XXXI.—Encystment of Tardigrada. By James Murray. (With Two Plates.)

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Introduction.

The object of this paper is to discuss the recently discovered encystment of Tardigrada, as far as our imperfect knowledge permits; to compare the process with that observed in certain low groups of the Acarina, in which the Tardigrada are generally considered to have their nearest relatives; then to inquire whether these new facts throw any light on certain puzzling facts in the physiology of the Tardigrada and Acarina, such as the retrogression which produces the simplex forms of Tardigrada, and which is said to accompany each moult of the Acarina; and lastly, if they contribute anything towards settling definitely the systematic position of the Tardigrada.

That water-bears encyst themselves was first ascertained by Professor Lauterborn, and a short note on the subject was published in 1906 (21).

It is now known that the cysts of Tardigrada are extremely common, and there is reason to believe that various naturalists have seen them, though without recognising their true nature.

Professor Richters, in a recent letter, suggests that some of Spallanzani's figures might possibly represent cysts (35).

Spallanzani's original figures I have not seen, but they are reproduced by Schultze in his "Macrobiotus hufelandi" (34). Those figures are so crude that it is only by making large allowances that they can be accepted as Tardigrada. Fig. 5 on Schultze's plate, representing the ventral view of the animal, shows five pairs of limbs, each ending in a single strongly hooked claw, two pairs of limbs terminating the body in a manner hardly possible in any natural position of a water-bear. But, granting that they are Tardigrada, as Spallanzani's experiments, and the situation in which he found the animals, render reasonably probable, then the elliptical body represented in fig. 7 as reproduced on Schultze's plate might well be a cyst.

Schultze's own figures 2 and 3 might be cysts, or merely contracted examples.

Doyère probably had cysts in view when he wrote, in his *Mémoire sur les Tardigrades*, in 1840 (4) p. 308, "J'eus d'abord quelque peine à reconnaître l'animal, dans la petite masse, inert, en apparence granuleuse et amorphe que je rencontrais parfois à l'intérieure de certaines peaux qui me semblait abandonnées."

No one appears to have suspected the true nature of these amorphous objects.

I learn from Professor Richters that he had seen some of Professor Lauterborn's cysts in 1906. For some years these sausage-like little yellow packages had been

familiar to me, but I had merely wondered at them till, in the beginning of winter 1906–7, an opportunity occurred of seeing how they were formed.

The cysts which I found in great abundance were of quite different forms from the familiar yellow objects, and were not at first suspected of being Tardigrada.

After watching them for some weeks and ascertaining a very remarkable series of changes, a preliminary note on the subject was published in *The Zoologist* for January 1907 (25).

While this was in press, Professor LAUTERBORN'S first short note on encystment reached me (21).

ENCYSTMENT OF M. MACRONYX.

Professor LAUTERBORN'S account of the process is brief, but of great interest. He observed a water-dwelling species, *Macrobiotus macronyx*, DUJ. On page 267 he tells how he found, in many ponds around Ludwigshafen, skins of *Macrobiotus* which appeared to be filled by a single immense resting egg. He saw also, on one occasion, the body of a *Macrobiotus* loosen itself from its cuticle, and contract within it into an elliptical body, which then secreted a closely fitting envelope. Within this shell there was at first a feeble movement, which ceased in about an hour. The rods in the pharynx remained visible.

At first the cuticle of the beast remained connected with the cyst by numerous folds, but later it shrank more and more together, till finally the surface of the cyst appeared covered with a maze of spines and ridges, like some winter-eggs of Rotifers. The stomach of the animal observed was colourless, instead of golden-brown as usual. The thick skin was disposed in numerous transverse folds.

Professor LAUTERBORN does not remark on retrogression, simplification, or liquefaction of organs taking place within the cyst.

In a ditch near the pond where he found the animal which he actually observed to encyst itself, he saw many females of *M. macronyx* with their eggs deposited in the moulted skin.

Since the discovery that Tardigrada encyst themselves, Professor RICHTERS has had cysts of various species under observation, and has made some interesting discoveries, which will no doubt be recorded at an early date.

ENCYSTMENT OF M. DISPAR.

My observations were made on another water-dwelling species, *M. dispar*, MURRAY (25). This is, like *M. macronyx*, DUJ., a very large animal, attaining to nearly a millimetre in length. A lateral view of the animal is given in fig. 1.

It is hyaline and yellow or brown, with a pair of dark eye-spots. The teeth and pharynx are of the same type as those of *M. hufelandi*, RICHTERS, but differ in many

details,—there is no "comma" in the pharynx,—the proximal end of the tooth has a very large "furca," the largest known in a tardigrade.

The claws are of a very distinct type, which is supposed to be that of *M. macronyx*. Each pair consists of two very unequal claws, united at the base, the longer claw strongly curved, and bearing at the back a strong bristle or supplementary point, which diverges from the main claw about a third of its length from the point, but can be traced down the back of the claw to its base. The lesser claws of the fourth legs are relatively much larger.

The eggs are spherical and are covered with little sharp conical processes, which do not touch at their bases. They are rare.

The species is common in Scotland, and has been found in England (York), Spitsbergen, and Franz Josef Land.

Professor Richters identified it at first as *M. macronyx*, but after the discovery of the egg he regarded it as a distinct species.

The two posterior dorsal processes, shown in fig. 1, vary greatly in size, and may be absent.

M. dispar had been known to me for a year or two before the cysts came to my notice. It had been found at the margin of Loch Tay, and in various ponds near Edinburgh and Glasgow.

The spiny egg had been seen on several occasions.

In the late autumn of the year 1906 I washed some moss from a shallow pond occupying an old quarry at Nerston, near East Kilbride, a few miles south of Glasgow.

M. dispar, not then named, was in very great abundance. No small examples were seen—all were large, and to all appearance full-grown, though there was no proof of full maturity, *i.e.*, no eggs were seen in the body. In one empty skin there was a spiny egg. I had hit upon the fortunate moment for observing them, as the great majority were in the act of casting their skins, and nearly all were at the same stage of the process—a very few had completed it or had not commenced. Even while I watched, many completed the moult, some leaving the old skin in the usual way, but most remaining in it.

The newly formed skin differed from the old skin; it was darker yellow, and dotted all over. The dots were probably pores from which a secretion exuded, as the surface appeared to be viscous, and extraneous matter adhered to it. The animal moved feebly and became gradually smaller; the amount of the secretion increased, and the adherent matter loaded the back with an umber-brown mass.

Still contracting and drawing in its legs, at the same time moving more and more feebly, it eventually became little more than half the original length, and assumed the appearance shown in fig. 2, which represents the cyst lying in the original skin.

At this stage it is quite rigid and dark-coloured, brown, purplish, or black. There is no appearance of extraneous matter, and the surface is even, but closely dotted. It is obscurely segmented, or divided by constrictions into four parts, which appear to

correspond to the four limb-bearing segments, the head and fourth legs being drawn in out of sight. There is a constriction in the centre of the body, and the anterior and posterior portions are very similar in form, but the posterior end is broader. There are deep constrictions towards each end, and the posterior one becomes important afterwards.

On the ventral side are six little conical stumps, the remnants of three pairs of legs (fig. 3). When the cyst has just been formed there are claws on those little stumpy legs, but after a short time it is found that they have been withdrawn through small openings at the ends of the legs (shown in fig. 3). I was never so fortunate as to witness the withdrawing of the claws.

The shagreening of the general surface continues on to the legs, but becomes more obscure towards the ends of the stumps, and the part surrounding the pores through which the claws are withdrawn is a clear membrane.

From the moment when it attains its final form, the skin of the cyst is brittle and so dark and opaque that its contents can only be dimly seen, and it is impossible to study the further developments within it. To do this it is necessary to break open the cysts.

When a cyst is broken open immediately after its completion, we find within it the animal much as it was before encystment, only smaller, and possessing all its organs, teeth, pharynx, claws, etc.

When a cyst is broken open at a somewhat later period, say after it has been formed for two or three days, the contained animal is found to have contracted and taken an elliptical form The shagreened skin remains as an *outer case*, within which the elliptical body lies loosely.

The elliptical cyst (fig. 4) is covered by smooth, yellow, transparent skin, without trace of external limbs, but still containing a complete animal, having legs, claws, teeth, and pharynx.

The origin of this inner covering is puzzling. The *outer case*, which appears to be secreted from the skin, retains traces of the form of the animal. The *inner case* appears to be of quite a different nature, and looks like a true skin, having no dots or evidence of having been secreted, and no trace of segmentation or limbs. Yet, if not secreted, how account for the complete animal within it; and, if secreted, how account for its regular egg-like form?

The most remarkable part of the process follows. Cases broken open about a week after their completion are found to enclose the elliptical yellow cysts as before, but these no longer contain complete animals.

If the contents of a cyst can be squeezed out without rupture, there appears an almost amorphous mass, without trace of limbs, claws, teeth, or pharynx. It is unfortunate that the opacity of the outer case prevents the study of this remarkable change in its various stages.

Professor RICHTERS has now under observation some cysts of species which have not

the opaque outer case, and it is hoped that he will soon observe the sequence of the changes.

Seen within the *inner case* (fig. 4), the contained animal shows a faint segmentation, three transverse furrows dividing it into four nearly equal parts. When the animal is squeezed out no segmentation can be noticed (fig. 5). It is covered by a very thin cuticle and has a somewhat undulate outline.

Even in this latest stage, the animal has not lost every trace of its former self. The eye-spots persist as long as an animal has been watched, and the fat cells in the blood continue recognisable. There are also some cells, with dark contents, in the centre of the body, which I take to represent the stomach. The eye-spots become very large and diffuse, of loosely agglomerated granules (fig. 5).

Animals squeezed from the cyst at an early stage showed, as above remarked, no trace of limbs. At a later period, but at an unknown interval of time from the formation of the cyst, a squeezed-out animal had obtuse papillæ for limbs, without trace of claws (as shown in fig. 14). There was at this stage a very thin cuticle, and underneath it a lax cellular tissue of large obscurely polygonal cells. The supposed cells of the stomach, with brown contents, were still conspicuous; the fat cells were few; there was no trace of pharynx or teeth.

I have no observations between this stage and the final emergence, shown in fig. 12.

This was witnessed on several occasions, and is sufficiently curious. The animals were remarkably large and lusty, considering the enormous expenditure of material in making the various cases and integuments, and the small cyst from which they issued. They were fully provided with all the organs they possessed when entering the cyst; well-grown claws, pharynx, teeth, etc.

The case splits at the posterior constriction before alluded to, and the end portion opens like a hinged lid, permitting the animal to walk out backward. The emergence occupied several hours, and after a severe struggle and the extrication of one pair of limbs, the animal would take a long rest before recommencing the struggle. Fully emerged, the creature did not appear greatly smaller or conspicuously different from what it was originally.

The process of encystment, as observed in *M. dispar*, and described above, differs in many respects from that sketched by Professor LAUTERBORN, yet there are many points of correspondence. *M. macronyx* and *M. dispar* are the only two species yet observed which secrete a special *outer case*. Those two species are of aquatic habit, while all others of which I have seen cysts are normally moss-dwellers.

CYSTS OF OTHER SPECIES.

Cysts of a good many species of Tardigrada have been seen, but I have had no opportunity to study any of them except *M. dispar*.

All the cysts which I have seen are a good deal alike, and none of them, except

M. dispar, had the special *outer case*. They are elliptical bodies, usually dark yellow in colour, but sufficiently transparent to allow the internal organs to be seen. Their surface is less regular than that of the *inner case* of *M. dispar*, with which they seem to be homologous; it is usually more or less wrinkled, and the wrinkles form a regular pattern, as in the cyst of *M. echinogenitus* (fig. 16*a*).

M. echinogenitus, RICHTERS.—Cysts were abundant in moss brought from Spitsbergen by Mr WM. S. BRUCE in August 1906. One cyst was found in a bog pond on Blantyre Moor, near Glasgow. In neither case did I notice any reduction of the internal organs. The example figured (fig. 16*a*), which I identify as *M. echinogenitus* by its claws, was found in a pond at Nerston, near Glasgow. It was empty when found, and is figured to illustrate the symmetrical wrinkling of the surface, which differentiates the cyst from an ordinary skin. Professor RICHTERS has sent me a photograph of the cyst of *M. hufelandi*, which appears to be wrinkled in a similar manner.

Claws were attached to this cyst, which rarely occurs, in my experience.

M. oberhäuseri, DOY.(?)—Two different cysts of animals resembling this species, but not positively identified, are figured. Fig. 15 is an oval cyst, narrowed towards the posterior end, with a smooth, unwrinkled skin, and the internal organs not reduced. The pharynx is quite like that of *M. oberhäuseri*, but the two pairs of claws are not so dissimilar as in that species.

Fig. 17 shows a slightly larger cyst, wrinkled, and not narrowed to one end. The claws are as in *M. oberhäuseri*, but the pharynx differs slightly, the second rod being slightly longer than the first (an unusual condition), and there is a comma.

Diphascon.—Cysts of this genus have been seen, but I have no notes throwing any light on this subject.

Echiniscus.—Cysts of this genus have long been known to me, but not being aware of their nature, no study was made of them. Since beginning the investigation of encystment, many cysts of one species, *E. arctomys*, EHR., have been found in moss from Uganda, sent to me by Mr N. D. F. PEARCE, of Cambridge. These cysts were similar to those of *Macrobiotus*, elliptical, and without limbs or processes. The contained animal was red.

E. perarmatus, MURRAY (27).—This species, recently discovered among moss sent to me by Mr WM. MILNE of Uitenhage, Cape Colony, is a common species in South Africa. In moss received from Mr MILNE in April 1907, I found several cysts. One is figured (Plate I. fig. 6). It is elliptical, and lies loosely within the ordinary skin of the animal. There is no *outer case*, as in *M. dispar*. There is no trace of external limbs on the cyst, and when subjected to pressure, it was found that there were within the case no limbs, pharynx, or other recognisable organs. The cyst is filled by a dark red granular mass, almost opaque, and in the centre is a darker, umber-brown tract (stomach ?).

Encystment of certain Acarina.

The account published by Michael in 1901 (23) of the encystment of certain Mites, of the family Tyroglyphidæ, indicates such a close correspondence with the process as observed in *M. dispar*, that it is thought desirable to give a pretty full account of it here.

The Tyroglyphidæ (or Cheese Mites, etc.), like most other Acarina, undergo a distinct metamorphosis, passing through the stages of *larva* and *nymph* before reaching the adult condition. They cast the skin three times before becoming adult, once in the larval state and twice as nymphs. In the course of growth they do not greatly alter in form, so that the species at the different ages is generally easily recognised; but the full complement of limbs is only acquired when the larval skin is thrown off, and the young nymph more nearly resembles the larva, while the old nymph (after the first nymphal moult) is more like the imago.

It has long been known that many Tyroglyphidæ have an immature condition in which there are special adaptations to assist distribution, and earlier authors founded on these immature forms the genera *Hypopus* and *Homopus*. These hypopial nymphs are active, but are said to be able to exist for a long time without food, and to survive great heat and drought.

Seeking for the hypopial nymphs of certain species of the genus *Glycophagus*, Michael discovered the rudimentary *hypopi*, of which the encystment so resembles that of *M. dispar*.

Michael (23), on p. 168, etc., tells how he found inert nymphs of *Glycophagus domesticus*, which had the cuticle thicker, whiter, and more opaque, the skin of the legs empty. These "cases," which are simply nymphal skins under peculiar conditions, contained each a protoplasmic mass, of the general form of a *Hypopus*, but without trace of legs, mouth, or other external organs. It was covered by a transparent, colourless, and almost structureless cuticle, and was rounded behind, and bluntly pointed in front.

Michael saw immature *G. domesticus* emerge from the cases "which did not split irregularly like ordinary nymphal skins, but usually opened by the posterior end of the case, which had been concave, being pushed out so as to become convex; and separating from the lateral and ventral parts of the case, but remaining attached to the dorsal. The cases, although open, were not entirely empty; I found that each contained a cast skin."

The nymphs which emerged from the cases became inert in about a week, and a few days later adults emerged. "It was thus ascertained that the cases were a penultimate-nymphal stage, *i.e.* the nymph which emerged from the case became adult at the first ecdysis."

A sketch copy of Michael's figure of the case containing the rudimentary *hypopus* is given in Plate II. fig. 18*a*, and fig. 18*b* shows the hinged lid by which the nymph emerged.

Encystment of Tardigrada and Acarina Compared.

In the foregoing accounts of the encystment of *Macrobiotus dispar* and *Glycophagus domesticus* there are many points of correspondence, as well as some important differences.

The essential points of agreement are the formation, within an ordinary skin of the animal, of an inert protoplasmic mass, protected by a peculiar skin, and which has lost by retrogression all trace of external organs, and apparently of most of the internal organs, and the final emergence, by the opening of a posterior trap-door in the case, of an animal having the full complement of organs.

The chief difference is that the case of the Mite is a real skin, that of the Tardigrade appears to be merely a dense secretion from the skin. This is not, however, demonstrated, and the *outer case* of the cyst may consist of a real skin, as well as the secretion. The reason for thinking that it is a secretion only is the withdrawal of the claws, which in ordinary changes of skin are thrown off with the rest.

Even if the case is only a secretion, there is the original skin of the animal to correspond to the hypopial case of the Mite, as encystment is always preceded by an ordinary moult.

The Mite is known to encyst, when it does so at all, at a definite stage in development, immediately before attaining to maturity. The Tardigrade is judged from its size to be full grown, but this also is not yet demonstrated, and it may be that here also it occurs at the corresponding stage of development, just before reaching sexual maturity.

Simplex Forms of Tardigrada.

We may now inquire whether the phenomena of encystment throw any light on the puzzling questions of *simplex* forms. As a simplification of the Tardigrade takes place during encystment, is it possible that the simplex forms are connected with this process?

Simplex forms are very common. They are known in nearly every species of *Macrobiotus*, in several of *Diphascon*, and it is likely that all Tardigrada possess them.

The name was given to them by Richters (31), in recognition of the fact that Plate's *Doyeria simplex* was nothing but a peculiar condition of some species of *Macrobiotus*.

The peculiarity of the simplex form is the reduction of the manducatory apparatus. The teeth are reduced in size, have no furca or bearers, and are simply little, straight, pointed stylets, which do not even reach the mouth or gullet, and therefore cannot be functional. The rods in the pharynx are usually quite abortive; the gullet becomes a very slender tube (fig. 7). This is the usual simplex state, but the reduction may go further, and the teeth, rods, gullet, and mouth may totally disappear; and though in these cases the muscular bulb of the pharynx usually persists, that is occasionally also

absent, and there is then no trace of the alimentary canal in front of the stomach (fig. 8).

This condition is found in large and strong animals, which have the appearance of having plenty of food in the stomach. I have seen no trace of reduction of any other organs. Simplex forms of several species have been seen in the egg.

I have very frequently noticed that animals in this condition were about to moult, though I doubt if this is invariably the case.

Richters (31) regards them as parallel forms, and thinks that some species at least (e.g., *M. hufelandi*) have peculiar forms of eggs from which the simplex individuals come.

The fact that the most fully reduced individuals have no anterior opening to the alimentary canal convinces me that the state is temporary. The alimentary canal is a *cul-de-sac*, opening only by the anus, and it is impossible that they can imbibe food.

As it is definitely known that some species at least undergo simplification in the course of encystment, it may be supposed possible that the simplex individuals may be about to encyst, or may just have emerged.

M. dispar retains all the parts of the manducatory apparatus till the inner case of the cyst is formed, and in the same species the individuals which were seen to emerge naturally from the cysts had also all their organs.

If this is the normal course in other species, we can only connect the ordinary simplex form with encystment by supposing that the absorption of the organs had been prematurely stimulated, or the moult and encystment somehow retarded.

The Moulting of Acarina.

A somewhat analogous simplification has been observed among certain of the lower Mites.

When moulting, these Mites become inert for a time, and return partially to an amorphous condition. Michael (23), p. 180, etc., quotes various authors who have written on the subject.

Gudden (18), p. 284, writing of certain parasitic Sarcoptidæ, states that at the moult the whole of the inner parts of the creature return to an amorphous mass, like the egg; and that from this the new creature is formed, as from an egg.

Megnin (22), p. 214, confirmed this view, and believed that at the ecdysis in all Acarina, all the internal organs liquefied and formed a sarcodic plasma, having a true blastoderm, which sprouted like that of an egg.

By this theory it appears almost as if the whole substance of the animal went to form a single egg.

Michael (23), p. 181, says that this theory has now been shown to be incorrect, "and that the return to a more or less amorphous condition is usually, and probably always, confined to the soft parts of the legs and trophi, and what may be described as appendages or external organs."

It is proven to be incorrect in various groups, so that MEGNIN's wide generalisation breaks down; but, considering the different extent to which retrogression goes during the hypopial encystment of such closely related species as *Glycophagus domesticus* and *G. spinipes*, it seems to me that each species must be independently investigated, and that it is not improbable that the change may go so far in some species as to reduce them to almost as simple a condition as an egg.

It may be that the reversion to an amorphous condition which GUDDEN and MEGNIN believe to accompany each moult, and the encystment in the penultimate-nymphal stage described by MICHAEL, may be simply different interpretations of the same facts.

THE MOULTING OF TARDIGRADA.

That Tardigrada cast their skins was known to GOEZE, the first naturalist who has recorded an observation of a Water-Bear. Most subsequent observers have confirmed the observation, and many of them noticed that some species deposited their eggs in the skin which they cast.

C. A. S. SCHULTZE is the first naturalist, so far as I know, who has paid any attention to the number of moults, and the only one who claims to have followed an individual from birth to death. He states (34), p. 4, that *M. hufelandi* moults twice, lays its eggs after the second moult, then dies. It must be remarked that an animal studied in this way cannot be under its normal conditions, and death might be premature.

DOYÈRE (4) gives a minute account of the casting of the skin, and says that the epithelium of the alimentary canal is also cast, as may be seen at both ends of the canal when the animal contracts greatly within the old skin. He states that they moult several times, but admits that he never watched an individual throughout life, and cannot state the number of moults.

I can find no one else who has even considered the question of the number of moults, though several have carefully studied a simple moult.

LANCE (20) considers DOYÈRE's observations and conclusions as incorrect in many particulars. He regards DOYÈRE's account of the shedding of the chitinous lining of the gullet and cloaca as greatly exaggerated, and on p. 44 he quotes ERLANGER (12) to show that a portion of the ectoderm is included in the anal invagination, and supposes that it is this part only, really belonging to the ectoderm, which is shed with the outer skin.

Except in the genus *Echiniscus*, no Tardigrada are known to undergo any metamorphosis. They are hatched in the final form and simply increase in size and attain to sexual maturity. On this account it would only be possible to ascertain the number of moults by watching the individual throughout life. In the Acarina, where each moult is characterised by a greater or less change of form, it is a simpler matter to count the moults.

The metamorphosis in the genus *Echiniscus* is a very slight thing. The larvæ are

hatched with only two claws on each leg, while the adults have four. They have sometimes also fewer or shorter setæ or other processes. They attain the four claws very early, probably at the first moult, and while still quite small. Unlike the Acarina, the larvæ have the full adult complement of limbs.

I have seen an individual of *E. granulatus*, Doy., which had been unable to completely throw off the old skin at the moult, and it remained adherent till the next moult. It was thus possible to compare three stages of the same individual. In these two moults there was very little increase in size, but some of the processes elongated considerably, and the straight spines on the outer claws increased in number.

There must in this case have been at least three moults—the one when it ceased to be a larva, and the two actually observed; but as the animal when first seen was very much larger than the larva, it is certain that there must have been one or more intermediate moults.

The rigid skin of *Echiniscus* may require to be more frequently cast than the softer skin of *Macrobiotus*, and Schultze may be right about the two moults of *M. hufelandi*.

The female does not ordinarily die after casting her skin and laying her eggs. One species at least carries the eggs about till they are hatched, and continues to live for some time after, though I have never been able to keep an individual under observation till a second lot of eggs was developed. On the whole, I am inclined to think that even after maturity the skin is changed more than once, and successive clutches of eggs are laid.

During encystment some species cast off at least three and possibly four coats, but these may not be true skins.

Systematic Position of Tardigrada.

The systematic position of the Tardigrada has given rise to much controversy. The only point on which there is general agreement is that they are Arthropoda, Dujardin alone among prominent naturalists assigning them a lower place—with the Rotifers in his class of the Systolides—an opinion which he afterwards modified.

It is not intended here to enter into the whole question, but merely to give a sketch of the history of the controversy, and to enquire whether the phenomena of encystment bring it any nearer settlement.

O. F. Müller (24) in 1785 first gave a scientific name to a Tardigrade, *Acarus ursellus*, which he thus included among the Acari. Müller's view is accepted by Gmelin, 1788, (13), Dutrochet, 1837 (9), Kauffmann, 1851 (19), etc. Schrank, 1804 (33), also put them near the Acari, in his *Insecta Aptera*, between *Pulex* and *Acarus*.

They were regarded as true Insects by Dutrochet in his earlier work, 1812 (8), Blainville, 1826 (2), etc.

They were reckoned among Crustacea by Nitzsch, 1820 (28), Schultz, 1834 (34), Ehrenberg, 1834 (10), Perty, 1834 (29), etc.

Other opinions had fewer adherents. DUJARDIN, 1841 (6), united them with the Rotifers to form his class of Systolides, a classification hardly accepted by anyone except DOYÈRE, 1840 (4), who afterwards abandoned it. DUJARDIN himself, in 1851 (7), admitted that they could not be classed with Rotifers, but contends that neither are they near the Acari.

GRAFF (15) makes a special order, Stelechopoda, of the Myzostomida, Linguatulida, and Tardigrada.

PLATE, 1888 (30), regards them as the lowest of the Tracheata, near the Onychophora.

LANCE, 1896 (20), places them between the Worms and the Tracheata, in the Protracheata, near *Peripatus*.

GREEFF, 1865 (17), admits that the general opinion places them with the Acari, and gives reasons against doing so, without committing himself to any more definite opinion.

BASSE, 1905 (1), denies any close affinity with *Peripatus*, and places them again in the Tracheata.

The fact that so many good zoologists have supported such different views of the affinities of the Tardigrada appears to indicate that their essential structure does not incline very markedly to any one group of the Arthropoda more than another. They form themselves a very distinct group.

This being so, such subordinate characters as the possession of four pairs of limbs, the absence of distinct abdomen, and the simplicity of the circulatory and respiratory arrangements, gain weight in indicating an affinity with the only other Arthropoda similarly characterised, viz. some of the lower Mites.

That the affinity is not really very close is, I think, indicated by the fact that, although the adults possess four pairs of limbs, they do not, like the Acari, at any stage in their development possess only three pairs.

We find among Tardigrada and certain Acarina a retrogression occurring at a certain stage in development, or under certain conditions, which results in an encystment having a marked analogy with that of such common occurrence among Protozoa (with which MEGNIN compares it), and which, outside of these two groups, has no known parallel among animals higher than the Protozoa.

The remarkable coincidence of even the secondary details of the process in *Macrobiotus dispar* and *Glycophagus domesticus*, even to the final leaving the cysts by a trap-door, can hardly be regarded as other than fortuitous.

The essential part of the process, however,—the formation of cysts, within which the animals return in a greater or less degree to an amorphous condition,—seems to me to strengthen the belief that there is a real affinity between the two groups.

Conclusions.

What purpose does encystment serve in the life-history of the Tardigrada? Encystment, if not accompanied by absorption of organs, might be regarded as simply a sort of hibernation. This view of its meaning is supported, in the case of *M. dispar*, by the fact that the encystment took place in the beginning of winter, just when the shallow ponds in which the animals live were beginning to skin over with ice on cold nights.

On the 18th November 1906 the pond was completely frozen over in the morning. At 11.30 A.M. there was open water at one side, having a temperature of 39°·0 F. At the other side the surface was still covered by ice, and the temperature under the ice was 36°·0. In moss taken from under the ice there were many Tardigrada (*M. dispar*) beginning to encyst. Later in the season, when the pond was frozen nearly to the bottom, the ice was broken and moss adhering to it washed. There were now numbers of cysts, but no active animals. When this moss had been kept in a warm room for a number of hours, active animals began to appear, and it was then that the emergence from the cysts was studied. In the course of a day or two the active animals became very numerous. These facts might indicate that the process is nothing but a hibernation. But the return to a simpler condition puts another aspect on the matter. In what way can it benefit a hibernating animal to absorb its legs and other organs, and afterwards grow a new set of them? There is surely waste here, while in the familiar instances of hibernation, physiological activity is so low that waste is reduced to a minimum.

Is there, then, in this absorption and regeneration of parts anything analogous to the rejuvenescence of lower forms? Does the animal retain its individuality throughout these changes?

If Megnin were right in his theory, that the sarcodic plasma formed during the ecdysis of Acarina was enveloped by a veritable blastoderm, the process might be considered a reproductive one.

In the Tyroglyphidæ studied by Michael the gradation which may be traced from species which have an inert, amorphous cyst, similar to that of Tardigrada, to those more closely resembling the nymph, and having rudimentary limbs, makes it clear that the cyst is merely a stage in the development of the individual, and by analogy we may suppose that this is the case with Tardigrada also.

The ordinary, active hypopi of the Tyroglyphidæ are adapted to secure distribution. Megnin suggests that the nymphal skins containing the cysts might be blown about by the wind, but Michael does not see why this should not as readily occur with ordinary inert nymphs before the ecdysis.

However it may be with Acarina and with Tardigrada living among terrestrial moss, the encystment of *M. dispar* cannot be supposed to assist distribution. It is an

aquatic species, living in shallow still water, and in the place where it was studied the cysts remained in the moss where they were formed, under the ice.

MEGNIN believed that the change of a nymph into a hypopus was caused by unfavourable conditions. MICHAEL thought it had no connection with unfavourable conditions.

With regard to the Tardigrada, I believe the evidence goes to show that the encystment is induced by unfavourable conditions. It may be the low temperature which is the unfavourable condition for aquatic forms, and the drying of the moss in the case of the terrestrial forms. The encystment of Protozoa appears to be frequently induced by adverse circumstances, though it may have other causes.

Further investigation will be necessary before it will be possible to draw any more definite conclusions as to the meaning of the encystment of Tardigrada.

NOTE ON M. MACRONYX, M. DISPAR, AND RELATED SPECIES.

M. macronyx and *M. dispar* appear to have much in common, although, if the two modes of disposing of the eggs really differentiate natural groups, they would be placed in different sections of the genus, or in different genera, if *Macrobiotus* were subdivided on that character.

I believe, however, that there has been much confusion over *M. macronyx*. Special biological studies have been made by men little acquainted with species, on animals supposed to be this species, and till quite recently few supposed that there were numerous species of Tardigrada. *M. macronyx* was supposed to be the only fresh-water species; therefore any species found in water must, it was thought, be that species.

M. macronyx, DUJ. (7), is very insufficiently described. DUJARDIN'S first description of the Tardigrade, published in 1838 (5), applies to an animal which he afterwards, in 1851 (7), named *M. lacustris*. The earlier description is of little value, as it undoubtedly confounds two or more species. He figures two sets of claws, totally distinct,—fig. 6 (5) shows claws which, I think, may be taken as like those which he (in 1851) ascribed to *M. macronyx*,—fig. 7 shows claws of the same type as *M. oberhäuseri*, DOY.

The description of *M. macronyx* given in 1851 (7), p. 163, is far from satisfactory. The animal is 1 mm. long, and the long claws are $\frac{1}{20}$ mm. long. The manducatory apparatus (mouth, teeth, gullet, and pharnyx) is nearly $\frac{1}{4}$ of the total length.

The pharynx forms half of the length of the manducatory apparatus. The mandibles (teeth) are larger and more curved than in *M. lacustris*, and are *not bifurcate* at the base. Of the eggs he says nothing.

As to the teeth, *not bifurcate* at the base, I have seen no *Macrobiotus* without a tooth furca, unless when in the *simplex* state; and, moreover, DUJARDIN shows a furca in his fig. 7.

Leaving that character aside, the animal is characterised by the peculiar form of

claws, the large size, and the habitat in water. The pharynx is figured with three slender rods, of which the first is longest.

M. dispar agrees closely with this description, except as to the pharynx, in which the first two rods are joined. It has a spiny egg (Plate I. fig. 11).

Another species, having the same type of claws, *M. ambiguus*, MURRAY (Plate I. fig. 10) (26), and resembling *M. dispar* in all other characters, has also a spiny egg.

M. furcatus, EHR. (11), another large species having claws of the *macronyx* type, likewise has a spiny egg (fig. 11*b*).

Considering that all the species known to me having claws of this particular type also had spiny eggs, and that DUJARDIN makes no mention of the egg of *M. macronyx*, I would have been inclined to recognise the animal which I have called *M. dispar* as the type of *M. macronyx*, and would have amplified the description by ascribing to it spiny eggs.

Various authors have, however, professed to recognise *M. macronyx* in an animal which lays smooth eggs in the cast skin.

GREEFF, in 1866 (16), p. 120,—PLATE, in 1888 (30), p. 536,—LANCE, in 1896 (20), p. 204,—LAUTERBORN (21), in 1906,—RICHTERS, in 1904 (32), p. 63, ascribe to the animal smooth eggs which are deposited in the skin at the moult. GREEFF and LANCE figure claws which agree with DUJARDIN'S figures of *M. macronyx*.

RICHTERS (32), p. 63, ascribes to DOYÈRE the assertion that the eggs are laid in the cast skin; but as DOYÈRE'S paper appeared in 1840, and *M. macronyx* was described in 1851, DOYÈRE can only be referring to DUJARDIN'S unnamed Tardigrade of 1838, afterwards called *M. lacustris*.

GREEFF (16), p. 105, probably originated the belief that *M. macronyx* laid the eggs in the skin by identifying *M. lacustris* as the young of *M. macronyx*, an absurdity on the face of it, as *M. lacustris* was said by DUJARDIN to lay the eggs in the skin, and he made no such assertion about *M. macronyx*.

It is not clear how far these various authors had for themselves verified the fact of the laying of smooth eggs in the skin, in association with claws of the *macronyx* type, or whether they were in some cases repeating statements made by others.

Professor RICHTERS has sent me preparations of *M. macronyx*, which agreed fully with *M. dispar* as to claws and pharnyx, but it was not demonstrated that the actual individuals mounted had either come from, or had deposited, smooth eggs.

In this unsatisfactory state of affairs, and in view of the large body of authority for the belief that *M. macronyx* lays smooth eggs, I judge it best in the meantime to retain *M. dispar*, though having a strong suspicion that it will prove to be DUJARDIN'S *macronyx*.

The species observed by Professor LAUTERBORN, whether it be the true *macronyx* of DUJARDIN or not, was at any rate a species which he believed to lay the eggs in the skin at the moult, and therefore quite distinct from that studied by me.

Note—Correction of Name.

Macrobiotus furcatus, Murray.

This name was given to a species collected by the Scottish National Antarctic Expedition in the South Orkneys ("Tardigrada of the South Orkneys," *Trans. Roy. Soc. Edin.* xlv., 1905, p. 327, Plate II. figs. 6*a* to 6*d*). I was then unaware that Ehrenberg had already used the name for an Alpine species in 1859 (11). *M. furcatus*, Ehr., appears to have escaped the attention of all subsequent writers on Tardigrada whose works I have been able to consult. My error was pointed out by Professor Hay of Washington, and I now correct it and give the species another name.

Macrobiotus furciger, n. sp. (Murray) (= *M. furcatus*, Murray).

Description.—Large (up to 600μ), hyaline. Claws of each pair united for about half the length of the larger claws; supplementary points very strong. Teeth strong, curved; gullet wide; pharynx shortly oval, with conspicuous apophyses on end of gullet, and three equal, separate rods in each row of thickenings, besides a large comma. Eggs spherical, spiny, about 83μ in diameter without the spines, 105μ over the spines; spines with bulbose bases, tapering upwards, and once, twice, or thrice forked at the tips; a circlet at the base as in *M. hufelandi*, Richters.

As remarked in the original description, this is the South Orkney representative of *M. hufelandi*. The most obvious distinction is the dichotomous processes of the egg, those of *M. hufelandi* ending in expansions, which may be likened to little funnels or discs. There are normally three distinct rods in the pharynx, while in *M. hufelandi* the two rods next the pharynx are normally joined, and when separate they remain close together or actually in contact.

Professor Richters has recently seen reason to believe that the rods in the pharynx of *M. hufelandi* vary greatly, and that there may be three quite distinct; he also finds that the degree of union of the claws varies so much as to offer a complete series from the V-shaped pairs of *M. echinogenitus* to the closely welded pairs of typical *M. hufelandi*.

My experience of Scottish examples has not yet confirmed Professor Richters' observations on those points, but has rather led me to regard most of the structures of Tardigrada as fairly constant. Species do, however, vary more in some regions than in others.

In the large numbers of examples of *M. furciger* examined I have never seen the first two rods in the pharynx united, nor an egg spine unforked. As no typical examples of *M. hufelandi*, nor of its egg, were found in the South Orkney collections, and as all the characters of *M. furciger* were very constant, it seems to me that, after

making all due allowance for variation, we must regard it as a good species, though closely related to *M. hufelandi.*

M. furciger, MURRAY, has no affinity with *M. furcatus*, EHR., which is related rather to *M. dispar*, MURRAY, and *M. ambiguus*, MURRAY.

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EXPLANATION OF PLATES.

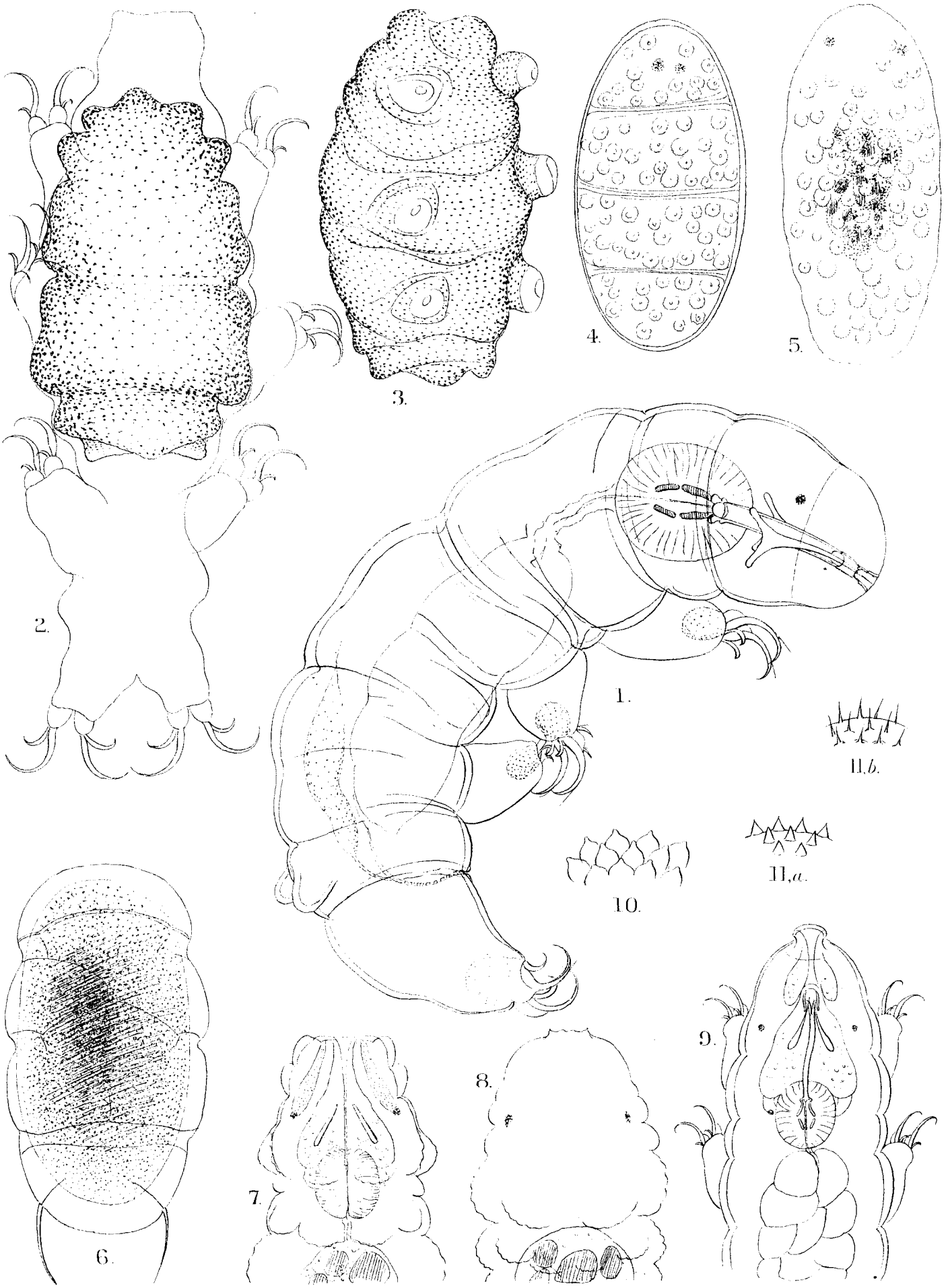
PLATE I.

1. *Macrobiotus dispar*, Murray. Side view.
2. ,, ,, cyst in the old skin.
3. ,, ,, oblique ventral view of cyst.
4. ,, ,, cyst, with outer case removed.
5. ,, ,, *simplex* animal squeezed out of cyst.
6. Cyst of *Echiniscus perarmatus*, Murray.
7. *Macrobiotus nodosus*, Murray. *Simplex* form.
8. ,, ,, further reduced state.
9. *M. dispar*, *diphascon-simplex* form.
10. *M. ambiguus*, Murray. Portion of surface of egg.
11*a*. *M. dispar*, portion of surface of egg.
11*b*. *M. furcatus*, Ehr. Portion of surface of egg.

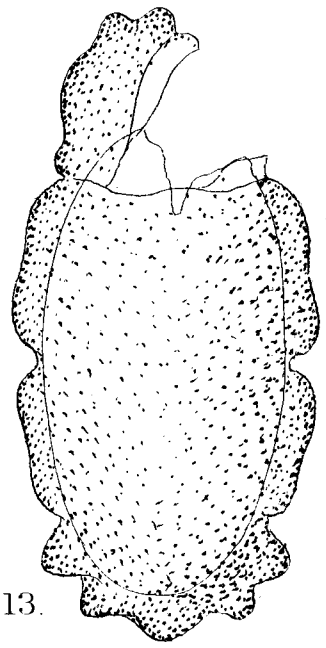
PLATE II.

12. *M. dispar*, emerging from cyst.
13. ,, empty case, with its trap-door.
14. ,, simplex individual, showing rudimentary limbs.
15*a*. *M. oberhäuseri*, Doy.? Cyst in skin.
15*b*. ,, teeth and pharynx in cyst.
15*c*. ,, one pair of claws.
16*a*. *M. echinogenitus*, Richters. Wrinkled cyst.
16*b*. *M. echinogenitus*, a pair of claws.
17*a*. *M. oberhäuseri*, Doy.? Wrinkled cyst.
17*b*. ,, teeth and pharynx.
17*c*. ,, a pair of claws.
18*a*. *Glycophagus domesticus*, De Geer. Rudimentary *Hypopus* (after Michael).
18*b*. The same. Posterior part of case, with its trap-door. (Compare 13.)

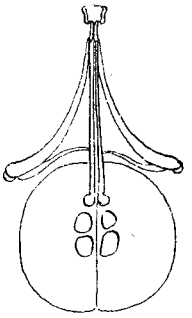
MURRAY: ENCYSTMENT OF TARDIGRADA.——PLATE I.



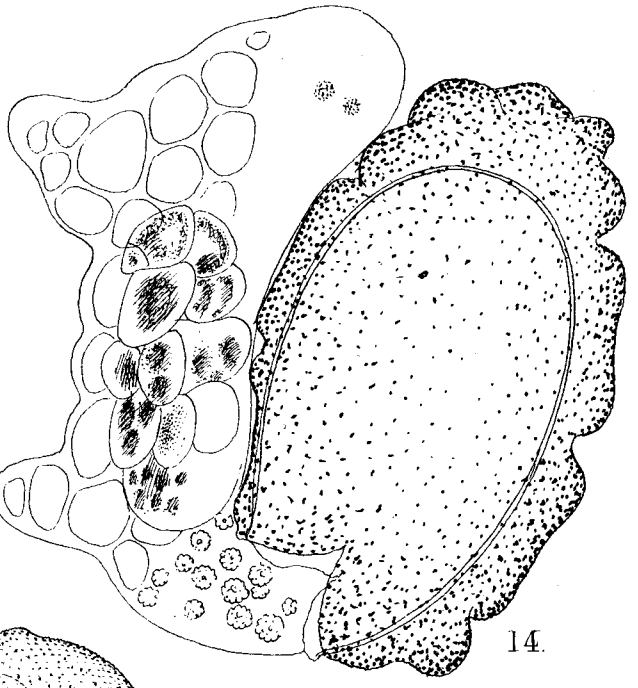
MURRAY: ENCYSTMENT OF TARDIGRADA.—PLATE II.



13.



15, *b*.



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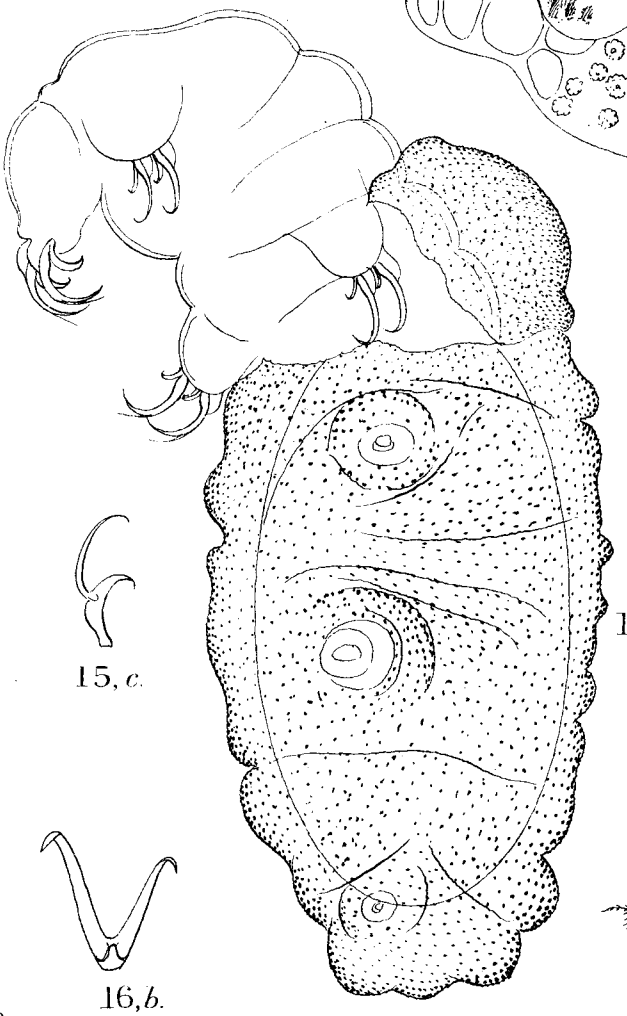
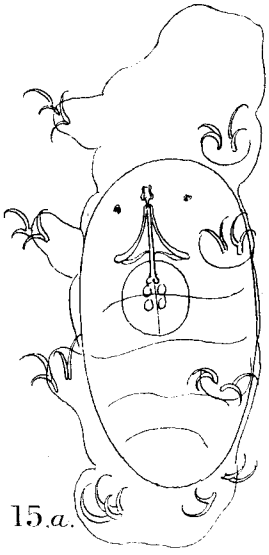
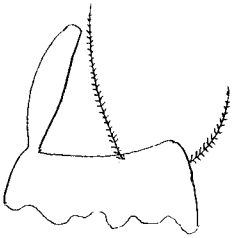


12.



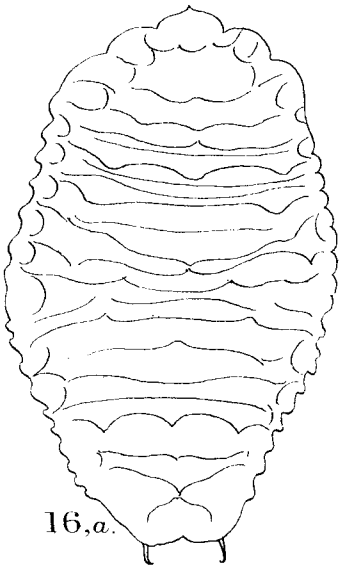
15.*a*.

15, *c*.

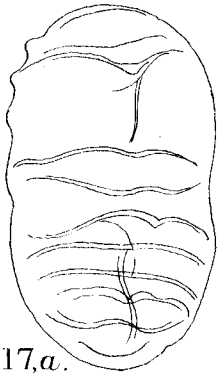


18, *b*.

16, *b*.



16, *a*.



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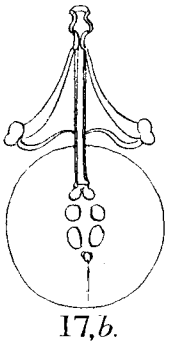
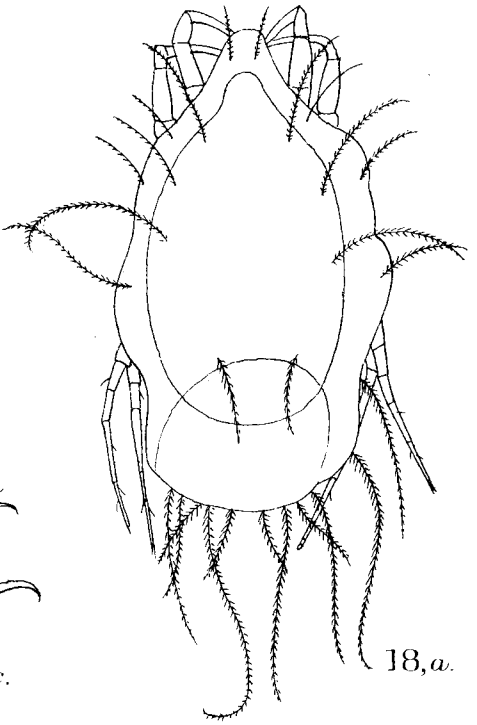


17, *b*.

17, *c*.



18, *a*.