

*Rhopalura granosa* sp. nov., an Orthonectid Parasite of  
a Lamellibranch *Heteranomia squamula* L., with a  
Note on its Swimming Behaviour.

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With 4 Figures in the Text.

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INTRODUCTION.

THE Orthonectida, a small group of rare parasites, have attracted considerable interest owing to their doubtful systematic position. In 1868 Keferstein (1868, pl. ii, fig. 8) figured, though he did not describe, a "problematic parasite"\* from the digestive tube of *Leptoplana tremellaris*, but it was not until about 1877 that a serious investigation of these forms was undertaken by Giard (1877), who gave to the group the name of Orthonectida. Our knowledge of the organisation and life-history of these parasites, however, is in great part due to the admirable researches of Caullery and his collaborators. The Orthonectida are forms in which the sexual generation is formed asexually from germ cells produced in a parasitic plasmodium.

Some eight species of *Rhopalura*, and one of *Stœcharthrum*, are now known.

*Rhopalura granosa* sp. nov., parasitic in *Heteranomia squamula* L. (= *Anomia aculeata* Müller), in the Plymouth area, is the first Orthonectid

\* This species was named *Rhopalura (Intoshia) leptoplanae* by Giard (1880, p. 236); it was rediscovered and described by Jourdain (1880)—under the name of *Prothelminthus hessi*—at Saint-Vaast-la-Hougue, and by Caullery and Mesnil (1901 c, pp. 399–400) in the bay of Saint-Martin, under the old fort of Saint-Germain-des-Vaux.

to be described from a Mollusc, members of the group being hitherto known from Annelids, Nemertines, a Planarian, and from an Ophiuroid, *Amphiura squamata*. The only previous record of an Orthonectid for the British Isles would seem to be that by McIntosh in 1873 (p. 129), when he described a curious parasite burrowing in the body wall of *Lineus gessnerensis*. The specific name *granosa* has been given on account of the presence of characteristic refringent bodies in the male (see p. 237).

#### DESCRIPTION OF *RHOPALURA GRANOSA*.

In *R. granosa* the sexes are separate, and, as in other species of *Rhopalura* where the male is known, exhibit sexual dimorphism. Sexual dimorphism is so marked in the genus that Giard (1879, 1880) originally referred the two sexes in *R. ophiocomæ* to two genera, *Rhopalura* and *Intoshia*.

#### *The Female.*

The female (Fig. 1, A and B) is cylindrical, tapering anteriorly and posteriorly, and about 190 to 210 $\mu$  long, and 60 to 75 $\mu$  broad, not including the cilia. Individuals, however, vary somewhat in shape, doubtless partly owing to their considerable powers of contraction; a narrow elongated female, ca. 230 $\mu$  long and 55 $\mu$  broad, is shown in Fig. 1, B, but this is an extreme form, and rarely seen.

Fig. 1, A and B, of *R. granosa* recall Julin's figures of "femelle aplatie" and "femelle cylindrique" of *R. ophiocomæ* (Julin, 1882, pl. ii, figs. 2 and 1). The type of female shown in Fig. 1, A, is not flattened, however, for there is no appreciable difference observable in the width as it rotates in swimming; the rings are no less clearly marked than in the elongated form (Fig. 1, B). Both forms are found among those issuing naturally from their hosts. Caullery and Mesnil (1901 c, pp. 395-397) think that possibly the cylindrical females of *R. ophiocomæ* are a temporary state, leading to the flattened females: their specimens were apparently taken artificially from the host.

The body of the female *R. granosa*, which is entirely ciliated, shows eight rings, of which the first is the anterior, and the last the posterior terminal cone. The fourth and sixth rings seem to be formed of two rows of cells, while the second, third, fifth and seventh are formed of one row each. The anterior and posterior cones are formed of several rows. Between the rings there is a row of tiny cells, as in the male. The rings are superficial, involving the ectoderm only, the body not being segmented: they are more evident in some individuals than in others. When the animal is swimming forwards the cilia on the anterior cone are directed forwards, and those on the body posterior to it backwards (see Fig. 1, A). When the animal is swimming backwards, however, those on the second ring, as

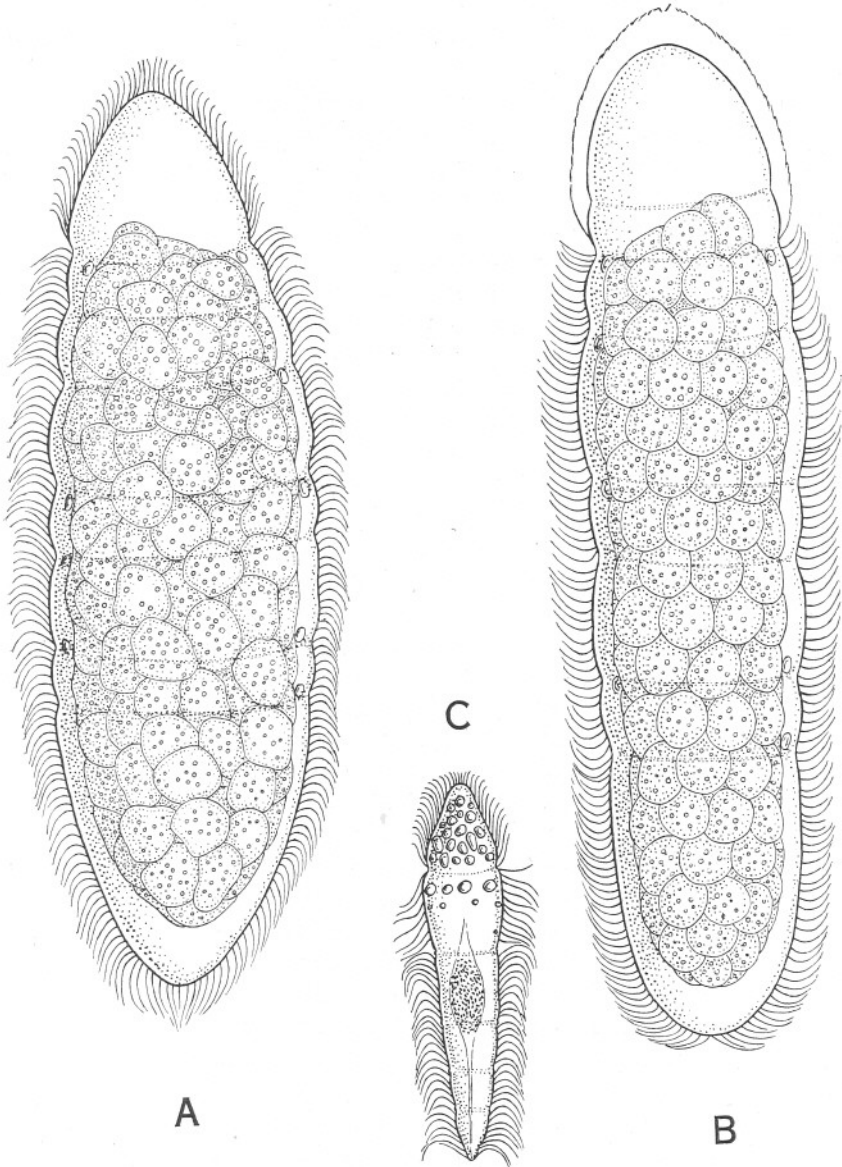


FIG. 1.—*Rhopalura granosa*. Sketches from life of individuals which had issued naturally from the host. The cilia are shown in profile only, although actually the animals are entirely ciliated: they are shown somewhat diagrammatically.  $\times 573\frac{1}{2}$ .

A. Female. The cilia are shown as they appear during slow forward movement.

B. Rare, elongated form of the female. The cilia are shown as they appear during reversed swimming. In both forms the asymmetrical anterior extension of the mass of ova is due to the presence of the problematical organ, which is not shown in the figures.

C. Male.

well as those on the anterior cone, become motionless and are applied closely to the surface of the body, the tips being directed anteriorly (see Fig. 1, B, and also p. 247).

The ectoderm cells of the anterior and posterior extremities are deeper than those covering the rest of the body, the depth of the anterior cells being especially noticeable. The latter, in particular, contain a number of granules towards their outer ends, which become red if a trace of neutral red is added to the water. In sections of animals preserved in Bouin's fixative and stained with Heidenhain's iron hæmatoxylin and acid fuchsin the ectoderm cells appear much vacuolated, the vacuolation occurring sometimes at the inner ends, and sometimes at the outer ends of the cells. A problematical organ is present in the anterior cone; narrow prolongations from the organ appear to encircle the cone. It has been suggested by Metschnikoff (1881, p. 285) that this structure may be a remnant of an alimentary canal, and by Caullery and Lavallée (1908 b, p. 465) a nervous ring. A few, one to four, large vacuoles are generally observable in the cells of the anterior cone.

A small number of refringent bodies occur at irregular intervals, in a position between the rings of the body. These become orange with neutral red, and pale blue with methylene blue *intra vitam* staining.

The ova are numerous, very roughly about two hundred. They extend only slightly into the region of the anterior cone. They are about  $14\mu$  in diameter, with transparent cytoplasm containing a number of refringent granules, which become red with neutral red used *intra vitam*: it would appear to be these granules which in sections of material preserved in Bouin's fixative stain black with iron hæmatoxylin.

The embryos develop in the body of the parent: they are closely packed, and no movement of the ciliated larvæ is observable, such as occurs in *R. pelseneri* (M. and C., 1905a, p. 429).

Embryos and ova have been observed, on several occasions, escaping from the parent in the region of the second and third rings. The fact that ova also have been seen to be expelled, points to the conclusion that these occurrences were not normal, but possibly due to unnatural conditions of observation; the position of emergence may, therefore, also be abnormal. A genital pore, such as described by Caullery and Mesnil (1901 c, p. 394) for *R. ophiocomæ*, could not be distinguished, at least in females sectioned while still within the host: no sections were made of those which had emerged.

#### *The Male.*

The male (Fig. 1, C) is cylindrical and slender, tapering anteriorly and posteriorly, the broadest region being the second ring. It is 87 to  $95\mu$  long and  $20\mu$  broad, not including the cilia. The body shows six super-

ficial rings, of which the first is the anterior, and the last the posterior terminal cone. Between the rings is a row of tiny cells, the nuclei of which show clearly in sections. The anterior cone is formed of several rows of ectoderm cells, as is also the second ring.

A characteristic of the male is the presence of large irregular refringent bodies in the cells of the anterior cone, as well as in the first row of cells of the second ring. Occasionally smaller refringent bodies occur in other parts of the second ring. In *R. ophiocomæ*, the only other species in which the males are known to have these curious large refringent bodies in the cells, they are present in the second ring only (Giard,

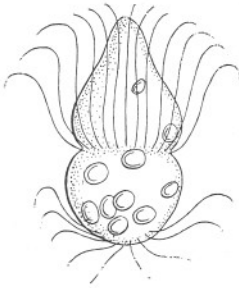


FIG. 2.—*Rhopalura granosa*. Ciliated larva, taken from the body of the parent.  $\times 1470$ .



FIG. 3.—*Rhopalura granosa*. Fragment of a male producing plasmodium.  $\times 70$ .

1880, p. 229; Julin, 1882, p. 11). In sections of *R. granosa* preserved in Bouin's fixative these bodies frequently stain lightly with a dark periphery with iron hæmatoxylin and acid fuchsin, but others stain uniformly black. Caullery and Mesnil (1901 c, p. 393) found those of *R. ophiocomæ* disappeared in preparations treated with alcohol and xylol, a vacuole occupying their place. The fixative they used chiefly was a saturated solution of sublimate in sea-water, with the addition of 1% acetic acid.

The male of *R. granosa* is entirely ciliated. The cilia on the anterior cone are rather shorter and denser than those on the rest of the body; they are directed forwards as in the female. Those on the second ring are much less closely set than those on the posterior rings, but appear to be as long. In *R. ophiocomæ*, the species which—of those so far described—seems most closely allied to *R. granosa*, the second ring is said to be unciliated, though Caullery and Mesnil (1901 c, p. 392 footnote) note that

some isolated cells exceptionally bear long cilia. In sections the basal granules of the cilia stain very darkly and clearly with iron hæmatoxylin.

The "testis" is present about the middle of the body. The spermatozoa are flagellated.

#### The Larva.

The ciliated larva (Fig. 2) forced from the body of the female, by gentle pressure on the coverslip, is somewhat acorn-shaped,\* being slightly constricted in the middle region, pointed anteriorly and broadly rounded posteriorly. (The pointed end is apparently anterior, as this is foremost when the larva swims.) It is about  $19\mu$  long. Ciliation is restricted to two bands of long cilia; one in the middle region about the slight constriction, and one posteriorly. Refrangent bodies are present in both regions of the larva, but chiefly posteriorly. Cells could not be distinguished in the living state; larvæ were not sectioned.

The larva of *R. granosa* differs considerably in appearance from those so far described, namely, that of *R. ophiocomæ* (C. and L., 1905, p. 266; 1908 b, p. 432, and pl. xv, figs. 43, 44) and *R. pelseneeri* (M. and C., 1905 a, p. 429, and fig. 1), which appear to have no regular arrangement of the cilia, though Caullery and Lavallée (1908 b, p. 432), speaking of the very small size ( $12-15\mu$  in diameter) and great transparency of the former larva, say: "La disposition des cils mêmes est à peu près impossible à fixer; ils paraissent longs et peu denses. Ils donnent à ces larves un mouvement rapide, souvent tourbillonnant."

#### Relationships.

Caullery and Mesnil (1901 c, p. 419) have distinguished three groups in the genus *Rhopalura*, characterised as follows:—

	Cilia limited to narrow rings, <i>R. pterocirri</i> St. J. (I).	
	Ova in compact mass. (II).	<i>R. ophiocomæ</i> Gd. <i>R. intoshi</i> Metchn.
Cilia entirely (or nearly) covering the body.	1 (or 2) linear row of ova. (III)	<i>R. leptoplanæ</i> Gd.
		<i>R. pelseneeri</i> C. and M.
		<i>R. linei</i> Gd. <i>R. metchnikovi</i> C. and M. <i>R. julini</i> C. and M.

*R. granosa* evidently belongs to the second group. It is intermediate in size in both sexes, between *R. intoshi* from *Lineus (Nemertes) lacteus* and *R. ophiocomæ* from *Amphiura squamata*. In the male it is clearly distinguishable from *R. intoshi* by the presence of large refrangent bodies, and from *R. ophiocomæ* by having these bodies in the anterior cone, and the

\* Shaped like an acorn in its cup.

first row of cells of the second ring, while in *R. ophiocomæ* they are restricted to the second ring.

The female of *R. granosa* has not been observed to be without cilia on the second ring, as occurs in certain individuals of *R. ophiocomæ* (Giard, 1880, p. 232; Julin, 1882, p. 16; C. and M., 1901 c, p. 393): it has eight rings, while that of *R. intoshi* has nine (Metschnikoff, 1881, p. 284).

The larva differs from that of *R. ophiocomæ* both in the shape and the arrangement of the cilia: the larva of *R. intoshi* is unknown.

#### THE PLASMODIA AND THEIR DISTRIBUTION IN THE HOST.

In *Heteranomia squamula* the parasite is found replacing the gonad; it also occurs in the blood lacunæ and vessels in the mantle and the suspensory membranes of the gills, even extending into the dorsal ends of the gill filaments. In one host sectioned, numerous young plasmodia were present in the mantle margin in the posterior region. In heavy infections the parasitic plasmodia, containing the sexual forms of the Orthonectid, entirely replace the gonad of the host, rendering the determination of sex impossible. A fragment of a male containing plasmodium is shown in Fig. 3.

It would appear to be more usual for the males and females to occur in separate hosts, though it is by no means rare for them to be found together (see p. 244); when this occurs one sex generally predominates. Observations were mostly made on living *Heteranomia*, only two specimens being sectioned. One of the sectioned individuals was parasitised by plasmodia containing males only, many of them being nearly mature. It was well infected, plasmodia occurring in the mantle, visceral mass, suspensory membranes of the gills and dorsal ends of the gill filaments, but much of the gonad remained, sperm being recognisable among degenerating cells in some regions.

The second *Heteranomia* sectioned was very heavily infected, no gonad being recognisable. The great majority of the plasmodia in this host contained female *Rhopalura*, but in four separate regions of the visceral mass and mantle, males were present, many being nearly mature, and in at least two of these regions males and females occurred together in the same plasmodium (see Fig. 4). In one region (a), several branches or lobes of a plasmodium contained males, but in a very small portion only were the two sexes present together. In a second region (b), a small island of well-formed males, together with numerous germ cells and groups of germ cells, was present among females, and the males were not segregated in separate lobes of the plasmodium. In a third region (c), a few males were present in a small, almost empty plasmodial lobe, which was rather doubtfully traced into a female plasmodium. The fourth region (d), near

the byssal muscle of the host, was by far the largest. This plasmodium appeared to contain males alone, though its considerable size made the tracing of its many branches difficult.

Where the males and females were present in the same plasmodium, the females yet appeared to contain unsegmented ova, so far as could be ascertained in the crowded condition of these. Caullery and Mesnil (1901 c, pp. 466-467) state that "dans des coupes d'une *Amphiura*, qui renfermait des plasmodes des deux sexes (of *R. ophiocomæ*), plusieurs femelles, à l'intérieur desquelles, au lieu d'ovules, on trouvait des corps plurinucléés ayant tout à fait l'aspect des embryons décrits ci-dessus, mais un peu moins avancés. Nous les interprétons comme tels. Il est parfaitement admissible que, lorsque les deux sexes de *Rhopalura* existent dans une même Ophiure, les femelles adultes puissent, avant d'arriver au dehors, être fécondées et renfermer des embryons."

It is impossible to be entirely certain that the presence of males and females in the same plasmodium is not due to the disappearance of host tissue dividing two originally separate plasmodia, but it seems not improbable that a plasmodium may produce males and females at different times, the two phases overlapping to some extent. When plasmodia are well established in the host, they ramify greatly, and it is practically impossible to determine their number and limits. The presence of plasmodia with males only in the less heavily infected host sectioned would seem to indicate that, if it should prove to be correct that the two sexes are produced by the same plasmodium at different times, the male phase precedes the female.

It may be noted that curious thread- or rod-like bodies, or regions, of darker staining protoplasm were present in some small portions of female producing plasmodia. Among masses of these occurred a few normal-looking groups of germ cells. The significance of these bodies is obscure; they may be a normal occurrence, or possibly parasites.

Caullery and Mesnil (1901 c, p. 384; C. and L., 1912, p. 159) have described plasmodia which produce one sex only, such as are usually found in *R. ophiocomæ* from *Amphiura squamata*, as "unisexual," and those in which males and females develop side by side in the same plasmodium, as in *R. metchnikovi* from *Spio martinensis* (C. and M., 1901 c, pp. 384, 402), as "hermaphrodite." In *R. intoshi* from *Lineus (Nemertes) lacteus* an intermediate condition, between that of *R. ophiocomæ* and *R. metchnikovi*, seems to obtain, "male," "female," and "hermaphrodite" plasmodia frequently occurring in the same host (Metschnikoff, 1881, p. 284).

Most known dicecious species of *Rhopalura* apparently have "hermaphrodite" plasmodia (*R. leptoplanae*, C. and M., 1901 c, p. 399, and *R. julini*, C. and M., 1901 c, p. 412, in addition to those already mentioned), *R. ophiocomæ* being the only one with generally "unisexual" plasmodia.



There appears, however, to be considerable variation in the condition of the plasmodia of this species in different localities. Giard (1880, p. 228) and Metschnikoff, 1881, p. 288) found that in *R. ophiocomæ* from *Amphiura squamata* from Wimereux, and from Naples and Spezzia respectively, a

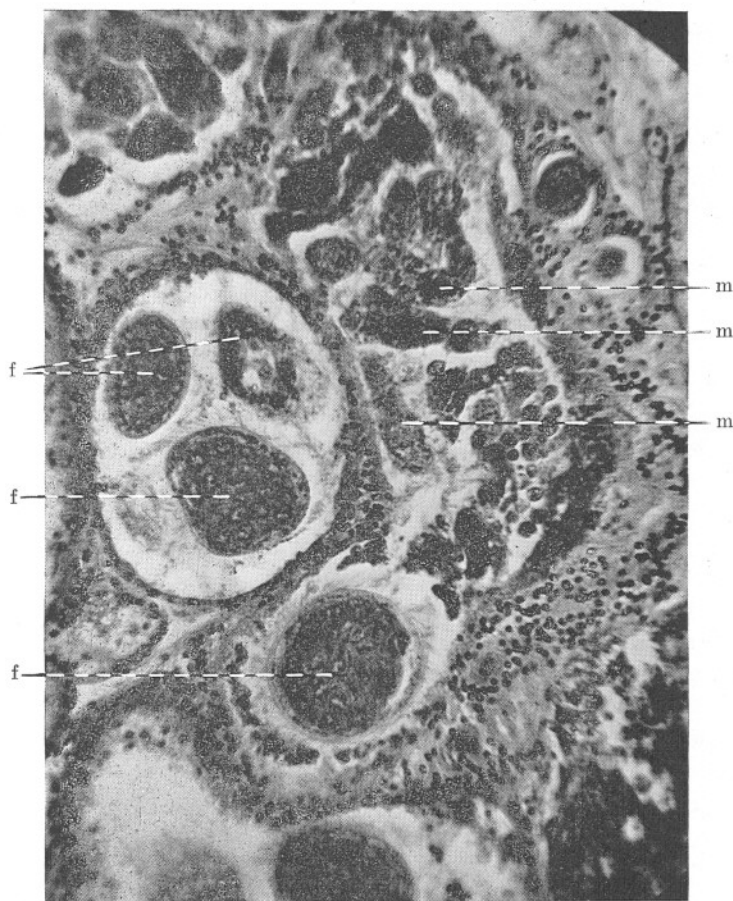


Photo.

D. P. Wilson.

FIG. 4.—*Rhopalura granosa*. Section of part of a plasmodium containing both males and females. F., female (transverse section); m., male (longitudinal section). Bouin's fixative: Heidenhain's iron hæmatoxylin and acid fuchsin.  $\times$  ca. 1012.

plasmodium produced Orthonectids of one sex only, males or females, though very occasionally the two sexes might be present in the same host. Julin (1882, p. 8), also working at Wimereux, says: "Je n'ai jamais rencontré dans le même hôte que l'une des deux formes, soit le mâle, soit la

femelle, . . ." Caullery and Mesnil (1901 c, p. 392) found " Dans le cas de la *Rh. ophiocomæ*, chaque plasmode ne renferme que des individus d'un seul sexe, et souvent dans une même Ophiure, tous les plasmodes sont du même sexe," and in a foot-note add: " Dans l'anse Saint-Martin, un tiers environ des Ophiures parasitées renfermait uniquement des femelles, un tiers uniquement des mâles, un tiers à la fois des mâles et des femelles." On the other hand Koehler (1886, p. 609) working at Cette on the same species very frequently found males and females in the same *Amphiura*, the latter always being less numerous than the males. He rarely found one sex only; in some fifty infected Ophiuroids only two contained males alone. He not only found males and females in the same host, but in the same plasmodium. Working in the same months of the year as Julin, he says (1886, p. 610) that the difference in their results can hardly be due to season, but that " Le genre de vie de ces remarquables parasites n'est donc pas le même à Cette qu'à Vimereux." In 1901 Caullery and Mesnil (p. 392) record that in an exceptional case they found adult males and females, together with developmental stages of both sexes, in the same plasmodium of *R. ophiocomæ*, thus confirming Koehler's observation of 1886.

In *R. ophiocomæ*, where the host is parasitised generally by Orthonectids of a single sex (with the exception of *Amphiura* at Cette), Caullery and Lavallée (1912, p. 163, foot-note) believe that the infection has mostly arisen from a single larva, and that the rare instances where males and females are present in the same host, have arisen from multiple infection by larvæ of different sexes. They think that one larva may possibly give rise to several amœboid germs, which spread the infection in the host (1912, p. 153).

When mature the males and females leave the host. Those from the gonad of *Heteranomia* would seem to pass out by way of the renal ducts, for in sections free forms have been recognised in them. The renal ducts opening into the exhalent chamber, the parasite will pass out safely in the exhalent current of the host. The way of escape of those parasites present in the blood-vessels and spaces has not been observed.

The males and females apparently meet outside the host and fertilisation is effected. The mode of fertilisation was not observed in *R. granosa*. It has, however, been studied in *R. ophiocomæ* by Caullery and Lavallée (1908 b, pp. 428-430). They found that on mixing mature males and females artificially taken from the host, " Au bout de 10 à 15 minutes, on observe très fréquemment que des mâles sont remorqués, aux flancs des femelles, dans la moitié postérieure de celles-ci, comme s'ils s'étaient accidentellement pris dans le revêtement ciliaire et n'avaient pu s'en dégager. Une femelle remorque parfois deux mâles; nous en avons même observé, une fois, trois.

Ce phénomène, extrêmement commun dans les premiers temps du mélange, ne se retrouve plus ensuite. Nous avons pu nous convaincre que ces rencontres des mâles et des femelles étaient les circonstances mêmes de la fécondation. Et cependant, la façon dont nagent les deux catégories d'individus n'indique nullement qu'ils se recherchent. On voit les mâles passer très près des femelles sans a'y fixer. Il semble que ce soit purement le hasard qui produise les couples observés. Le contact des deux sexes n'est d'ailleurs jamais très long. Au bout de quelques minutes, les mâles se sont dégagés, les femelles sont de nouveau solitaires. Ce contact n'est jamais non plus intime. Le mâle paraît simplement retenu par sa ciliation à celle de la femelle.

. . . nous avons constaté, d'une façon indiscutable, que les spermatozoïdes sont émis, à ce moment, au dehors. . . .

Caullery et Mesnil (1901, p. 394, pl. x, fig. 2, og.) ont signalé, sur la surface de la femelle, un pore, appelé par eux *pore génital*; . . . Nous avons lieu de croire que c'est par là que les spermatozoïdes pénètrent."

The embryos develop while in the body of the parent, being liberated as ciliated larvæ. To Caullery and his collaborators is due the discovery that in the Orthonectida the ciliated larvæ carry infection to fresh hosts; Caullery and Lavallée (1910, 1912) have caused experimental infection of *Amphiura squamata* by *R. ophiocomæ*.

They (C. and L., 1912, p. 140) have discovered that in the life-history of the Orthonectida two generations alternate regularly:—

- (1) a sexual generation, constituted in most species of males and females (some species, e.g. *R. pelseneri*, are hermaphrodite) formed asexually from germ cells produced in the parasitic plasmodium.
- (2) a generation produced from the fertilised ova of the preceding generation and carrying infection in the larval state to new hosts, where these larvæ are transformed into plasmodia, which give birth to the sexual generation.

It is puzzling to imagine how minute ciliated larvæ can effect safe settlement in a Lamellibranch. In the Anomiidæ the action of the lateral cilia on the gills is particularly furious, and the inhalent current rapid. One would not expect larvæ of not more than  $19\mu$  in length to be sufficiently strong swimmers to resist such a current, though it is possible that their cilia may interlock with those on the gills. If carried to the dorsal groove between the two demibranchs of each side they would in all probability eventually reach the mouth. It is possible, however, that after being thrown against the gills, they may be carried to the free edges of these, and then posteriorly in the rejection current, and dropped on the mantle margin in the posterior region. It may be noted that in one of the two *Heteranomia* sectioned, numerous young plasmodia were found in the

mantle margin in this region, but the actual path of infection remains obscure.

If a number of *Heteranomia* from an infected batch be placed in a finger-bowl, numerous specimens of *R. granosa* may be obtained after a time. These will live quite happily in sea-water, whereas those obtained by opening the hosts, being not yet fully mature and ready for their free life, very quickly die. Even the cilia of immature forms frequently break down into droplets. It is remarkable that males, sufficiently mature for the sperm to be active, yet, when artificially liberated from the host die after a very few minutes in sea-water. In well-formed males obtained in this way sperm is frequently seen issuing from about the middle of the body (see also Julin, 1882, pp. 13-14), but this is almost certainly abnormal and not the true method of emission.

If a jet of air be passed through the bowl, the *Heteranomia* will live for weeks, and a supply of *Rhopalura* be available. A number of *Heteranomia* have been kept in this way for some fourteen weeks, though towards the end of that time specimens of *Rhopalura* were difficult to find, and females with segmented ova rare.

*R. ophiocomæ* issues from *Amphiura squamata* chiefly in the late afternoon; Caullery and Lavallée (1912, p. 143) suggest that the more or less fixed hour of emergence, providing for the simultaneous emission of the males and females, brings about the meeting of the sexes. No observations were made on the time of emergence of the *Orthonectids* from *Heteranomia*.

#### NUMBER AND DISTRIBUTION OF THE HOSTS INFECTED.

The infected *Heteranomia*, with one exception, have been obtained from masses of *Lepralia foliacea*. The *Rhopalura* was first found on November 25, 1932, infecting *Heteranomia* taken from a mass of *Lepralia* turned out of the Plymouth Aquarium, but which had most probably come from off Revelstoke Point or Stoke Point. Ten out of twenty-two (45.4%) of the Lamellibranchs were found to be infected. Of these seven were infected with plasmodia containing females, and three with plasmodia containing males. In an unnoted number of *Heteranomia* infected with female forms, males were also present. This high percentage of infection, which has so far not been reached in material examined direct from the grounds, may possibly have been artificially induced by favourable conditions in the tanks.

On February 2, 1933, 149 *Heteranomia* from fragments of *Lepralia* dredged from off Revelstoke Point were examined. Of these twenty-four (16.1%) were found to be infected; ten with males, ten with females (in one the plasmodia contained only immature forms, which from their size

were probably female), and four with both males and females. When both sexes were present together the males were the more numerous, but as they are so much smaller than the females it does not necessarily follow that the plasmodia producing males occupied more space in the host than those producing females. The hosts varied from 3 to 10 mm. in diameter: that of 3 mm. was parasitised by male-bearing plasmodia.

The following animals, living either attached to, or sheltering in the crevices of the *Lepralia* obtained on February 2, were examined, but unsuccessfully, for the presence of Orthonectids: ten *Chlamys distorta*, ten *Ophiothrix fragilis*, five *Ophiocomina nigra*, and one *Antedon bifida*.

On February 6, 1933, 165 *Heteranomia* from fragments of *Lepralia* trawled from off Revelstoke and Stoke Points were examined. Of these only eleven (6.6%) were infected; four with males, five with females, and two with both males and females. In four the infection was slight. The hosts varied from about 6 to 12 mm. in diameter.

On February 13, 1933, all the *Heteranomia* of any size from three large pieces of *Lepralia* trawled from off Stoke Point were examined. Of the ninety obtained fourteen (15.5%) were infected; nine with males, two with females, and three with both males and females. Where the two sexes were present together the males were much more numerous than the females. The hosts varied from about 5 to 14 mm. in diameter.

The *Heteranomia* obtained on February 2, 6, and 13 were opened and carefully examined at a magnification of about 140, so that it is unlikely that even small numbers of well-developed sexual forms would have been overlooked, though tiny plasmodia most probably would have been.

Thirty-eight *Heteranomia* from twenty-four *Chlamys opercularis* from the "Corner" Ground off the Mewstone were examined on January 20, 1933: none were found to be infected.

An examination of seventy-three *Heteranomia* taken from the carapace of a single *Maia squinado* from the Mewstone Ground on March 20, 1933, showed only one to be infected, and that with plasmodia containing males alone.

It is possible that only those *Heteranomia* which occur in large communities, such as on masses of *Lepralia*, will be found to be generally infected. The branching colonies of the Polyzoan would also provide shelter from dispersing currents for the Orthonectids emerging from their hosts, thus facilitating the meeting of the males and females. Colonies of *Lepralia foliacea* were dredged and trawled chiefly off Revelstoke and Stoke Points at a depth of from 15 to 22 fathoms.

*R. ophiocomæ*, a species on which most work has been done, has been found to infect 2.5% to under 10% of *Amphiura squamata* (Giard, 1880, p. 227; Julin, 1882, p. 9; Caullery and Lavallée, 1908 b, p. 425), varying widely in different parts of the same locality (Caullery and Mesnil, 1901 c,

p. 391 ; Koehler, 1886, p. 609), and in different years (Metschnikoff, 1881, pp. 287, 288).

#### THE SWIMMING BEHAVIOUR OF *Rhopalura granosa*.

The Orthonectida were so named by Giard from their habit of swimming in a straight line. Giard states (1877, translated 1878, p. 182): "By the name of Orthonectida I have desired to recall their progression, which is so characteristic that it would of itself suffice for their recognition among the parasites with which they might be confounded."

In *R. granosa* this habit would seem to be very generally confined to animals travelling relatively slowly, as they frequently do on being artificially liberated from the host. It was apparently on individuals of *R. ophiocomæ* and *R. linei* obtained in this way that Giard made his observations. Observations on *R. granosa* have been made on individuals which had issued naturally from the hosts ; mostly on females because of their larger size. The males swim more rapidly than the females, for although there is little difference in the length of the cilia in the two sexes, the males are less than half the size of the females.

The females, when normally active, continually take short flights upwards, often touching the bottom of the watch-glass or finger-bowl only to leave it almost immediately : the males less frequently touch the bottom. They turn in all directions, this apparently being due to bending of the body. It is only when their activity becomes much reduced that they travel largely in contact with the substratum, and then nearly always in a straight line. When swimming at speed these Orthonectids mostly, though not invariably, follow a gently spiral path. They may, on occasion, swim almost perpendicularly upwards, and on reaching the surface film (in a watch-glass of water) swim beneath it for a short distance, before diving downwards again. It is particularly when an animal is swimming perpendicularly upwards that it may be observed to follow a spiral path, for the anterior end of the animal is seen to describe tiny circles. *R. granosa* is symmetrical, with apparently no longitudinal differentiation of cilia, but any slight bending of the body out of a straight line—such as might well occur in an animal capable of muscular contraction—would, owing to the rotation of the animal on its own axis, result in a spiral path being followed.

The cilia on the body do not beat directly backwards, but obliquely, and the animal moves forwards, rotating on its own axis to the left. This is actual, and not apparent rotation due to the appearance of metachronal waves. During backwards swimming the animal rotates to the right.

Caullery and Mesnil (1901 c, p. 402) noted that the males of *R. metchnikovi* while in the plasmodia "montrent une assez grande mobilité ; ils tournent sur leur grand axe à la façon d'une toupie." It is also of interest that they

remark of the male, which is globular, measuring  $40\mu$  by  $30\mu$ , with the anterior extremity larger than the posterior, "En raison sans doute de sa forme globuleuse, il n'a pas le mouvement en ligne droite, si général dans le groupe et que nous avons constaté, en particulier, pour la femelle. Il est extrêmement mobile et il décrit des sortes de cercles; il ressemble beaucoup à un Infusoire holotriche." It should be noted that Caullery and his collaborators—in common with previous investigators—in most of their work prior to that recorded in the 1912 paper, apparently used material obtained by opening the hosts.

As in other species of *Rhopalura*, there is in both sexes clear differentiation of the ciliation of the anterior cone from that of the rest of the body, in that when the animal is swimming forwards the cilia on the anterior cone appear to be directed forwards, while those on the rings posterior to it appear to be directed backwards (see Fig. 1, A). Over both regions, however, the effective beat is backwards, in spite of the difference in appearance. The appearance of the cilia on the anterior cone may be due to a restricted amplitude of beat; when an animal is swimming slowly and the beat can be seen, they then certainly appear to beat through a small angle. That the effective beat is backwards over the anterior cone may be observed by the movement of particles caused by the action of these cilia in animals artificially liberated before maturity from their host, and in consequence have lost the rest of the cilia while in sea-water (see p. 244). It is of interest that the cilia on the anterior cone resist disintegration considerably longer than those on the rest of the body, and may frequently be seen intact and active, when the others have been shed. These cilia beating alone appear unable to move the animal. It is possible that they have a sensory function in addition to a locomotory one; their differentiation from those on the rest of the body suggests this.

When the *Orthonectids* are swimming slowly forwards the appearance of the cilia is as shown in Fig. 1, A and C.

The cilia on the anterior cone, and in addition those on the second ring—though the latter during activity appear to be directed posteriorly with the cilia on the posterior rings—can be suddenly applied so closely to the surface that even at a magnification of 500 they appear as a thick, and but slightly striated cuticle (Fig. 1, B); during reversed swimming they were observed always to be motionless. In animals swimming forwards very slowly the cilia on the anterior cone have been observed, on occasions, to be motionless, but it is doubtful whether this occurs under normal conditions.

The cilia on the body posterior to the second ring rarely, if ever, become motionless, but the rate of beat may be much reduced temporarily so that the animal remains almost stationary. Females have been observed to remain for a time practically stationary, but with the body rotating.

This is apparently due to change of direction of beat of the cilia. They have also been seen to stand on end and rotate, being evidently attached posteriorly owing to some viscid property of the cilia in that region.

From observations it seems that the direction of beat of the cilia may be changed, for the Orthonectids are capable of swimming forwards and backwards, though the forward movement is the more usual. Frequently, however, females artificially liberated from their host may continue swimming backwards for the few minutes they live. McIntosh (1873, p. 129), one of the first observers of an Orthonectid, noticed the backward swimming of the parasite he found in *Lineus gesserensis*.

The change in direction of movement from forward to backward swimming is abrupt, the animal giving a sudden dart backwards as the cilia on the anterior cone and second ring are closed down; during backward swimming these cilia have never been observed to beat, and would, therefore, seem to be incapable of reversing the direction of their beat.

In reversed swimming the cilia posterior to the second ring appear to be directed forwards (see Fig. 1, B)—that is in the opposite direction to that obtaining when the animals are swimming forward. Although it has been impossible to make observations on the movement of individual cilia, the change in direction of swimming would seem to be due to reversal of the effective beat of the cilia.

This power of sudden reversal of direction of movement is probably of value to the animal in rapid retreat from danger. On running into an obstacle, they have been observed frequently to swim in the reversed manner for a certain distance, then to turn round by muscular action, and continue the retreat in the same direction, but with the anterior end foremost. This reaction, however, does not invariably occur, for females at least appear to have a tendency to collect round debris, with the anterior end pushed against it, and the body cilia beating, though not at full speed. They frequently rub backwards and forwards against debris, at the same time contracting and expanding the body, and if the posterior extremity should come in contact, they have been observed to become caught—apparently by some viscid secretion or thread—and unable to free themselves, though the cilia, including those on the anterior cone, beat rapidly. The production of this viscid thread may perhaps be abnormal, due to the unnatural conditions of observation; a female on one occasion was seen towing a male by such a thread, though at some considerable distance, and on other occasions females have been seen attached to each other posteriorly by a thread and tugging against each other, and so held stationary. It is just possible, however, that the viscid property of the cilia may play some part in the pairing of the males and females.

Males may frequently be observed continually reversing the direction of swimming, at very short intervals of time (a second or less). A



characteristic movement of the male is that of sharply striking the water with the slender posterior region of the body: it is to this that the name *Rhopalura* refers (see Giard, 1880, p. 231).

I wish to thank the British Association for granting me the use of their table at Plymouth, and the Director and Council of the Marine Biological Association for facilities. For the microphotograph (Fig. 4) I am indebted to Mr. D. P. Wilson.

#### SUMMARY.

A new species of Orthonectid, *Rhopalura granosa* from *Heteranomia squamula* L., is described. The female is fusiform, and about 190 to 230 $\mu$  long, and 55 to 75 $\mu$  broad. The male is also fusiform, but less than half the size of the female, being only 87 to 95 $\mu$  long and 20 $\mu$  broad. *R. granosa* is distinguished from all other known species of *Rhopalura* by the presence in the male of large refringent bodies in the anterior cone, and in the first row of cells of the second ring. The male of *R. ophiocoma* Giard, a species closely allied to *R. granosa*, has the refringent bodies in the cells of the second ring only.

The infected *Heteranomia* have been obtained from *Lepralia foliacea* trawled and dredged from off Revelstoke and Stoke Points. In *Heteranomia* examined direct from the grounds the infection in February, 1933, varied from about 6.6% to 16%, but in a number taken from *Lepralia* from the Plymouth Aquarium in November, 1932, the percentage of infection was as high as 45.4%.

The parasite is found replacing the gonad of the host; it also occurs in the blood lacunæ and vessels in the mantle, and in the suspensory membranes of the gills, even extending into the dorsal ends of the filaments. In heavy infections the Orthonectid may entirely replace the gonad of the host.

Male and female containing plasmodia usually occur in separate hosts, but it is not uncommon for the two sexes to be found together. From sections it would seem that males and females may on occasion be produced in the same plasmodium.

The swimming behaviour of *R. granosa* is described.

#### APPENDIX.

##### OTHER ORGANISMS OBSERVED IN *Heteranomia squamula*.

Few parasites and commensals, other than *Rhopalura*, were noticed in some four to five hundred *Heteranomia* examined. Those seen were:—

- (1) Rounded masses, ca. 60 to 80 $\mu$  in diameter, of tiny spores in the gonad of two individuals.

- (2) A Coccidian in the kidney. The number of *Heteranomia* infected with this parasite was not noted.
- (3) A Rhabdocœle from each of six individuals of the seventy-three taken from the carapace of a single *Maia* (see p. 245). This Rhabdocœle almost certainly was not *Graffilla gemellipara* Linton, which is common in the mantle cavity and in the gut of *Cardium edule* from the Yealm Estuary and Millbrook. Although the specimens were large they did not contain viviparous young as is characteristic of *G. gemellipara*, and were more opaque than that species: they were broadly rounded anteriorly and pointed posteriorly. In addition to these six specimens another two or three have been seen at different times.
- (4) A few larval Trematodes were seen in two or three *Heteranomia* taken from the *Maia* mentioned previously.
- (5) In a number of *Heteranomia* a ciliate, possibly a species of *Boveria*, was present on the mantle.

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