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BY

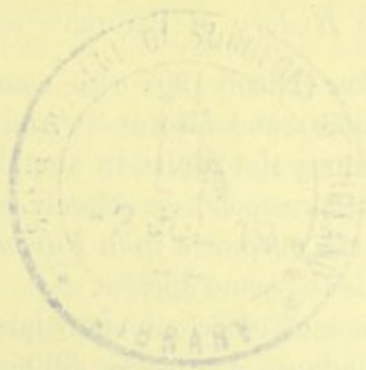
MARIE V. LEBOUR, M.Sc.,

Assistant Lecturer and Demonstrator in Zoology, Leeds University.

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SOME LARVAL TREMATODES FROM MILLPORT.

By MARIE V. LEBOUR, M.Sc.,

Assistant Lecturer and Demonstrator in Zoology, Leeds University.

(With Plate I.)

INTRODUCTION.

THE following work was done chiefly on material collected during a month's stay at Millport in August 1913 whilst working at the Marine Station. I have to thank Mr Elmhirst for much help in obtaining material and also for giving me the first specimens of the larval *Maritrema* encysted in *Ligia oceanica* which he discovered in the genital ducts of that Isopod. This is specially interesting as it is the first undoubted *Maritrema* larva known.

For the first specimens of the encysted Trematode from the Haddock's skin I am indebted to Dr Jas. Johnstone of Liverpool. From further material obtained at Millport this was found to be a larval *Opisthorchis*, again the first step towards the life history of any species of the genus as yet described¹.

In finding the young stages of *Parorchis acanthus* Nicoll, in the cloaca of the Herring Gull, together with the adults, it has been possible to identify the first larval stage as *Cercaria purpuræ* sp. inq. (Lebour 1912) from *Purpura lapillus*, a specially interesting fact as it bears out the view that the genus *Parorchis* is closely allied to *Echinostomum*.

¹ Lühe, *Die Süßwasserfauna Deutschlands*, Heft 17, Trematodes, states that the young form of *O. felineus* (Riv.) encysts in the muscles of *Leuciscus idus* (L.) and *L. rutilus* (L.) undescribed at that time (1909).

The Life History of Parorchis acanthus Nicoll.

Parorchis acanthus (Nicoll 1907 a) is common in the cloaca of the Herring Gull at Millport and also at St Andrews where Nicoll got his type specimens. Among the adults in the Millport Gulls were several young forms which correspond so closely with the cercariae which I have named *Cercaria purpurae* from *Purpura lapillus* that there can be no doubt that it is the same species.

This cercaria is one of our commonest larval Trematodes and occurs in extraordinary abundance completely filling the spine of the mollusc when present and giving a curious swollen pale yellowish appearance to the digestive gland, so that it can at once be recognised. Some years ago Dr Nicoll told me he thought that this was the larva of *Parorchis acanthus* but it has not been possible to prove this until now. The intermediate host is still unknown in spite of constant searchings on my part in the animals living side by side with *Purpura*. I have found these cercariae in *Purpura* in the following localities: Fenham Flats, Budle Bay and Cullercoats in Northumberland (Fenham Flats being muddy ground with few rocks, Budle Bay rocky surrounded by sand, Cullercoats very rocky), Robin Hood's Bay in Yorkshire (rocky), the shores of Loch Ryan on the west coast of Scotland (muddy and sandy ground with stones) and on the coast round about Millport in Cumbrae (rocks surrounded by muddy sand). It occurs therefore in various habitats. The Herring Gull (*Larus argentatus*) is a constant feeder in all these places.

The animals almost always to be found with *Purpura* are Sea Anemones (*Actinia mesembryanthemum*), Green Crabs (*Carcinus moenas*), Rock Barnacles (*Balanus balanoides*), Limpets (*Patella vulgata*), Periwinkles (*Littorina littorea*, also frequently *L. rudis* and *L. obturata*) and very often Mussels (*Mytilus edulis* and *Modiola modiola*). All these I have examined for the encysted stages but with no success. I have also examined various worms from the mud or sand below the rocks and *Mya arenaria* from Millport and *Saxicava rugosa* from Robin Hood's Bay, all without success. The cercaria has a powerful tail which points to its leaving its first host and swimming to its second so that it seems unlikely that it ensysts in the *Purpura* or in an animal that feeds on *Purpura*. I should expect to find it in some animal living near and most probably in a mollusc as the cercaria closely resembles an *Echinostomum* cercaria, and those members of this genus whose complete life histories are known encyst in molluscs (*E. secundum* in *Mytilus edulis*,

E. leptosomum in *Scrobicularia tenuis*). The cercaria is developed in long rediae very like those of *Echinostomum* and its general form, suckers, alimentary canal, excretory system, tail and head spines (although the latter are somewhat different in form) remind us of this group. The excretory vesicle however does not extend into the tail, which is solid except for a sucker-like structure at the hinder end (Lebour 1912, pp. 446-7, Figs. 1-3, Plate XXIX). From these notes there seems to exist a close relation between *Parorchis* and the *Echinostomum* group, as is pointed out by Odhner who regards the genus as an aberrant form of an Echinostomid (Odhner 1913).

The length of a full-sized cercaria before it emerges from its first host is 0.64 mm. It has, when of this size, large spines on its body and spines round its head. It may be found, when full grown, either free in the digestive gland having come out of the redia, or in the mantle cavity near the gill, having migrated from the digestive gland on its way out of the body. This is as far as I can follow it until we find it again in the cloaca of the Herring Gull. The smallest worm from the cloaca measured 2.4 mm. (Plate I, fig. 1), so that a large increase in growth has taken place. The form of the body is identical, the body covered with thick spines dwindling towards the posterior end and a crown of spines forming a sort of collar interrupted ventrally by the oral sucker. The alimentary canal has the same form and likewise the excretory vesicle with its lateral branches, which however are much more conspicuous and complicated than in the tailed cercaria. The suckers have both increased in size especially the ventral sucker. In the smallest the oral sucker measured 0.16 mm. and the ventral 0.40 mm. (oral sucker and ventral sucker of tailed cercaria from *Purpura* 0.06 mm. and 0.09 mm. respectively). In larger forms the increase is greater. In specimens 2.8 mm. long the oral sucker was 0.32 mm. and ventral sucker 0.60 mm. across. Size in mature worm, oral sucker 0.50 mm., ventral sucker usually more than twice as great (Nicoll).

It is interesting to find this intermediate form as it indicates the first stage in the life history of *Parorchis acanthus* and also shows that at any rate in its early life it is exceedingly like *Echinostomum*. I have included it in this group when trying to classify the cercariae and these young stages help greatly in the elucidation of the relationships and proper systematic position of the adults.

A Larval Maritrema encysted in Ligia oceanica.

Specimens of *Ligia oceanica* were sent to me in October 1912 by Mr Elmhirst from Millport in which species he had found an encysted Trematode occurring abundantly in the genital ducts of both male and female. The *Ligiae* live just above high water mark in the tidal drift below the laboratory, generally under stones.

In both oviducts and vasa deferentia the cysts were scattered about, sometimes also occurring in the other organs, more particularly in the muscles. The cysts were either colourless or a bright yellowish brown, the latter probably being older, and inside each cyst was curled up a larval Trematode which undoubtedly is a species of the genus *Maritrema* Nicoll.

The cysts (Plate I, fig. 4) are oval and measure 0.36–0.45 mm. by 0.24–0.32 mm. and with a thick cell wall measuring 0.02 mm. in thickness. Each cyst has very much the appearance of a Trematode egg. I have called this worm *Cercaria ligiae* sp. inq.

Cercaria ligiae. Inside the cyst the curled-up larva is visible through the transparent wall. The oral and ventral suckers, alimentary canal and excretory vesicle are easily made out. The head and posterior end are folded in towards each other in the cyst. During the encysted stage the excretory organs of the posterior end have a peculiar structure. The vesicle is much dilated and full of conspicuously refractive granules. If by any chance the cyst is ruptured accidentally these granules always come out quickly from the excretory pore which is at the extreme posterior end. The granules are irregular in shape and perfectly clear.

The most successful way to get the worm out of the cyst is to leave it in normal salt solution over night. The larval worm will have emerged by the next morning and will be still living and can be examined alive. The refractive granules of the excretory system however will have all disappeared.

The whole body (Plate I, figs. 2 and 3), is quite colourless and transparent, very much flattened dorso-ventrally and with the sides having a tendency to fold over ventrally. The first two-thirds are covered with small spines and scattered about at much greater intervals are conspicuous unicellular glands. It is more or less tongue-shaped with the anterior end slightly more pointed than the posterior and usually narrow in the middle region. When contracted it is more oval. The length of the worm is 0.60 mm.–0.66 mm. and the breadth

0.28 mm.–0.32 mm., the greatest breadth usually being at the beginning of the posterior third of the body. The oral sucker is smaller than the ventral, measuring 0.04 mm.–0.06 mm. across, the ventral sucker measuring 0.06 mm.–0.08 mm. Both are circular in outline. The oral sucker is situated at the extreme anterior end of the body and leads by a circular aperture to the prepharynx which may be hardly recognisable when contracted but may measure as much as 0.04 mm. in length. The pharynx is large and muscular and measures 0.03 mm. in length and is rather longer than broad. The oesophagus is slightly longer than the pharynx and forks at a distance of 0.06 mm. in front of the ventral sucker. The intestinal caeca reach to the level of the posterior end of the ventral sucker. The ventral sucker is situated some way in front of the centre of the body and has a circular aperture.

The excretory system consists of a posterior vesicle which is Y shaped and gives off lateral ducts, the flame cells at the ends of the smaller branches being very conspicuous. The reproductive organs are far advanced in development. The testes lie just behind the ventral sucker and are large, lobed organs of a roundish form. Each measures about 0.13 mm. in diameter. Ducts arise from these but could not be followed; they spring from the anterior margins slightly to the inner side. The cirrus pouch lies in front of the ventral sucker and is well developed although the genital pore is not open and apparently the right end is not yet joined by the vasa deferentia. It is full of large nucleated cells and the vesicula seminalis is not formed. The ovary is conspicuous and lies on the right of the ventral sucker, none of it extending behind. It is roughly three-lobed and gives off a winding duct which loops behind the ventral sucker and then runs upwards, curving as it goes and enlarging to reach the left side of the cirrus pouch. The shell-gland complex could not be made out and probably is not completely developed. The vitellaria are of the typical *Maritrema* form and make a nearly complete wreath round the region behind the ventral sucker, running round the lateral margins and the posterior margin and then bending inwards in front of the testes and nearly joining, but leaving a small gap anteriorly. No vitellarian receptacle is as yet formed. The vitellaria consist of small rosettes of yolk material.

Hitherto no larval *Maritrema* has been known for certain, although *Cercaria oocysta* sp. inq. and *Cercaria pirum* sp. inq., both from *Paludetrina stagnalis*, seem to belong to this genus or to one closely allied (Lebour 1912 and Nicoll 1907 b). My suggestion that the habit of encysting in sporocysts (as these cercariae do) may distinguish this

genus from *Spelotrema*, has turned out to be erroneous as we see this present form encysting in a Crustacean.

So far the first larval stage of this species has not been found although much search has been made. The first host must almost certainly be a mollusc and the only mollusc living with *Ligia* below the laboratory at Millport is *Littorina rudis*. I have examined a great number of these but have not found a cercaria which certainly corresponds with this *Maritrema*. The *Littorinae* live under the same stones with *Ligia* as well as in small shallow pools and on the rocks close by, and also extend further down into the *Pelvetia* zone and even into the zone of *Fucus vesiculosus*. The *Littorina rudis* from all these parts contained several larval Trematodes the most frequent being the sporocysts and cercariae of what I believe to be *Spelotrema excellens* Nicoll, which are perhaps identical with the *Cercaria brevicauda* of Pelseneer from the same mollusc (Pelseneer 1906). This cercaria encysts in the Green Crab (*Carcinus moenas*), which does not live so high up as *Ligia* but does live with *Littorina rudis* lower down. I tried to find differences in these sporocysts and cercariae from the *Littorinae* living with *Ligia* but failed.

Three other species of cercariae were found in *Littorina rudis* living with *Ligia*:—*Cercaria linearis* Lespés. This occurred in one specimen together with the sporocysts of *Spelotrema excellens*. The cercariae are contained in sporocysts in the kidney whilst those of *S. excellens* are in the digestive gland. This is a new host for this species as hitherto it has only been known from the common Periwinkle *Littorina littorea* (Lespés 1857 and Lebour 1912).

Of the other two, one is a *Monostomum* cercaria in rediae, probably new, and the other is apparently identical with *Cercaria emascuans* Pelseneer (1906).

Cercaria emascuans Pelseneer. This cercaria occurred in sporocysts in the genital gland of *Littorina rudis* living with *Ligia* and also in that of *L. littorea* from Balloch Bay, Millport. It occurred more frequently in *L. rudis* which is the original host for it, *L. littorea* being new. The sporocysts are very like those of *Spelotrema excellens* and in many ways the cercaria resembles a *Spelotrema* cercaria. This made me think it might be the first stage of the *Maritrema*. The shape and size of the excretory vesicle however prevent my being in any way certain of this, although it is conceivable that the worm may grow very much larger without a proportionate growth in the excretory system. Some infection experiments were tried with this larval form and some *Ligiae* from

Plymouth which have not got this parasite, but these were unsuccessful.

The genital gland of *Littorina rudis*, when this cercaria is present, is white and very much swollen; in *L. littorea* it is orange-coloured although the colour only affects parts of the sporocyst and the tissues surrounding them, not the cercariae which are colourless.

The sporocysts (fig. 6) are long and thin-walled, 0.40 mm.–0.80 mm. long and 0.20 mm.–0.35 mm. broad. All were full of cercariae in various stages of development.

The cercaria (figs. 5, 6) is extremely active with a long tail which is continually in motion and the whole worm is so constantly changing its shape that its structure is very difficult to see when alive. The body measures 0.38 mm. in length without the tail, but can be much shorter or longer when contracted or expanded. The tail is nearly the length of the body. The breadth of the body is about a third of the length. The whole surface is covered with minute spines dwindling posteriorly. The oral sucker measures 0.03 mm. across and is circular with a circular aperture. On its dorsal surface is a single straight stylet measuring in length not quite half the width of the sucker. The ventral sucker is peculiarly prominent protruding as a great bulb from the ventral surface. It is situated almost exactly in the centre of the body and is the same size as the oral sucker or slightly larger. The aperture which is round is set at an oblique angle to the body.

The oral sucker leads to a somewhat obscure alimentary canal with no apparent pharynx (Pelseneer however shows a pharynx in his figure so my specimens may be younger than his). In some the intestinal caeca could be made out, with the fork well in front of the ventral sucker and the caeca reaching to just behind the level of the ventral sucker and slightly swollen at the ends, reminding one very much of the intestine of the encysted *Maritrema*.

The excretory vesicle is Y shaped, the forks reaching to the level of the anterior margin of the ventral sucker. The tail is very transparent and extremely contractile with striations both transverse and longitudinal, the transverse being the more conspicuous.

I think it is possible that this cercaria may prove to be the first stage of the *Maritrema* from *Ligia* but this is as yet not at all certain.

With regard to the adult stage of this *Maritrema* it does not agree exactly with any known species and is probably a new one.

The species of *Maritrema* all inhabit birds and Nicoll (1907) describes three British species occurring in the Dunlin (*Pelidna alpina*),

Ringed Sand Plover (*Aegialites hiaticula*), Black-Headed Gull (*Larus ridibundus*), Herring Gull (*Larus argentatus*), Oyster Catcher (*Haemalopus ostralegus*), and Redshank (*Totanus calidris*). Of these the only one which has the ventral sucker greater than the oral is *Maritrema humile* from the Redshank but in other ways this does not agree with our species.

Jägerskiöld (1908) gives a summary of the characters of *Maritrema* and divides up the species of the genus into groups, also describing some new species. Of these his *Maritrema subdolum* from the Common Sandpiper (*Actitis hypoleucos*) comes very near the present species, although I do not think they are identical.

At Millport I have examined many birds for the adult worms but as yet without success.

A larval Opisthorchis encysted in the skin of Gadoids.

In September 1912 Dr Jas. Johnstone kindly sent me pieces of a Haddock (*Gadus aeglefinus*) the skin of which was spotted at frequent intervals with black, each spot marking the presence of an encysted larval Trematode. Owing perhaps to the preservative it was difficult to make out the structure of these worms, the cyst being extremely tough and difficult to break through. Enough was seen however to show that it was a Distomid. Gamble and Drew (1912) noticed the same appearance in a Whiting (*Gadus merlangus*) but in this the parasite was identified by them as probably a species of *Holostomum*. It is evident that many Trematodes cause the segregation of pigment granules in the neighbourhood of the cysts. Nicoll (1907 c) found an encysted Trematode in a *Cottus bubalis* also with black pigment. Williamson (1911 a and b) notices the same thing in the "Spotted Haddock" but believes his specimens to be *Gasterostomum gracilescens*, i.e. the same species that inhabits the nerves of that fish. There are also several examples from American fish, notably the Trout (Linton 1911, p. 252).

It was not until this summer (1913) that I managed to study a Trematode in the skin of a Lythe from Millport which evidently belongs to the same species as those in the Haddock sent to me by Dr Johnstone. Lythe (*Gadus pollachnis*) and Saithe (*Gadus virens*) are frequently spotted in this way at Millport and they come quite close to the shore so long as the water is fairly deep. In Jan. 1914, Mr Elmhirst sent me portions of the skin of a Cod with similar spots which contain the same worm.

The fish in question was spotted at infrequent intervals dorsally, some spots being below the lateral line and more above it, many occurring near and on the fins. A fair number of cysts were found in the muscles below the skin and even here there was a small amount of pigment present. The fish was quite healthy and seemed not at all inconvenienced by the cysts. The skin was quite clean and to a casual observer the spots might have seemed natural to the fish.

From its habit of attracting pigment I propose for this worm the name *Cercaria chromatophila*.

Cercaria chromatophila sp. inq. The cyst is oval and measures 0.36 mm. \times 0.26 mm. which is very much the same size as Williamson's cysts (1911). Round it is deposited the blackish-brown pigment either completely covering it or leaving a small area on the outside uncovered. The pigment is arranged in closely aggregated star-like masses close together near the cyst and gradually dwindling as they get farther away from it (Plate I, fig. 9). A fibrous covering containing the pigment can be removed from the cyst by careful manipulation with needles and the worm is then seen coiled up inside. The cyst is formed of two coats both colourless and transparent, the first thick and extremely tough and elastic, the second, immediately internal to it and closely pressed to its walls, is very thin and easily ruptured. Great difficulty was experienced in opening the cyst without damaging the worm; the most successful method was found to be to soak the cyst in normal salt solution for a day and then under a dissecting microscope prick it with a fine needle. By this means it was possible to get the worm to emerge still alive (figs. 7-8).

Fully extended it measures 0.70 mm. in length, the greatest breadth being 0.24 mm. at the anterior third of the body which is pointed at each end but more blunt anteriorly, colourless and transparent.

It is covered completely with small spines which dwindle posteriorly although they do not entirely disappear.

The oral sucker is circular with a circular aperture at the extreme anterior end. It measures 0.06 mm. across, the aperture being 0.03 mm. across. A very small prepharynx 0.01 mm. in length leads to a broad and muscular pharynx 0.03 mm. long and this leads to the oesophagus 0.03 mm. long which branches into two long caeca which are narrow and bend inwards in front of the ventral sucker, curve outwards again and inwards with the tapering of the body and end at the extreme posterior end. Inside the intestine are numerous colourless refractive granules. The ventral sucker is situated very far back at the junction between

the middle third and posterior third of the body, and 0.23 mm. from the intestinal fork. It measures 0.04 mm. across. Just in front of it the oviduct runs up for about 0.02 mm. beyond it.

The excretory vesicle is Y shaped, opening at the posterior end of the body. The stalk measures 0.11 mm. in length, each fork measuring 0.05 mm. The vesicle also contains a refractive substance which is very conspicuous in the encysted worm.

The testes are oval and occur obliquely one on each side of the excretory vesicle, the left in front of the right. Each measures 0.04 mm. \times 0.02 mm.

It is evident from the arrangement of the organs, especially the excretory vesicle and the testes, that this is a species of *Opisthorchis* as, so far as the anatomy can be made out, it agrees very well with the diagnosis of that genus (Looss 1899, p. 564), although it is described as without spines except in one species. It is interesting to note that the encysted worm found by Nicoll in the eyes etc. of *Cottus*, although not fully described, agrees in all the points he mentions with this worm.

The final host will in all probability be found to be a fish-eating bird. The group to which *Opisthorchis* belongs includes forms from mammals, birds and fishes, chiefly birds. Odhner (1912) describes an *Opisthorchis* from the gall bladder of the Electric Eel (*Gymnarchus niloticus*). Braun (1902) describes one from a Heron (*Ardea stellaris*) from Brazil which is much like the present worm. So far as I am aware no adult *Opisthorchis* has as yet been recorded from Britain and this is probably a new species. It seems to be the first record of a larval form of this genus and is interesting on that account.

DESCRIPTION OF PLATE I.

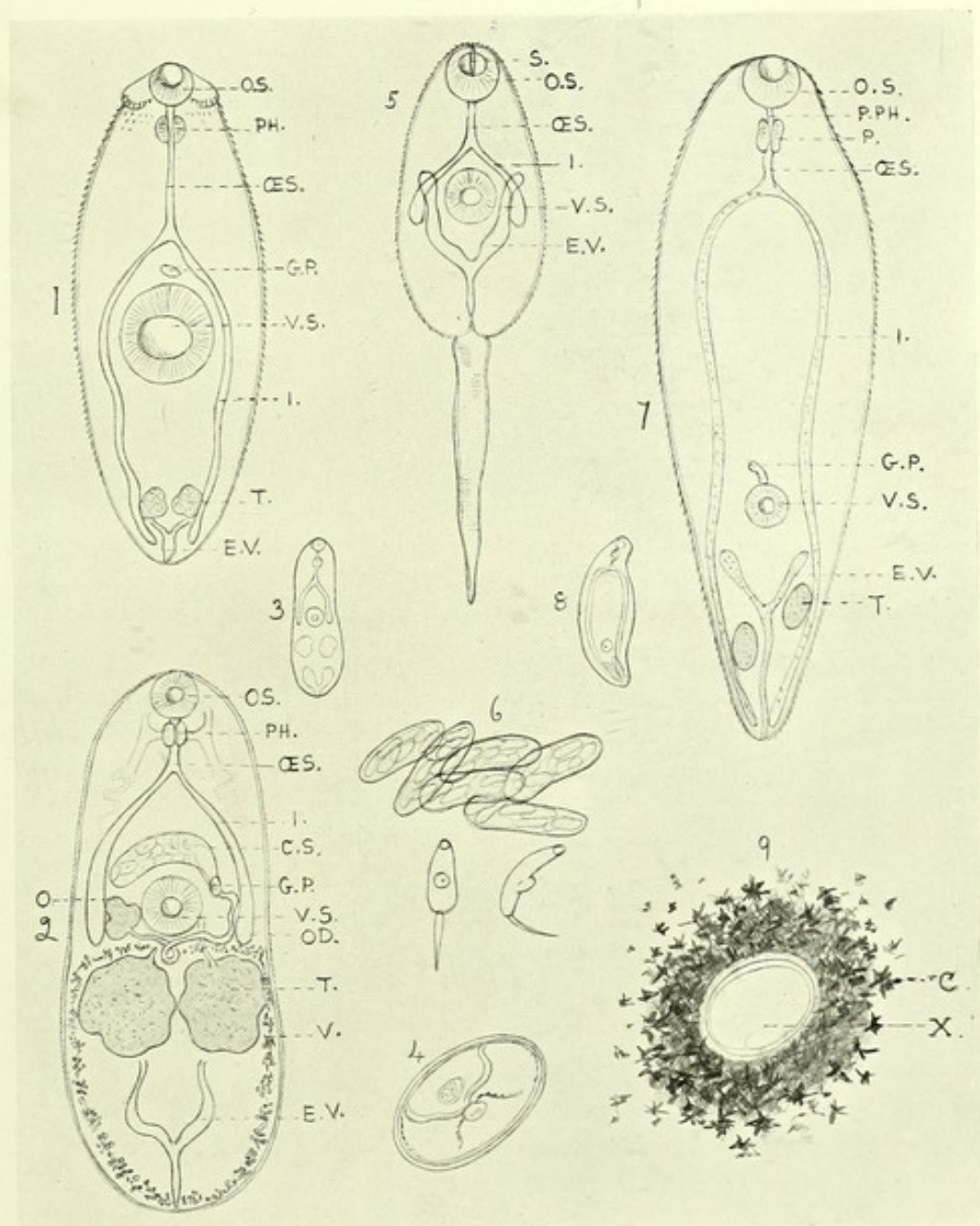
The following letters apply to all the figures :

C. pigment	O. ovary
C.S. cirrus sac	P. pharynx
E.V. excretory vesicle	PPH. prepharynx
G.P. genital pore	T. testis
I. intestine	V. vitellaria
O.S. oral sucker	V.S. ventral sucker
ŒS. oesophagus	X. worm in cyst
OD. oviduct	

Figs. 1, 3, 6 and 8 are on the same scale.

Fig. 1. Young stage of *Parorchis acanthus* Nicoll, from cloaca of Herring Gull. Size 2.4 mm.

Fig. 2. *Cercaria ligiae* sp. inq., larva pressed out of cyst. Size 0.65 mm.



M. V. Lebour, del.



- Fig. 3. The same under low power.
 Fig. 4. The same encysted. Size 0.36 mm. \times 0.24 mm.
 Fig. 5. *Cercaria emascuans* Pelseneer. Size 0.38 mm. without tail.
 Fig. 6. The same showing sporocysts.
 Fig. 7. *Cercaria chromatophila* sp. inq. Size 0.70 mm.
 Fig. 8. The same under low pressure.
 Fig. 9. The same in cyst with surrounding pigment. Size of cyst 0.36 mm. \times 0.26 mm.

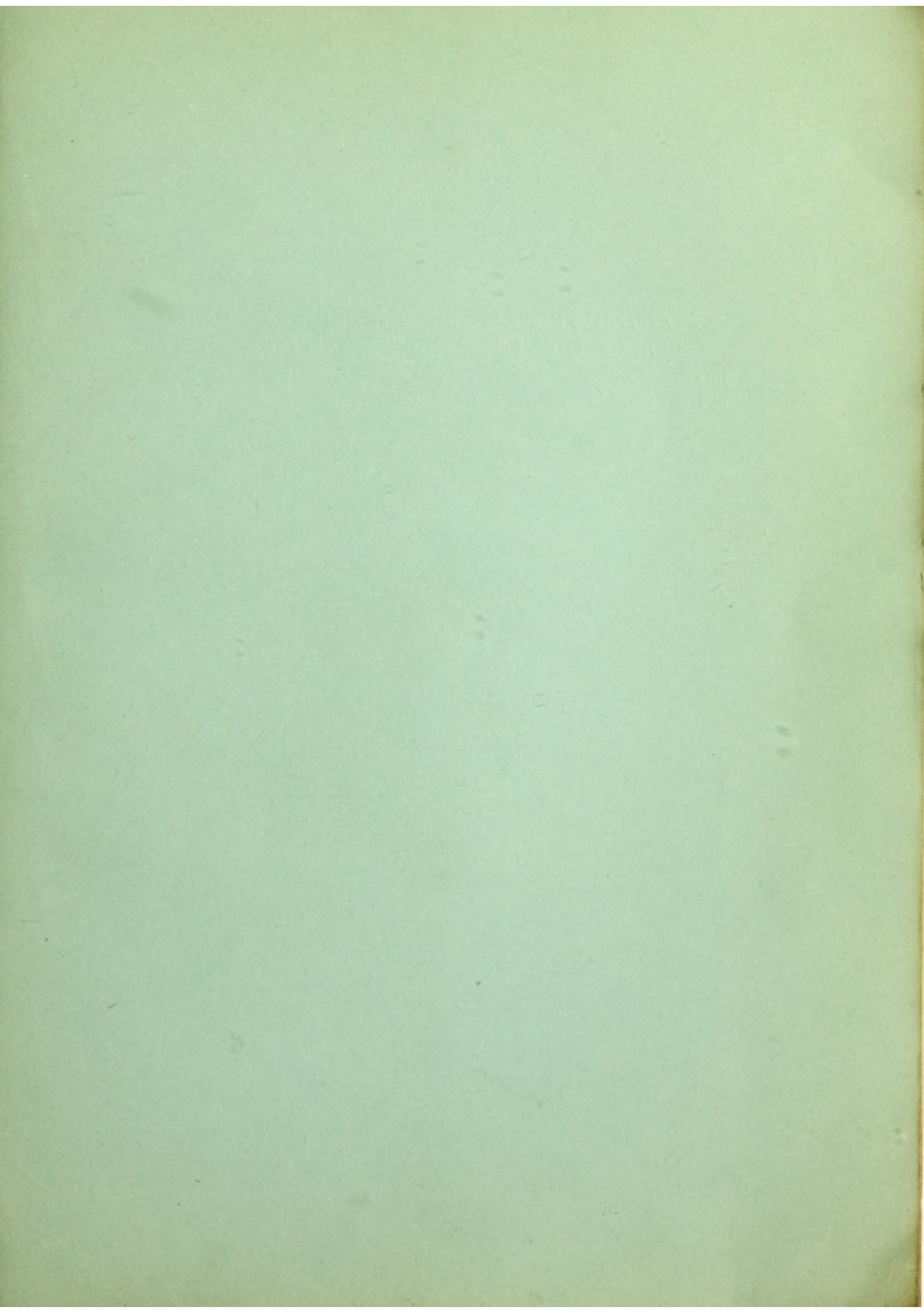
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