (never in formalin).

Trematodes.

After the first shaking up in salt solution the dirty liquid is poured off, and a small quantity of fresh salt solution introduced. The worms are shaken vigorously in this, and an equal quantity of saturated solution of corrosive sublimate added quickly, the shaking being then continued for several minutes. This should kill and fix the worms in an extended position. They may be left in the fluid for several days, or transferred at once to water, in which, in any case, they should be washed for about twelve hours. After this they may be stored in 70% alcohol as usual.

[Formalin may be used instead of corrosive sublimate, in a solution of 10% strength, followed by a weaker solution (about 3%) as a storing fluid.]

Cestodes.

Wash gently in salt solution;† then fix in a solution containing equal parts of saturated corrosive sublimate and 70% alcohol, to which add a few drops of glacial acetic acid—the whole heated to about 50° C. The Tapeworms should remain in the fixing fluid till it is cold; they are then washed gently in running water for twelve hours, and stored in 70% alcohol.

PACKING OF SPECIMENS.

The separate small bottles containing worms should be filled as full as possible with the preserving fluid (alcohol or formalin), and damage to the specimens by shaking prevented as far as possible by a light packing of cotton-wool or tissue-paper. Care must, however, be taken to insert the wool in such a way that the

* In the case of Nematodes with a mouth-capsule, a thorough shaking-up is necessary to remove parts of the tissues of the host, or other *débris*, from it.

† When the Tape-worms are long and tend to become much tangled they should be washed in a shallow dish, and picked out one by one on a splinter of wood (not a metal instrument) and transferred to the fixing fluid.

small specimens cannot get entangled in it. The liquid must on no account be allowed to dry up. When they are to be forwarded all the small bottles should be placed in a large, wide-mouthed bottle with a good sound cork or a glass stopper, or better still, a screwed-on lid with rubber ring. In this the small bottles should be packed lightly with wool, horse-hair, or soft paper screwed up into loose balls, and the whole filled up with the fluid. It is a good plan, if the large bottle or jar is well stoppered and protected against breakage, to leave the small bottles without corks, simply plugging their necks with a pellet of cotton-wool or a stopper of pith—*e.g.* elder-pith—and then stand them upside-down in the large jar. There is then much less risk of the fluid in the small bottles getting dried up, so long as there is plenty in the big jar. Air-bubbles in the small bottles should be avoided as far as possible.

N.B.—Small bottles packed in this way, immersed in fluid, must have their labels *inside*, and not gummed on the outside.

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