THE ENCYSTMENT OF *MACROBIOTUS*.

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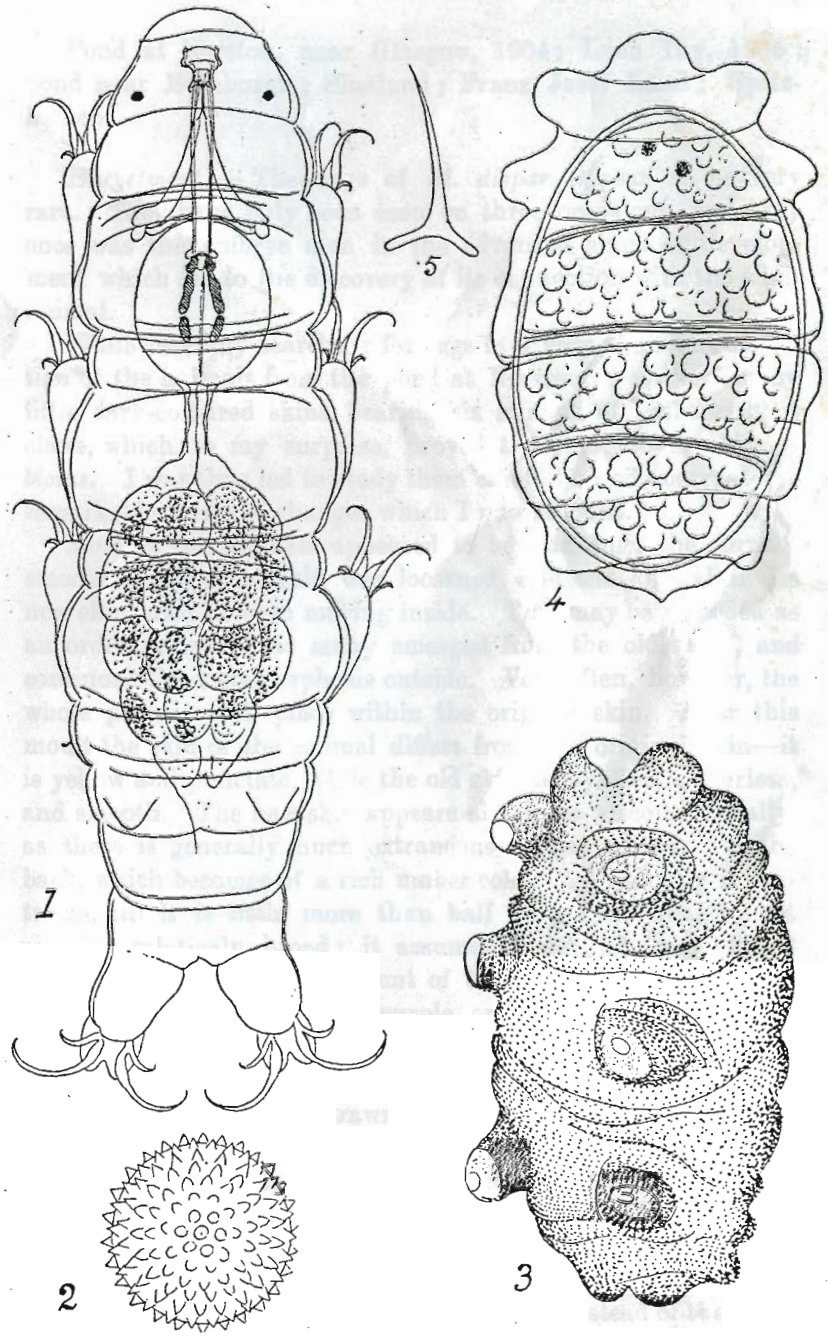
Many of the smallest animals, in common with some of the largest, experience regularly at some period of the year unfavourable conditions which threaten the destruction of the individual and the extermination of the race. This fate is avoided by one of two means—either the individual perishes, and the race is saved by the production of what are called winter-eggs or resting-eggs, or the individual protects itself in some way, and lies dormant till better times, or, as it is called, hybernates.

It is usually the cold of winter which constitutes the threatening condition, hence the terms winter-egg and hybernation; but it may be quite the converse, and it may be the heat or drought of summer which has to be guarded against. Similar means of protection may be used against either evil. Many resting-eggs are laid in summer, and many animals, including Water-Bears and Bdelloid Rotifers, lie dormant during droughts. Using the term in the wider sense to cover all means taken to tide over untoward seasons, large numbers of animals hybernate—Bears, Tortoises, Water-Bears, Bdelloid Rotifers, Nematodes, &c.

Bdelloid Rotifers resist desiccation by coating themselves with an air-proof varnish. Water-Bears (or Tardigrada) are equally well protected against desiccation, though the means by which they protect themselves are not understood. There is no coating of varnish in this case. The Bears are found among dry moss, rigidly extended as though in *rigor mortis*, but they quickly revive when placed in water.

The process now to be described is of a totally different kind. Its occurrence at the beginning of winter suggests that it is a sort of *hybernation*; the profound changes in the organization of the animals which characterize it suggest the name *encystment*.



J. Murray del. ad nat.

Macrobiotus dispar, n. sp.

For some years I have frequently seen, in the moss-washings where I seek for Water-Bears (Tardigrada), little yellow elliptical or sausage-shaped packages, from which I could squeeze out animals of the genus *Macrobiotus*, in a quiescent state, but alive. I thought to connect these in some way with imperfect moults, but the packages differed from the moulting animal in that the yellow skin tightly enclosed the animal within, suggesting a comparison with pupæ, or with mummies, and there were no limbs on the outer skin. The origin of these curious bodies was quite unknown, and the matter rested thus till I had recently the good fortune to watch the process of formation in one species which abounded in a shallow pond near Glasgow.

The species observed was long supposed to be *Macrobiotus macronyx*, but the eggs have recently been found, and as these are spiny and are laid free, while those of *M. macronyx* are smooth and are laid in the moulted skin, the species would have to be placed in a different section of the genus. Prof. Richters agrees that the species is one hitherto undescribed, and I now give a description of it:—

Macrobiotus dispar, n. sp. (figs. 1 to 5). — Large, hyaline, with yellow or brown stomach. Eyes dark. Claws of each pair very unequal, one very large, the other very minute, the disproportion less in those of the last legs. Pharynx large, shortly oval; thickenings three in each row—first a nut joined to the gullet, second a long narrow rod, third a rod half as long as the second. Teeth curved, with very wide furca. Eggs spiny, laid free; spines little sharp cones, separated by interspaces about equal to the diameter of the cones. Length up to 600 μ; pharynx, 80 μ long; larger claws, 48 μ long; diameter of egg, over spines, 90 μ. There are generally two large conical processes on the back (fig. 1), between the third and fourth legs. These are variable, and may be apparently quite obsolete. There appears, however, to be always some trace of them when the animal moults.

This species was supposed by Prof. Richters to be *M. macronyx*, which the adult closely resembles, and under that name I have recorded it in various papers. That species lays smooth eggs in the moulted skin, and has not the conical processes so frequent in *M. dispar*.

Pond at Nerston, near Glasgow, 1904; Loch Tay, 1905; pond near Edinburgh; Shetland; Franz Josef Land; Spitzbergen.

Encystment. — The eggs of *M. dispar* appear to be very rare. They have only been seen on three occasions, and only once was the embryo seen in the advanced stage of development which led to the discovery of its connection with the adult animal.

While carefully searching for eggs in a very abundant collection of the animals from the pond at Nerston, I noticed many little dark-coloured skins, bearing six stumps of limbs without claws, which, to my surprise, proved to belong to the *Macrobiotus*. I was thus led to study them carefully, and observed the remarkable series of changes which I now recount.

Most of the animals appeared to be moulting; the normal, smooth, transparent skin was loosened, and the animal in its new skin could be seen moving inside. This may be regarded as an ordinary moult, as many emerged from the old skins, and continued the metamorphosis outside. Very often, however, the whole process took place within the original skin. After this moult the skin of the animal differs from the original skin—it is yellow and punctate, while the old skin is hyaline, colourless, and smooth. The new skin appears also to be viscous dorsally, as there is generally much extraneous matter adhering to the back, which becomes of a rich umber colour. Gradually it contracts, till it is little more than half its original length, but remains relatively broad; it assumes a definite form (figs. 3 and 4), only slightly reminiscent of the adult form; the colour darkens till it becomes grey, purple, or well-nigh black; finally it becomes quite rigid, with brittle shagreened skin, and six little stumps of legs, without claws (fig. 3). This I call the *outer cyst*. The last legs have been drawn in before the hardening of the cyst, so that they do not show conspicuously like the others, and the claws of the other legs have also been withdrawn. If one of these cysts is squeezed shortly after its formation it breaks up and reveals a normal adult, having all the usual organs—claws, pharynx, teeth, stomach, eyes, &c. If it is examined at a somewhat later period it is found that it contains, instead of the adult

animal, an elliptical, thick-skinned, yellow body (fig. 4), which I call here the *inner cyst*.

This inner cyst is smooth, and has no trace of limbs. It is considerably less in size than the outer cyst, in which it lies loosely. Just after it is formed it contains a complete animal, possessed of all the normal organs. This may be squeezed out, and may be seen to move feebly.

When a large number of cysts were broken up, after the lapse of about a week from their first observation, it was found that a large proportion of the inner cysts had undergone further change. The cyst no longer contained a complete animal. Most of the conspicuous organs had disappeared—claws, pharynx, teeth, &c.—and the animal would not have been recognized as a Tardigrade had its antecedents not been known. Only the pigment-spots, commonly called eyes, and the fat-cells in the blood were recognizable. There was a faint segmentation within the cyst, three transverse furrows dividing the body into four nearly equal parts. The pigment-spots were very diffuse.

The animal contained in the inner cyst is so unlike a Tardigrade that I have entertained the idea that it might be some parasite—possibly a Turbellarian or other worm. The complexity of the series of changes by which the final cyst is produced, the constant form and size of the outer cyst, and the presence of all the organs till the last stage is reached—all tell against the theory of parasitism, and lead to the conclusion that we have to do with a normal process in the life-history of the animal.

What may be the meaning of these remarkable changes we need hardly attempt to guess till the further stages have been observed. Cysts of other species have been seen, in which there were active animals which I supposed were about to emerge, but in view of the history of *M. dispar*, partly traced above, we may rather suppose that these cysts had just been formed, and that the process of simplification had not yet taken place.

Till the full history is known it will remain inexplicable why the double moult should take place, the animal at each moult assuming a different form; and, finally, why the individual should undergo such a profound simplification. The curious outer cyst may be regarded not as the result of a moult, but as

the product of a secretion from the skin. Fragile though it is, it may be a sufficient protection to the inner cyst against the only enemies likely to be attracted by Water-Bears.

Extraordinary though it may appear that an animal so high in the scale as an Arthropod should lose all its internal organs in the course of encystment, a well-known phenomenon in the same group (Tardigrada) supports the belief that this really happens. Nearly all species of *Macrobiotus* are known to have what Richters calls "simplex" forms. The teeth in these are reduced in size, and the rods in the pharynx are absent. Prof. Richters appears to have seen no further simplification, but in Scotland it is common for the whole alimentary canal in front of the stomach, with all its adjuncts, to be absent. There is no mouth, no gullet, no teeth, but some trace of the pharyngial bulb usually remains. We find large, strong, active animals whose stomachs appear distended with food, yet which possess no organs for imbibing food. No conclusion seems tenable but that reduction must have taken place since the food was imbibed, but this explanation is itself inexplicable, and we are further puzzled when we find that some eggs produce simplex forms.

The applicability of the term "encystment" to the process which has been described may be questioned, but the word "cyst" well describes the bodies formed; and, moreover, the formation of outer envelopes, within which the whole substance of the animal passes into an apparently simpler condition, offers sufficient analogy with encystment as we find it in the Protozoa to justify the adoption of the term.

The formation of the cysts was going on in the beginning of winter, in October and November. This may indicate that it is, like the production of winter-eggs by various animals, a means of preserving the species through the rigours of winter.

M. dispar is the only species in which the complex process has been traced so far as the simplex form within the inner cyst. Cysts similar to the inner cysts of *M. dispar* are known in several species. Among moss brought by Mr. W. S. Bruce from Spitzbergen in August, 1906, and examined in September, there were many cysts which were shown by the pharynx and claws of the contained animal to belong to *M. echinogenitus*, Richters. More recently the formation of the cyst of this species has been seen

in examples from a bog-pool in Scotland. There is no outer cyst like that of *M. dispar*. The cyst formed within the moulted skin resembles the inner cyst, and contains a complete animal. The reduction to the simplex state has not in this instance been seen.

M. dispar is one of the few Tardigrada which have their usual habitat in ponds. It is also the most boreal of known species, as it was found at an elevation of several hundred feet in Franz Josef Land by Mr. Bruce. Whether it is exclusively a northern species remains to be seen. At present its southern known limit is Scotland, but, in view of the close resemblance of the adult animal to *M. macronyx*, I suspect that some of the records made under that name really refer to *M. dispar*.

In this country it lives in ponds liable to freeze in winter, occasionally at the margins of lakes, and it may be that the encystment is correlated with its life in permanent waters. The case of *M. echinogenitus*, cited above, suggests the possibility that species which normally live in moss may be induced to form cysts when they find themselves amid the different conditions of a pond.

The publication of these observations in their incomplete state is made in the hope that other students of the Tardigrada may interest themselves in the "encystment," and that we may thus hope for an earlier solution of the problems involved.

Explanation of Figures (p. 5).

1. Adult, dorsal view, showing the conical processes.
2. Egg.
3. Outer cyst, showing shagreened surface, and legs.
4. Inner cyst, shown within outline of outer cyst; dorsal view.
5. Tooth, showing the very wide *furca*.

(All the figures are drawn to the same scale.)

Since writing these notes I have received from Prof. Lauterborn an interesting little paper, in which he describes a similar encystment of *Macrobiotus macronyx* (Verhand. d. Deutsch. Zool. Ges. 1906, p. 267). Prof. Lauterborn's observations differ from mine in many respects, and it may be that the cycle of changes is not identical in the two species. It is curious that these two species, which are believed by Prof. Richters to very closely resemble each other, yet differ profoundly in the details of reproduction, as shown by the recent discovery of the spiny eggs of *M. dispar*. Prof. Lauterborn also refers to this remarkable process as *encystment* (Encystierung). Both *M. macronyx* and *M. dispar* live in ponds, and we might find in this the cause compelling to the encystment, but *M. echinogenitus*, which also encysts, is not especially a pond-dweller.