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AN ADULT DIGENETIC TREMATODE FROM AN INVERTEBRATE HOST: *PROCTOECES SUBTENUIS* (LINTON) FROM THE LAMELLIBRANCH *SCROBICULARIA PLANA* (DA COSTA)

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(Plates I and II and Text-figs. 1 and 2)

Adult digenetic trematodes are typically parasites of vertebrates. Sexually mature trematodes occurring in invertebrate hosts are usually regarded as precocious last-larval stages, to which the term 'progenetic' is applied. These progenetic forms are often encysted, and can usually only contribute to the further life history of the species, and, in many cases, can only attain the definitive form of the adult trematode, after transference to a vertebrate host.

Specimens of the mud-burrowing lamellibranch *Scrobicularia plana*, collected from the region of the Thames estuary, were found to be infected with unencysted, sexually mature trematodes. These parasites occurred in the kidneys of the molluscs, and, as will be discussed later, there seems no reason to regard them as other than adult digenetic trematodes. They were identified as specimens of *Proctoeces subtenuis* (Linton, 1907) Hanson, 1950. This species has been recorded, hitherto, only as a parasite of the hind-gut of marine fishes belonging to the families Labridae and Sparidae, from the Red Sea, New Zealand, and the eastern seaboard of America.

In this paper an account is given of the occurrence, distribution, environment, morphology, and taxonomy of *Proctoeces subtenuis* from *Scrobicularia plana*, and the possible life history of this trematode in the Thames estuary is discussed with reference to the phenomenon of progenesis.

OCCURRENCE AND DISTRIBUTION

Specimens of *Proctoeces subtenuis* in the kidney of *Scrobicularia plana* were first observed in 1954 in animals collected from the mud flats at Chalkwell in Essex. During the subsequent three years nearly a thousand specimens of *S. plana* from this location were examined. Every single specimen was

infected. The degree of infection, estimated from examination of fifty-one hosts, varied from one to thirteen parasites per host, with an average of between four and five. There appeared to be no correlation between degree of infection and size of host.

The distribution of *S. plana* at Chalkwell has already been discussed by Freeman & Rigler (1957). The mollusc is plentiful at mid-tide level, but absent from the sandy deposits lower down the shore. It lies in a burrow 6–10 in. below the surface of the mud, maintaining contact with the overlying water through long, extensible siphons. Spooner & Moore (1940) record population densities of *S. plana* of up to 1000 per m^2 in the Tamar estuary at Plymouth, and Green (1957) gives 500–1025 per m^2 as the population density in the Gwendraeth estuary in South Wales. The impression derived from collecting at Chalkwell on numerous occasions is that similar population densities occur there also. No attempt has been made to delimit the occurrence of the parasite along the Thames estuary to the east and west of Chalkwell, but infected specimens of *S. plana* have been collected from stations over a distance of about a mile of the mud flats in the neighbourhood of Chalkwell. The total population of *S. plana* in this area must number many millions, and the evidence is consistent with the view that all of them are infected with the trematode. The only occurrence from a locality other than Chalkwell was at Whitstable in Kent where three specimens of *S. plana* of about 150 examined were infected. Specimens of *S. plana* have been examined from the Rivers Tamar and Tavy (*ca.* 80), the Gwendraeth (*ca.* 100), the Rivers Dart and Teign in south Devon (*ca.* 40), the Butley river in Suffolk (7), the Essex rivers Blackwater (*ca.* 50) and Colne (7), and Conway in North Wales (*ca.* 100), but no trematodes have been found. Specimens of the lamellibranchs *Macoma balthica* (L.) and *Mya arenaria* L., which occur alongside *Scrobicularia plana* in the mud flats at Chalkwell, have also been examined, but were found to be uninfected.

In addition to living specimens of *Proctoeces subtenuis*, the kidney of infected *Scrobicularia plana* was observed often to contain dead parasites. These varied in colour between light brown and almost black, and also varied in texture, the darker specimens being harder and even stone-like. These dead specimens clearly showed the external form of *Proctoeces subtenuis*, the oral and ventral suckers being evident, and, in some cases, the cirrus was extruded (Pl. I, fig. 1). The dead specimens occurred in about one in ten of all infected *Scrobicularia plana* examined, and as many as six were found in a single host. They always occurred together with living trematodes in the same host. It is known that some nematode parasites become calcified after death (von Brand, 1952), and a phenomenon comparable to that described above has been reported for another trematode by Macfarlane (1939).

ENVIRONMENT OF THE PARASITE

Proctoeces subtenuis has previously been reported as a parasite of the hind-gut of certain marine fishes. The present work is concerned with the occurrence of this trematode in the kidney of an estuarine lamellibranch. It is very probable that the conditions of the immediate external environment of the parasite in such different situations, in such different hosts, are widely dissimilar. A brief account will therefore be given of some aspects of the environmental conditions of a parasite within the lamellibranch kidney, with particular reference to the kidney of *Scrobicularia plana*.

The lamellibranch kidney consists essentially of a proximal, glandular, ciliated tubule (the organ of Bojanus) and a ureter which conveys the excretory products to the supra-branchial space, and thence to the outside world. The organ of Bojanus receives fluid through a ciliated coelomostome from the pericardium. This fluid is derived from the blood by ultra-filtration through the heart wall. The tubular filtrate is isotonic with the blood, but differs slightly from blood in its ionic composition (Robertson, 1949). Modification of the filtrate occurs in the tubule by the addition of nitrogenous excretory compounds, of which urea and the amino acids taurine and creatine have been identified in twenty-two lamellibranchs (Letellier, quoted by Delaunay, 1927). In the freshwater mussel, *Anodonta cygnea*, as much as 91·6% of the total nitrogen in the tubular fluid can be protein nitrogen, and this can represent a concentration of eight times the blood plasma protein nitrogen level (Florkin & Duchateau, 1948).

The infected population of *Scrobicularia plana* at Chalkwell is exposed to considerable seasonal variations in the tonicity of the overlying water, and these variations are accompanied by corresponding changes in the tonicity of the blood (Freeman & Rigler, 1957). Since the tubular filtrate in other lamellibranchs has been shown to be isotonic with the blood, it seems likely that the osmotic pressure of the tubular fluid will vary with changes in external salinity. This was examined by equilibrating specimens of *S. plana* to sea water and to 70% sea water, and, using the method described by Freeman & Rigler, comparing the depression of the freezing-point of fluid drawn directly from the organ of Bojanus with that of the external medium. No significant difference was found between the osmotic pressure of the tubular fluid and that of the external medium.

It seems certain, therefore, that the trematodes are exposed to an environment which is variable in osmotic pressure, and, in this respect, they resemble free-living estuarine turbellarians (Krogh, 1939) rather than adult trematode parasites in vertebrate hosts. It also seems possible that the fluid in which the trematodes live in the kidney of *S. plana* has an electrolyte composition similar to that of sea water, but has a high concentration of proteins and/or amino acids.

DESCRIPTION OF THE PARASITE

Size and shape.

Proctoeces subtenuis is generally cylindrical, but tapers at each end. The mouth is subterminal, and the ventral sucker is situated at about one-third of the total body length from the anterior end. The integument is without spines. Although living specimens are cylindrical, the general practice in studies of the Digenea of illustrating a well-flattened specimen has been followed here (Text-fig. 1). Some measurements, in mm, of ten such specimens, randomly chosen from a collection of permanent preparations, are given in Table 1. The first figure given is an arithmetic mean for the sample, and the figures in parentheses indicate the range for the sample. The egg capsule measurements were made on samples of five eggs from each of twenty adults, i.e. 100 eggs in all.

TABLE 1. MEASUREMENTS OF *PROCTOECES SUBTENUIS*

See text	Millimetres
Length	2·92 (1·52 – 4·80)
Maximum width	0·96 (0·56 – 1·40)
Oral sucker length	0·20 (0·14 – 0·32)
Oral sucker width	0·21 (0·14 – 0·30)
Prepharynx length*	0·03 (0·01 – 0·05)
Pharynx length	0·15 (0·10 – 0·19)
Pharynx width	0·15 (0·12 – 0·25)
Oesophagus length	0·22 (0·01 – 0·45)
Ventral sucker length	0·34 (0·24 – 0·57)
Ventral sucker width	0·39 (0·26 – 0·61)
Anterior end of body to anterior border of ventral sucker	0·90 (0·40 – 1·40)
Cirrus sac length	0·54 (0·42 – 0·70)
Cirrus sac maximum width	0·13 (0·10 – 0·20)
Anterior testis diameter	0·19 (0·10 – 0·28)
Posterior testis diameter	0·21 (0·12 – 0·32)
Germarium diameter	0·16 (0·05 – 0·27)
Egg capsule length	0·042 (0·026 – 0·073)
Egg capsule width	0·024 (0·015 – 0·030)

* Visible in only seven of the ten specimens in the sample.

When freshly collected specimens of *P. subtenuis* were transferred to dishes of sea water, they became quite active, but the muscular contraction and stretching was confined almost entirely to the region of the body anterior to the ventral sucker (Pl. 1, figs. 2A, B). In contracted specimens the genital pore was situated approximately midway between the oral and ventral suckers, but in stretched specimens it was that region anterior to the genital pore that was most elongated, so that the genital pore came to lie relatively nearer to the ventral sucker. This variation assumes special significance when it is considered that the position of the genital pore has sometimes been used as a diagnostic character of some digeneans.

Colour

Perhaps the most immediately noticeable feature of the specimens of *P. subtenuis* from the kidney of *Scrobicularia plana* is that they are red or pink. This coloration is not obviously restricted to any particular organ-system of the trematode but is generally distributed. The intensity of the colour varies with the size of the animal; even specimens about 1 mm long are noticeably pink, and large (3–4 mm) specimens are distinctly red. As is shown in Table 4, *Proctoeces* species have previously been described from the hind-gut of fishes on fourteen occasions, and in none of these descriptions was it observed that the specimens were red in colour. The name *Proctoeces erythraeus*, given by Odhner (1911) to a species from the Red Sea, is apparently a reference to the geographical location, rather than to the colour, of this trematode (cf. *Tomopteris erythraea* Caroli, 1928).

Red coloration in several other trematodes is known to be due to the presence of haem pigments (von Brand, 1952). Haem pigments react with certain nitrogenous compounds to form the appropriate haemochromogen, the absorption bands of which can be recognized by spectroscopic examination. Observations were made with a microspectroscope on specimens of *Proctoeces subtenuis* mounted in sea water in an attempt to characterize the pigment. No absorption bands could be recognized on examination of untreated specimens, but, after addition of Takayama's fluid (Hawk, Oser & Summerson, 1954), the characteristic absorption bands of pyridine haemochromogen were readily observed. The α-band at about 558–560 mμ was particularly evident, and a less well-defined β-band at about 525 mμ was also observed. Pieces of tissue taken from the host, *Scrobicularia plana*, including the wall of the kidney and the brown-coloured pericardial gland, gave no indication of the formation of pyridine haemochromogen when treated with Takayama's fluid.

The red coloration of specimens of *Proctoeces subtenuis* inhabiting the kidney of *Scrobicularia plana* is, therefore, due to a native haem pigment, whereas there is no evidence that members of the same species inhabiting the hind-gut of fishes are similarly pigmented.

It is not possible, on the basis of the present information, to suggest whether this pigment plays any part in the respiratory processes of the parasite, nor why it should be developed by specimens in the kidney of *S. plana* whilst apparently being absent in specimens in the hind-gut of fishes. It is, however, interesting to note that the only other adult platyhelminth parasite occurring in *S. plana*, the viviparous rhabdocoele *Paravortex scrobiculariae* (Graff), is red in colour (Freeman, 1957), whereas the closely related species *P. cardii* Hallez, occurring in cockles and some other lamellibranchs, is colourless. It is hoped in the near future to be able to determine whether this red coloration in *P. scrobiculariae* is also due to a haem pigment. A study of this assumption

of a red coloration by platyhelminth parasites of *Scrobicularia plana* might throw some light on the metabolism of this lamellibranch as well as on that of the parasites.

Alimentary canal.

The mouth opens into the oral sucker which is succeeded by a pre-pharynx, which in turn communicates with the pharynx. In whole mount preparations, the posterior end of the oral sucker often overlies the anterior end of the pharynx, thus completely obscuring the pre-pharynx (Pl. I, fig. 5A), but in sections the pre-pharynx is always readily evident (Pl. I, fig. 4). Posterior to the pharynx is the oesophagus, and the junction between these two regions receives the openings of a ring of gland cells. The oesophagus itself is provided with well-developed circular and longitudinal muscles, and, depending on the state of contraction of these muscles, the length of the oesophagus may vary very considerably. Thus, in some whole mount preparations the oesophagus may appear long (Pl. I, fig. 5B), and in others very short (Pl. I, fig. 5A), and so the presence or absence of the oesophagus, based on examination of whole mount preparations, is particularly unreliable as a diagnostic character. All the regions of the alimentary canal mentioned so far, namely the oral sucker, pre-pharynx, pharynx and oesophagus, are lined by a cuticle (Pl. I, fig. 4), which is continuous with that covering the external surface of the body. Posterior to the oesophagus the alimentary canal bifurcates into two simple intestinal caeca which reach almost to the posterior end of the body. This intestinal region is lined by an epithelium of columnar cells, each with a basal nucleus (Pl. II, fig. 4A). In living specimens the alimentary canal invariably contains an abundance of refractile globules (Pl. II, fig. 3) that readily take up fat stains and blacken with osmium tetroxide, but the kidney tissue of the host lacks such droplets.

Excretory system.

The most prominent feature of the excretory system is the Y-shaped bladder (Pl. II, fig. 1), the paired arms of which extend as far forwards as the posterior limit of the pharynx. Posteriorly, at about the level of the testes, these arms join to form the median stem that runs backwards as a relatively wide tube. Peristaltic waves were observed to pass posteriorly along this median stem of the excretory bladder. The median stem narrows posteriorly to join a cuticular-lined terminal duct (Pl. II, fig. 5), the junction receiving the ducts from a surrounding collar of gland cells (Pl. II, fig. 5). Similar glands have been illustrated in other trematodes, but their function appears not to be known. Except in its extreme posterior region, the Y-shaped excretory bladder lies in the dorsal half of the body in a plane immediately ventral to the alimentary canal. The anterior extremities of the bladder lie to the outside of the oesophagus, then, as they run posteriorly, they pass beneath the intestinal

limbs to appear on the inside of these structures at about the level of the germarium.

Numerous tributary ducts open into the anterior extremities of the arms of the Y, at least two prominent vessels on each side coming from the anterior region of the trematode, and one on each side from the posterior region. The tributary vessels were best seen in well-flattened living specimens (Pl. II, fig. 3) and were very difficult to observe in fixed specimens whether sectioned or mounted whole. Attempts were made to inject pigmented latex through the excretory pore in order to trace the tributary vessels but these attempts were unsuccessful.

In sections near to the anterior limits of the excretory bladder, the tributary ducts appear to have walls composed of 'fibrillar cytoplasm', i.e. they are non-cellular, and thus differ from the walls of the bladder itself, which are lined by a cellular epithelium (Pl. II, figs. 4A–C). The bladder epithelium, as seen in sections, consists of somewhat hemispherical or conical cells with large nuclei (Pl. II, fig. 4B). The cytoplasm often contains an abundance of refractile spherical bodies about 1–2·5 μ in diameter (Pl. II, fig. 4C), which readily take up haematoxylin, and, only a little less readily, acid stains such as eosin and Orange G. These spherical bodies are insoluble in xylene and do not take up Sudan dyes or blacken with osmium tetroxide. They may also occur freely in the lumen of the excretory bladder. The fibrous nature of the walls of the tributary ducts was described for *Proctoeces ostreae* by Fujita (1925), who also observed, 'la paroi de la vessie consiste en une très mince membrane pourvue de noyaux et de corpuscles d'excrétion, en particulier dans la paroi des rameaux'.

The excretory system of *P. subtenuis* (and also apparently of *P. ostreae*) assumes a special taxonomic significance in view of La Rue's (1957) recent classification of the Digenea based on the structure of the wall of the excretory bladder. La Rue divides the Digenea into two super-orders, which he characterizes as follows—Anepitheliocystidia: 'primitive excretory bladder retained, i.e. not replaced by cells from mesoderm, hence definitive bladder not epithelial'; and the Epitheliocystidia: 'primitive excretory bladder surrounded by, and then replaced by, layer of cells derived from mesoderm, hence definitive bladder thick walled and epithelial'. The Fellodistomatidae (which includes *Proctoeces*) is placed in the Anepitheliocystidia, its position there being thought to have been firmly established by the work of Cable. However, as described above and illustrated in Pl. II, fig. 4A–C, the excretory bladder in our specimens of *P. subtenuis* is lined by a well-developed epithelium.

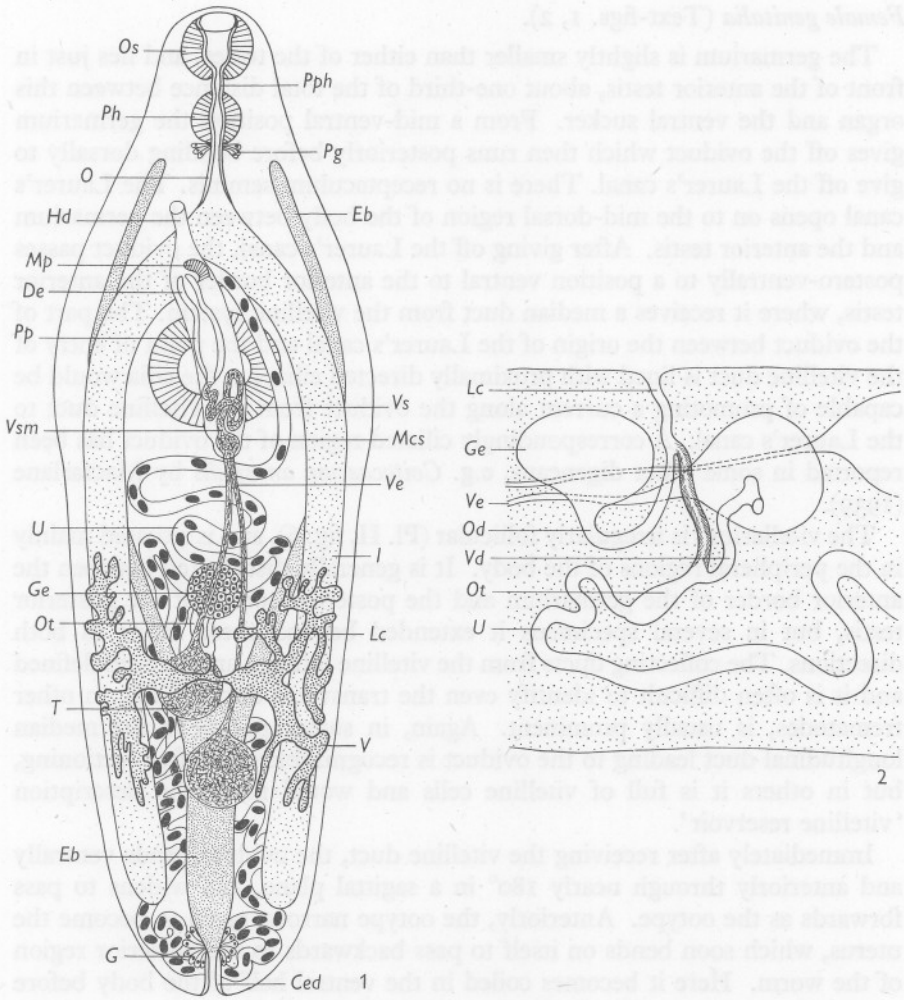
Occasionally the excretory bladder of *P. subtenuis* contains about five to twenty spherical bodies (Pl. II, fig. 2), each about 50 μ (35–66 μ) in diameter, and which in section may be seen to consist of several concentric, radially striated layers. These bodies are stained intensely by haematoxylin. Similar structures have been recorded from the excretory bladder of other trematodes (von Brand, 1952).

Male genitalia (Text-fig. 1)

The two spherical unlobed testes are situated in slightly oblique tandem in the anterior region of the posterior third of the body. Each testis gives rise anteriorly to a vas efferens, the two ducts running forwards fairly directly and joining with each other at their point of entry into the cirrus sac. When at rest this sac lies longitudinally in the body and overlies the ventral sucker, overlapping it both anteriorly and posteriorly, but pressure during histological processing may force it to lie entirely behind or in front of the ventral sucker. The posterior end of the cirrus sac is situated near the sagittal plane of the worm, but anteriorly it curves a little to the left to become confluent with the uterus to form the hermaphrodite duct.

The united vasa efferentia (there is practically no vas deferens) lead immediately to a much-coiled vesicula seminalis which may fill the posterior half of the cirrus. In ten of a sample of twenty flattened specimens the posterior region of the vesicula seminalis was somewhat conical as illustrated in Text-fig. 1, but in the remaining ten specimens such a conical region was not evident. It is possible that in living specimens a proximal conical region of the vesicula seminalis is demarcated, but that it may become obliterated during histological preparation.

The vesicula seminalis leads anteriorly to a 'pars prostatica' which is surrounded by unicellular glands, and which contains villous projections into its lumen. The pars prostatica leads in turn to a muscular ejaculatory duct, and this opens into the relatively narrow hermaphrodite duct. Active sperms were seen in the ejaculatory duct of pressed living specimens, even in specimens less than 1 mm long which contained no egg capsules. Lying sometimes alongside and sometimes anterior to the ejaculatory duct is a 'muscular papilla' (Pl. I, fig. 3A, B; Text-fig. 1). When lying alongside the ejaculatory duct, this papilla forms part of the wall of a pouch that is completed by the ejaculatory duct itself (Text-fig. 1). Such a muscular papilla has been reported in other species of *Proctoeces* (Fujita, 1925; Yamaguti, 1934, 1938), and a somewhat similar structure was described in the gasterostome *Bucephalopsis tylosuris* by Ozaki & Ozaki (1952) as a 'genital tongue'. By applying pressure to the cover-glasses of whole mount preparations of living *Proctoeces subtenuis*, the cirrus was sometimes caused to extrude, and, during this extrusion, the muscular papilla was seen to lie anteriorly to the muscular end of the ejaculatory duct. The muscular papilla preceded the cirrus proper in its passage out of the cirrus sac and was of such size as apparently forcibly to enlarge the normal diameter of the cirrus sac and hermaphrodite duct. The function of this muscular papilla is not known, but its topographical relationships and intimate association with the male intromittent organ suggest that it could play some part in the 'geometrical fitting' of co-copulants, as has been shown by Ullyot & Beauchamp (1931) to occur in certain turbellarians.



Text-figs. 1, 2. *Proctoeces subtenuis.* Text-fig. 1. Whole animal in dorsal view. (Diagram based on microprojections of whole mount preparations, with adjustments so that dimensions agree with the mean measurements included in Table 1.) Fig. 2. Proximal regions of the genitalia in side view. (Reconstructed from serial longitudinal sections.) *Ced*, cuticular-lined terminal excretory duct; *De*, ductus ejaculatorius; *Eb*, epithelial-lined excretory bladder; *G*, gland cells surrounding junction of epithelial- and cuticular-lined regions of excretory system; *Ge*, germarium; *Hd*, hermaphrodite duct; *I*, intestine; *Lc*, Laurer's canal; *Mcs*, muscular wall of cirrus sac; *Mp*, muscular papilla; *O*, oesophagus; *Od*, oviduct; *Os*, oral sucker; *Ot*, ootype; *Pg*, pharyngeal glands; *Ph*, pharynx; *Pp*, pars prostatica; *Pph*, prepharynx; *T*, testis; *U*, uterus; *V*, vitelline follicles; *Vd*, vitelline duct; *Ve*, vas efferens; *Vs* ventral sucker; *Vsm*, vesicula seminalis.

Female genitalia (Text-figs. 1, 2).

The germarium is slightly smaller than either of the testes, and lies just in front of the anterior testis, about one-third of the total distance between this organ and the ventral sucker. From a mid-ventral position the germarium gives off the oviduct which then runs posteriorly before bending dorsally to give off the Laurer's canal. There is no receptaculum seminis. The Laurer's canal opens on to the mid-dorsal region of the body between the germarium and the anterior testis. After giving off the Laurer's canal, the oviduct passes postero-ventrally to a position ventral to the anterior border of the anterior testis, where it receives a median duct from the vitelline system. The part of the oviduct between the origin of the Laurer's canal and the point of entry of the vitelline duct is lined with proximally directed cilia, i.e. the cilia would be capable of promoting a current along the oviduct from the vitelline duct to the Laurer's canal. A correspondingly ciliated region of the oviduct has been reported in some other digeneans, e.g. *Coitocaecum anaspidis* by Macfarlane (1939).

The vitellarium is irregularly follicular (Pl. II, fig. 6), and is situated mainly in the peripheral regions of the body. It is generally distributed between the anterior border of the germarium and the posterior border of the posterior testis, but in several specimens it extended beyond these limits in both directions. The collecting ducts from the vitelline follicles are not well defined and it is often difficult to identify even the transverse duct, which, in other trematodes, is usually prominent. Again, in some specimens the median longitudinal duct leading to the oviduct is recognizable only after sectioning, but in others it is full of vitelline cells and would merit the description 'vitelline reservoir'.

Immediately after receiving the vitelline duct, the oviduct bends ventrally and anteriorly through nearly 180° in a sagittal plane, and widens to pass forwards as the ootype. Anteriorly, the ootype narrows again to become the uterus, which soon bends on itself to pass backwards to the posterior region of the worm. Here it becomes coiled in the ventral half of the body before eventually passing anteriorly, finally to take a fairly straight course alongside or beneath the cirrus sac, to open into the hermaphrodite duct ventral to the cirrus.

The hermaphrodite duct is provided with strongly muscular walls and leads to the genital pore, which, in fixed specimens, lies midway between the oral and ventral suckers to the left of the mid-line of the body (Text-fig. 1).

Egg capsules.

In a sample of 176 specimens of *Proctoeces subtenuis* from forty-one hosts, all but one of the trematodes had egg capsules in the uterus. This single specimen was very small, measuring 0·72 mm when flattened and fixed, and,

although no egg capsules were present, the vesicula seminalis contained active sperms. The larger specimens each contained at least one to two thousand capsules.

The egg capsules were oval, and about twice as long as wide ($42 \times 24\ \mu$). In some cases there was considerable variation in the size of capsules. Those measured for Table 1 varied in length from 26 to 73 μ, but there were only three longer than 52 μ. The capsules were light brown, with relatively thin, elastic walls which were capable of being stretched by muscular movements of a contained embryo. No operculum could be seen, even in capsules which contained active embryos and which had been freely liberated by the trematode. Furthermore, when capsules were pressed under cover-glasses until they burst, the lines of fracture did not reveal any incipient operculum. It was, however, possible to recognize a polarity in most capsules, a small part of the wall being thickened to form a disc at that end nearest to the posterior end of the embryo.

In a sample of twenty specimens of *P. subtenuis*, collected from hosts that contained more than one parasite per host, all twenty were found to have egg capsules enclosing active embryos. Only one of over 200 specimens of *Scrobicularia plana* examined contained only a single trematode, and, although this parasite possessed numerous egg capsules, they contained no embryos.

Attempts to obtain embryos freed from their capsules were unsuccessful. Capsules which had been freely liberated from the trematodes were kept in sea water for periods of up to 7 days, but no hatching took place. Pressure was applied to the capsules in an attempt to release the embryos, but, although the capsules were ruptured, the miracidia were always damaged. Therefore, it was possible to make observations on embryos only while they were still enclosed in their capsules. The embryos (Pl. II, fig. 7) were seen to be ciliated, and ciliary activity was sometimes manifested by a rotation of the embryo alternately to the left and right about the long axis, and also by the agitation of hyaline droplets, presumably residual yolk material, lying freely in the capsule, particularly around the equator of the embryo. The orientation of the cilia suggested that the posterior end of the embryo lay near to the thickened disc in the capsule wall. A constant feature to be seen at the other end of the embryo was a prominent refractile body. The only other internal structures that could be identified were two flame cells situated about mid-way along the length of the embryo. It is possible, however, that more flame cells may have been present.

Evidence was obtained that egg capsules are normally liberated by the trematodes while in the kidney of *S. plana*. Twenty-five hosts were carefully examined and egg capsules were found lying freely in the kidneys of three of them. Two of these hosts contained two capsules each, and the third over 500. A further twenty-five hosts were kept in separate dishes of filtered sea water and the contents of the dishes examined daily. Free egg capsules were

found in two of them. An estimate of the rate of egg laying was obtained by isolating freshly collected trematodes in filtered sea water at 19° C, and making daily counts of the egg capsules in each dish. The numbers of eggs laid on each of 5 days after isolation are given in Table 2. It is possible that eggs liberated immediately after removal of the trematode from the host were forcibly discharged as a result of mechanical stimulation involved in the act of removal, but the figures in the table show that eggs continued to be liberated for several days while the trematodes were left undisturbed.

TABLE 2. RATE OF EGG LAYING OF *PROCTOECES SUBTENUIS*

Days after isolation ...	1	2	3	4	5
Specimen A	123	9	10	5	8
B	17	0	Specimen died		
C	1	0	0	14	Died
D	37	8	0	0	7
E	82	3	51	4	5

It may thus be concluded that this parasite regularly produces large numbers of fertile eggs, and that these are liberated into the kidney and pass outside the body of the host. The ciliation of the embryo suggests the occurrence of a free miracidial stage, but there was no evidence of a special operculum to facilitate hatching. It is interesting to note the observation by Baer (1952, p. 127): Curiously enough, this ciliated coating is also found in miracidia that remain within the egg and hatch only when the latter is swallowed by the snail.'

The eggs are enclosed in a thin, light-brown capsule. The hardening of trematode egg capsules has recently been reviewed by Johri & Smyth (1956), and may be summarized as follows:

Dihydroxy-phenols are oxidized, through the agency of a phenol-oxidase, to the corresponding quinone, which combines with protein to form a tanned protein or sclerotin. The histochemical techniques used by Johri & Smyth have demonstrated that the vitellaria are the site of the protein, phenol, and phenol-oxidase involved. Stains such as malachite green have an affinity for the vitellaria and are believed to be specific for the protein component localised there. Diazo-reagents, such as Fast Red B, are specific for phenolic substances, and the localization of these phenols can also be demonstrated by use of ammonium molybdate. The presence of a phenol-oxidase acting on certain dihydroxy-phenols can be demonstrated by incubating alcohol-fixed specimens at 37° C for about 2 h in catechol, protocatechuic acid or dihydroxy-phenyl-alanine (DOPA).

These techniques were applied to specimens of *Proctoeces subtenuis*, and also, for comparison, to *Gastrocotyle trachuri*, a monogenetic trematode from the gills of the horse mackerel *Trachurus trachurus*. A specimen treated with Fast Red B is shown in Pl. II, fig. 6. The results (Table 3) refer in each case to the intensity of reaction localised in the vitellaria.

These show the presence in the vitellaria of *Proctoeces subtenuis* of the phenol and protein components of the egg tanning processes, but negative results were consistently obtained for techniques which, under identical conditions, demonstrated the presence of a phenol-oxidase in *Gastrocotyle*.

TABLE 3. EGG TANNING PROCESSES OF *GASTROCOTYLE TRACHURI* AND *PROCTOECES SUBTENUIS*

For explanation see text

	G. trachuri	*P. subtenuis*
Malachite green	+	+
Fast Red B	++	++
Ammonium molybdate	++	+
Catechol	++	o
Protocatechuic acid	++	o
DOPA	++	o

The use of catechol, protocatechuic acid, and DOPA as substrates will necessarily reveal only the occurrence of an enzyme acting on these dihydroxy-phenols, which may possibly be derived from tyrosine. Pryor (1955) has suggested the role of amino-phenols derived from some other source, probably tryptophan, in the hardening of some dipterous puparia. Perhaps a similar mechanism may be operative in *Proctoeces subtenuis*.

TAXONOMY OF THE PARASITE

Our description clearly identifies this trematode as belonging to the genus *Proctoeces* Odhner, 1911, of the family Fellodistomatidae, subfamily Haplocladinae. Yamaguti (1953) lists seven species of *Proctoeces*, together with a larva of which specific identification was not possible. Winter (1954) proposed another new species. Several of the species are based on very few specimens, sometimes only one, and their distinction rests on differences of relative body proportions and on egg size. We could not, however, refer our trematodes exclusively to any one of these species. Several hundred specimens were examined, and it is apparent that many of the characters thought to indicate specific differences probably represent intraspecific variations of the kind emphasized by Stunkard (1957). The present species are *P. maculatus* (Looss, 1901) Odhner, 1911; *P. erythraeus* Odhner, 1911; *P. subtenuis* (Linton, 1907) Hanson, 1950; *P. insolitus* (Nicoll, 1915) Dollfus, 1952; *P. ostreae* Fujita, 1925; *P. major* Yamaguti, 1934; *P. magnorus* Manter, 1940; and *P. macrovitellus* Winter, 1954.

Proctoeces was established by Odhner (1911) for *Distomum maculatum* Looss, 1901 and *Proctoeces erythraeus*. He distinguished *P. erythraeus* by its smaller ventral sucker (at least one-third smaller), its smaller eggs, and the shorter extent of the vitellaria. These differences are best illustrated by comparing the following measurements, in mm, taken from Odhner (1911):

	P. maculatus	*P. erythraeus*
Length	2·5	3·0
Ventral sucker	0·42 – 0·70 × 0·28 – 0·42	0·38 × 0·40 (about 0·3 in life)
Vitellaria	Extend behind posterior testis	Extend to anterior border of posterior testis
Eggs	0·072 – 0·079 × 0·027	0·045

Dawes (1946) lists *P. erythraeus* as a synonym of *P. maculatus* and says (p. 245) that only the difference of egg size is significant, 'but this is marred by the fact that only a solitary mature specimen was found'. *P. erythraeus* was reinstated by Manter (1947) who found six specimens in *Calamus* spp. at Tortugas, Florida. He gave the egg size as 46–53 × 19–24 μ and concluded that 'the extent of the vitellaria varied some but never reached past the posterior testis as it does in *P. maculatus*'.

Hanson (1950) collected two trematodes from *Calamus* sp. at Bermuda which agreed with *Proctoeces erythraeus* in sucker ratio, extent of vitellaria, and egg size, but she argues that Linton's (1907) description of *Distomum subtenue*, also collected from *Calamus* sp. at Bermuda, agrees with *Proctoeces erythraeus* sufficiently for them to be considered the same species. Linton's measurements, in mm, of two specimens of '*Distomum subtenue*' are:

Length	3·6	2·07
Ventral sucker	0·68	0·30 × 0·48
Eggs	0·05 × 0·02	0·042 × 0·015

He illustrates the vitellaria extending behind the posterior testis. Whereas Hanson's two specimens were said to agree with *Proctoeces erythraeus* in all three characters of egg size, sucker ratio, and extent of vitellaria, one of Linton's specimens has a considerably larger ventral sucker (the measurement of 0·68 mm was from a living specimen) and the vitellaria extend behind the posterior testis. Our material from *Scrobicularia plana* showed that the vitellaria could stop short of the posterior testis in some specimens while extending behind it in others. Examination of only a few specimens would permit a conclusion that would not have been true for all the specimens available. It seems best, therefore, to recognize the general similarity of *Distomum subtenue* and *Proctoeces erythraeus*, particularly because of identical hosts in Bermuda, and regard them as the same species, *P. subtenuis* (Linton, 1907) (syn. *P. erythraeus* Odhner, 1911), as was proposed by Hanson.

Comparison of *P. erythraeus* (as described by Odhner, Manter and Hanson) and *Distomum subtenue* (as described by Linton) illustrates the amount of individual variation within the same species. It also shows the difficulty of reaching any firm statement of differences between related species. On the evidence of size of ventral sucker and extent of vitellaria at least one of Linton's specimens agrees with Odhner's description of *Proctoeces maculatus*. This adds emphasis to Dawes's (1946) observation that only the difference of egg size is significant in separating *P. maculatus* from *P. subtenuis* (= *P. erythraeus*). The average egg dimensions (42 × 24 μ) of our trematodes agree with those of *P. subtenuis*, but the largest egg measured (73 × 30 μ) is within the size range given by Odhner for *P. maculatus*. While it seems generally true that the characters of sucker size, extent of vitellaria, and size of eggs are sufficient to distinguish *P. maculatus* and *P. subtenuis*, intraspecific variation may lead to some overlap in all three characters. Odhner, the only author

who had the opportunity of directly comparing *P. maculatus* and *P. subtenuis* (=*P. erythraeus*), considered them to be separate species. On the evidence available then it seems advisable to follow Odhner, but a critical re-examination of numerous specimens of these two species is obviously desirable.

Nicoll (1915) collected five trematodes from the rectum of *Sparus australis* and named them *Xenopora insolita* n.g., n.sp. This species was later placed in *Proctoeces* by Dollfus (1952). In egg size, ventral sucker size, and extent of vitellaria, *P. insolitus* agrees with *P. subtenuis*, but differs in the extension of the hermaphrodite duct a short distance behind the ventral sucker, the situation of the cirrus pouch entirely behind the ventral sucker, and in the vesicula seminalis being mainly external to the cirrus pouch. Odhner comments that in Looss's specimens of *P. maculatus* the cirrus sac was displaced behind the ventral sucker by pressure, the genital sinus (hermaphrodite duct) being stretched to twice its normal length. In some of our preparations of *P. subtenuis* the cirrus sac was similarly situated almost entirely behind the ventral sucker. Differences in extent of the hermaphrodite duct and the position of the cirrus sac are not in themselves, therefore, sufficient specific characters. The extension of the vesicula seminalis outside the cirrus sac is contrary to Odhner's diagnosis of *Proctoeces* and, as Yamaguti (1953) observes, this feature of *P. insolitus* requires confirmation. In all other respects *P. insolitus* agrees with *P. subtenuis*, but, pending further examination of the vesicula seminalis, it is felt that *P. insolitus* must remain a separate species.

The specific status of *P. ostreae* Fujita, 1925, has been discussed by Dollfus in his addenda to Fujita's paper. He concludes that it is a progenetic metacercaria that has not yet attained complete maturity nor definitive size, and declines to come to any firm conclusion whether it is a separate species or merely a metacercaria of *P. maculatus*. Its validity must therefore remain in doubt.

P. major Yamaguti, 1934, shows some resemblances to *P. maculatus*, but differs significantly in the size of the body, the trilobate shape of the ovary, and the size of the eggs. To these might be added the position of the genital pore to the right of the mid-line, not, as usually in *Proctoeces*, to the left.

The final species in Yamaguti's list is *P. magnorus* Manter, 1940, based on one specimen from Cerros Island, Mexico. Manter considered it most similar to *P. erythraeus* as described by Odhner (1911), but differing in having a larger oral sucker and smaller eggs. He says that it is also probable that *P. magnorus* differs from *P. erythraeus* in having shorter vitellaria, an acetabular stalk, a shorter oesophagus, a cirrus sac not reaching the ovary, and a longitudinal groove within the acetabular cavity. It is difficult to know how Manter reached these conclusions, since Odhner nowhere describes or figures the oral sucker, oesophagus, or cirrus sac of *P. erythraeus*. In view of the variability we have shown in extent of vitellaria and in egg size it would seem unwise to base a new species on such characters when only a single specimen has been

examined. *P. magnorus* must, therefore, be considered a synonym of *P. subtenuis* (syn. *P. erythraeus*).

P. macrovitellus Winter, 1954, was based on two specimens from the posterior part of the intestine of *Cymatogaster aggregatus* Gibbons from Southern California. This was the first record of *Proctoeces* from a fish of the family Embiotocidae, and also the first from the west coast of America. Many of the features of *P. macrovitellus*, as described and figured by Winter, are in disagreement with the original generic diagnosis by Odhner (1911) and the modified diagnosis by Yamaguti (1953). *P. macrovitellus* has the genital pore to the right of the mid-line at the level of the pre-pharynx, the vitelline follicles

TABLE 4. A REVISED LIST OF THE SPECIES OF THE GENUS *PROCTOECES* AND THEIR FISH HOSTS

Host	Family	Location	Authority
P. maculatus (Looss, 1901)			
Labrus merula L.	Labridae	Trieste	Looss, 1901
Julis pavo (L.) (= *Crenilabrus pavo*)	Labridae	—	—
Symphodus (= *Crenilabrus*) *griseus* (Gmelin)	Labridae	—	—
Blennius ocellaris L.	Blenniidae	Naples	Odhner, 1911
Milio (= *Sparus*) *macrocephalus* (Basilewsky)	Sparidae	Inland Sea	Yamaguti, 1934
Sparus sarba Forskål (= *S. aries*)	Sparidae	—	—
Pagrosomus auratus (Bloch & Schneider)	Sparidae	—	—
Epinephelus aka-ava Bleeker	Serranidae	—	—
Semicossyphus reticulatus (Cuvier & Valenciennes)	Labridae	Tarumi	Yamaguti, 1938
Crenilabrus sp.	Labridae	Black Sea	Wlassenko, 1931
Duymaeria flagellifera (Cuvier & Valenciennes)	Labridae	Hamazima	Yamaguti, 1953
P. subtenuis (Linton, 1907)			
Calamus calamus (Cuvier & Valenciennes)	Sparidae	Bermuda	Linton, 1907
Bodianus rufus (L.) (= *Harpe rufa*)	Labridae	—	—
Iridio bivittata (Bloch)	Labridae	—	—
Lachnolaimus maximus (Walbaum)	Labridae	—	—
Calamus sp.	Sparidae	Bermuda	Hanson, 1950
Latridopsis ciliaris (Forster)	Latridae	Wellington, N.Z.	Manter, 1954
P. subtenuis (but described as *P. erythraeus*)			
Sparus (= *Chrysophrys*) *bifasciatus* (Forskål)	Sparidae	Red Sea	Odhner, 1911
Thalassoma lunare (L.) (= *Julis lunaris*)	Labridae	—	—
Calamus calamus (Cuvier & Valenciennes)	Sparidae	Tortugas	Manter, 1947
C. bajanado (Bloch & Schneider)	Sparidae	—	—
P. subtenuis (but described as *P. magnorus*)			
Caulolatilus anomalus (Cooper)	Sparidae	Mexico	Manter, 1940
P. insolitus (Nicoll, 1915)			
Sparus australis (Günther)	Sparidae	North Queensland	Nicoll, 1915
P. major Yamaguti, 1934			
Pagrosomus auratus (Bloch & Schneider)	Sparidae	Tarumi	Yamaguti, 1934
Unknown species or species of doubtful validity; in molluscan hosts			
P. ostreae Fujita, 1925			
Ostrea gigas Thunberg	Ostreidae	Hiroshima	Fujita, 1925
Proctoeces larva, Yamaguti, 1938			
Brachiodontes senhausi (Reeve)	Mytilidae	Lake Hamana	Yamaguti, 1938

situated wholly in front of the testes, thick-shelled eggs, and the cirrus sac not parallel to the long axis of the body but acutely twisted in the first third of its length. Winter also describes and figures an ovoid receptaculum seminis, measuring 0·079 by 0·095 mm, situated immediately in front of the ovary. The presence of a receptaculum seminis would, in itself, exclude Winter's species from *Proctoeces* as defined hitherto. We consider that Winter's species cannot be included in *Proctoeces* without drastic revision of the limits of this genus as at present understood.

A revised list of the species of *Proctoeces*, based on the foregoing systematic considerations, with the modern names of their hosts, is given in Table 4. It is now possible to assign the trematodes from *Scrobicularia plana* exclusively to the species *Proctoeces subtenuis* (Linton, 1907).

DISCUSSION

There seems little doubt from their morphology that our specimens of *Proctoeces subtenuis* are adult digenetic trematodes. They exhibit, in all significant features, the same structure as other members of the species described from fish hosts, and the availability of very large numbers has, indeed, allowed a more detailed account of the structure and variability of this species than was hitherto possible.

All possible adult forms of this trematode have been found, from the small immature form, through larger sexually mature individuals, to dead specimens present in the same kidney with living individuals. Their unencysted condition in the kidney makes copulation and fertilization possible: the only specimen found singly in a host had undeveloped eggs. There is evidence that eggs containing active, ciliated miracidia are liberated from the trematode and passed out of the host. Such eggs were found free in the kidney and also in dishes in which infected hosts had been isolated. The eggs presumably pass from the kidney by the urinary pore into the supra-branchial space and reach the exterior by the exhalant siphon.

The very heavy infection of *Scrobicularia plana* at Chalkwell, and the isolated occurrences of the trematode at Whitstable, must have been initiated, and must be maintained, by larval stages developing in an invertebrate host. The life history of at least one member of the Fellodistomatidae is known (Palombi, 1934), and a cercaria undoubtedly belonging to this family has been described by Martin (1945) and redescribed, without reference to Martin's work, by Cable (1954). In both, the cercaria was non-ocellate and trichocercous, and developed in sporocysts in a marine lamellibranch. The life cycle studied by Palombi was of *Bacciger bacciger* (Rud.), subfamily Fellodistomatinae, and Martin's cercaria can almost certainly be referred to the same subfamily. *Proctoeces* belongs to the subfamily Haplocladinae, and Cable (1953) suggests that the cercariae of this subfamily may be furcocercous or

trichofurcocercous. We have found furcocercous cercariae developing in sporocysts in *Scrobicularia plana* at Chalkwell, but the fact that they occurred also in *S. plana* from the Gwendraeth and Tavy rivers where *Proctoeces subtenuis* was absent suggested that they were not related to this trematode. This impression that adult and cercarial stages of *P. subtenuis* did not both occur in *Scrobicularia plana* was confirmed by the negative results of numerous attempts to infect *S. plana* with these furcocercous cercariae, and the failure to find post-miracidial stages in *S. plana* into which fertile eggs of *Proctoeces subtenuis* had been introduced.

There is some evidence that *Proctoeces* in Japanese waters has an unencysted metacercaria in marine lamellibranchs. As already noted, the trematode described by Fujita (1925) as *P. ostreae* from the gonad of the edible oyster of Japan, *Ostrea gigas*, was considered by Dollfus to be a metacercaria of an undetermined species of *Proctoeces*, and similarly Yamaguti (1938) described a single immature stage of *Proctoeces* sp. from the digestive gland of the mytilid *Brachiodontes senhausi*. The normal life cycle of *Proctoeces* thus appears to be as follows: adult trematodes live in the hind-gut of labrid or sparid fishes; cercariae, which may be furcocercous, trichofurcocercous or trichocercous, develop in sporocysts in marine lamellibranchs; and unencysted metacercariae also occur in marine lamellibranchs. These metacercariae are almost certainly transferred to the fish which is the definitive host when it eats the lamellibranch (Yamaguti, 1938).

Our evidence indicates that this life cycle of *Proctoeces* must be modified for *P. subtenuis* in British waters. The apparent limitation of the species to the region of the Thames estuary, in a lamellibranch widely distributed in estuarine muds around the British Isles, strongly suggests that the introduction of *P. subtenuis* to this country is fairly recent. It is typically a parasite of fish of Asian, Australasian or eastern American waters. It could possibly have arrived through the accidental introduction of a normal fish host, or, more probably, in the marine lamellibranch that is the first intermediate host. The cercariae issuing from this mollusc could have entered *Scrobicularia plana* and, now being outside the range of the normal fish host, have achieved maturity in the kidney of this lamellibranch. It may be noted that a trichocercous cercaria has been found in the lamellibranch *Petricola pholadiformis* Lamarck at Whitstable (M. Duval, personal communication). *P. pholadiformis* is a recent introduction from the east coast of North America (Newell, 1954), and has a limited distribution in the southern and eastern parts of Britain, including the Essex coast of the Thames estuary (Cole, 1903). Work is now being done to determine whether the cercaria from *P. pholadiformis* is a stage in the life cycle of *Proctoeces subtenuis* in the Thames estuary. The evidence so far available suggests that the fish host has been eliminated from the life cycle. No sparids have been recorded from the Thames estuary, and only two members of the Labridae occur there, namely *Labrus bergylta* Ascanius (three specimens up to

1903 (Laver, 1903; Murie, 1903)), and *Crenilabrus melops* (L.) which is common. Any final statement on the life cycle of *Proctoeces subtenuis* in the Thames estuary must, of course, include consideration of the only common labrid in the area, but transference of the trematode from its last molluscan host to a fish must almost certainly be effected by the fish eating the mollusc, and there is no evidence that a lamellibranch such as *Scrobicularia plana* is eaten by *Crenilabrus melops*. The nature of the habitat of *Scrobicularia plana* makes it unlikely that it is readily available as food for fish, although shells have occasionally been found in the stomach of some flatfish (J. E. Forrest, personal communication). The limitation of *Proctoeces subtenuis* to the mouth of the Thames also suggests that a fish host is not involved in the life cycle. At Chalkwell, every specimen of *Scrobicularia plana* examined was infected, and yet the trematode appears to be absent from *S. plana* in the neighbouring Essex rivers, the Blackwater and Colne. It seems unlikely that mobile fish, acting as hosts to breeding trematodes, would not have produced a wider distribution of the parasite while the intensive infection of *S. plana* at Chalkwell was taking place.

If this view is correct then the trematodes in *S. plana* are directly comparable, structurally, functionally, and in their status in the life history, with the adults found in the hind-gut of fishes in other parts of the world. They enter the kidney of *S. plana* as immature stages, achieve maturity there, and eventually die there. They certainly differ from the stages of *Proctoeces* previously described from lamellibranchs. The single immature stage described by Yamaguti (1938) from *Brachiodontes senhausi*, and termed by him an adolescaria, had undeveloped female genitalia. Dollfus relegated Fujita's *Proctoeces ostreae* to the status of progenetic metacercariae on the grounds that only a few eggs were present, even these being abnormal, and that the animals did not appear to have attained complete maturity nor definitive size. In these respects our *P. subtenuis* clearly agrees more closely with previous descriptions of any of the adults of this trematode from fish hosts than with any metacercarial stage of related species. There seems no reason to modify the emphasis on their adult nature by invoking the phenomenon of progenesis.

The term progenesis was first used by Giard & Bonnier (1887) to describe the assumption of sexual maturity by male parasitic isopods while still in a larval condition. It has since been applied to numerous digenetic trematodes which become sexually mature as cercariae or metacercariae in an intermediate host. Many examples are listed by Wu (1938) and Dawes (1946). The only significant question from the point of view of this study is that posed by Dollfus (1929) in the title of his paper: 'Existe-t-il des cycles évolutifs abrégés chez les trématodes digénétiques?' This question is further discussed by Joyeux, Noyer & Baer (1930) and again by Wu (1938). Wu concludes that 'up to the present we still have no definite proof that there is an abridged life cycle or omission of a final host in the life history of a digenetic trematode'.

This proof was provided by Serkova & Bychowsky (1940). They exposed uninfected *Bithynia tentaculata* (L.) to members of the same species of snail which contained mature *Asymphylodora progenetica*, and obtained experimental infections. They identified rediae and also mature adults in the previously uninfected snails. In this abbreviated life cycle the rediae develop in one specimen of *Bithynia tentaculata* and the adults achieve maturity in another specimen of the same species. Serkova & Bychowsky assume that an alternative life cycle of *Asymphylodora progenetica* is possible, in which the parasite of the mollusc enters a fish and there attains sexual maturity. The importance of Serkova & Bychowsky's work, in relation to our study of *Proctoeces subtenuis*, lies in the precedent it established that abbreviated life cycles of digenetic trematodes are possible. More recently, Buttner (1955) has shown that *Ratzia joyeuxi* (Brumpt, 1922) and *Paralepoderma brumpti* (Buttner, 1950), both normally parasites of snakes, can show abbreviated life cycles in which the final host is omitted.

We suggest that the facts presented in this paper provide strong circumstantial evidence that *Proctoeces subtenuis* in the Thames estuary displays a similar phenomenon of a life cycle restricted to invertebrate hosts. A final decision on this question must, however, await the results of further investigations.

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SUMMARY

The digenetic trematode *Proctoeces subtenuis* (Linton, 1907) is recorded from the kidney of the lamellibranch *Scrobicularia plana* (da Costa). At Chalkwell in Essex all of nearly a thousand *S. plana* examined were found to be infected, as were three of approximately 150 *S. plana* at Whitstable in Kent. The average number of parasites per host was between four and five. No *Proctoeces subtenuis* were found in *Scrobicularia plana* examined from other localities in Essex, Devon, Wales and Suffolk. The environmental conditions of a parasite in the lamellibranch kidney are discussed. It was found that the kidney fluid surrounding the parasites varies in osmotic pressure.

The anatomy of the parasite is described. The excretory bladder has a cellular wall, thus disagreeing with the inclusion of *Proctoeces subtenuis* in La Rue's superorder Anepitheliocystidia. The red colour of the parasite is due

to a native haem pigment. The egg capsules are hardened by a quinone-tanning mechanism, but incubation with catechol, protocatechuic acid, and dopa failed to reveal the presence of a phenol-oxidase.

The species of *Proctoeces* are reviewed, and some revisions suggested. *P. magnorus* Manter, 1940 is considered a synonym of *P. subtenuis*, and the synonymy of *P. erythraeus* Odhner, 1911 with *P. subtenuis* is confirmed. The differences between *P. insolitus* (Nicoll, 1915) and *P. subtenuis*, and between *P. maculatus* (Looss, 1901) and *P. subtenuis*, require re-examination. It is considered that *P. macrovitellus* Winter, 1954 should be excluded from the genus *Proctoeces*. A definitive host list of *Proctoeces* spp. is given.

The adult nature of *P. subtenuis* from *Scrobicularia plana* is discussed, and it is suggested that *Proctoeces subtenuis* in the Thames estuary shows an abbreviated life cycle restricted to invertebrate hosts.

REFERENCES

BAER, J. G., 1952. *Ecology of Animal Parasites*. Urbana, Illinois.

BRAND, T. VON, 1952. *Chemical Physiology of Endoparasitic Animals*. New York: Academic Press.

BUTTNER, A., 1955. Les distomes progénétiques sont-il des pré-adultes ou des adultes véritables? Valeur évolutive de la progénèse chez les Digenea. *C.R. Soc. Biol., Paris*, T. 149, pp. 267–72.

CABLE, R. M., 1953. The life cycle of *Parvatrema borinqueñae* gen. et sp.nov. (Trematoda: Digenea) and the systematic position of the subfamily Gymnophallinae. *J. Parasit.*, Vol. 39, pp. 408–21.

—— 1954. A new marine cercaria from the Woods Hole region and its bearing on the interpretation of larval types in the Fellodistomatidae (Trematoda: Digenea). *Biol. Bull., Woods Hole*, Vol. 106, pp. 15–20.

CAROLI, A., 1928. Tomopteridi del Mar Rosso, con considerazioni sulla loro distribuzione geografica. *Ann. idrogr.*, Vol. 11, pp. 1–23.

COLE, W., 1903. Marine Mollusca. In *The Victoria History of the County of Essex* Vol. 1. London: Constable.

DAWES, B., 1946. *The Trematoda*. Cambridge University Press.

DELAUNAY, H., 1927. Recherches biochimiques sur l'excrétion azotée des invertébrés. *Bull. Soc. sci. Arcachon*, T. 24, pp. 95–214.

DOLLFUS, R. P., 1929. Existe-t-il des cycles évolutifs abrégés chez les trématodes digénétiques? Le cas de *Ratzia parva* (Stossich, 1904). *Ann. Parasit. hum. comp.*, T. 7, pp. 196–203.

—— 1952. Miscellanea helminthologica maroccana. IV. Affinités naturelles de *Pseudochetosoma salmonicola* R. Ph. Dollfus 1951 (famille Steganodermatidae nov.). Emendation de la superfamille Haploporoidea W. Nicoll 1935. *Arch. Inst. Pasteur Maroc*, T 4 (5), pp. 369–86.

FLORKIN, M. & DUCHATEAU, G., 1948. Sur l'osmorégulation de l'anodonte (*Anodonta cygnea* L.). *Physiol. comp.*, Vol. 1, pp. 29–45.

FREEMAN, R. F. H., 1957. *Paravortex scrobiculariae* (Graff) in Great Britain. *Nature, Lond.*, Vol. 180, pp. 1213–14.

FREEMAN, R. F. H. & RIGLER, F. H., 1957. The responses of *Scrobicularia plana* (da Costa) to osmotic pressure changes. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 553–67.

FUJITA, T., 1925. Études sur les parasites de l'huitre comestible du Japon *Ostrea gigas* Thunberg. Traduction accompagnée de notes, de diagnoses et d'une bibliographie par Robert-Ph. Dollfus. *Ann. Parasit. hum. comp.*, T. 3, pp. 37–59.

GIARD, A. & BONNIER, J., 1887. Contributions à l'étude des bopyriens. *Trav. Sta. zool. Wimereux*, T. 5, pp. 1–252.

GREEN, J., 1957. The growth of *Scrobicularia plana* (da Costa) in the Gwendraeth estuary. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 41–7.

HANSON, M. L., 1950. Some digenetic trematodes of marine fishes of Bermuda. *Proc. helm. Soc. Wash.*, Vol. 17, pp. 74–89.

HAWK, P. B., OSER, B. L. & SUMMERSON, W. H., 1954. *Practical Physiological Chemistry*. 13th edition. London: Churchill.

JOHRI, L. N. & SMYTH, J. D., 1956. A histochemical approach to the study of helminth morphology. *Parasitology*, Vol. 46, pp. 107–16.

JOYEUX, C., NOYER, R. DU & BAER, J. G., 1930. L'activité génitale des métacercaires progénétiques. *Bull. Soc. Pat. exot.*, T. 23, pp. 967–77.

KROGH, A., 1939. *Osmotic Regulation in Aquatic Animals*. Cambridge University Press.

LA RUE, G. R., 1957. The classification of digenetic Trematoda: a review and a new system. *Exp. Parasit.*, Vol. 6, pp. 306–49.

LAVER, H., 1903. Fishes. In *The Victoria History of the County of Essex*, Vol. 1. London: Constable.

LINTON, E., 1907. Notes on parasites of Bermuda fishes. *Proc. U.S. nat. Mus.*, Vol. 33, pp. 85–126.

LOOSS, A., 1901. Über einiger Distomen der Labriden des Triester Hafens. *Zbl. Bakt.*, Bd. 29, pp. 398–405.

MACFARLANE, W. V., 1939. Life cycle of *Coitocaecum anaspidis* Hickman, a New Zealand digenetic trematode. *Parasitology*, Vol. 31, pp. 172–83.

MANTER, H. W., 1940. Digenetic trematodes of fishes from the Galapagos Islands and the neighbouring Pacific. *Rep. Hancock Pacific Exped.*, Vol. 2, pp. 325–497.

—— 1947. The digenetic trematodes of marine fishes of Tortugas, Florida. *Amer. Midl. Nat.*, Vol. 38, pp. 257–416.

—— 1954. Some digenetic trematodes from fishes of New Zealand. *Trans. roy. Soc. N.Z.*, Vol. 82, pp. 475–568.

MARTIN, W. E., 1945. Two new species of marine cercariae. *Trans. Amer. micr. Soc.*, Vol. 64, pp. 203–12.

MURIE, J., 1903. *Report on the Sea Fisheries and Fishing Industries of the Thames Estuary. I. The Thames Estuary and Leigh-on-Sea Fisheries*. Kent and Essex Sea Fisheries Committee, London.

NEWELL, G. E., 1954. The marine fauna of Whitstable. *Ann. Mag. nat. Hist.*, Ser. 12, Vol. 7, pp. 321–50.

NICOLL, W., 1915. The trematode parasites of North Queensland. III. Parasites of fishes. *Parasitology*, Vol. 8, pp. 22–41.

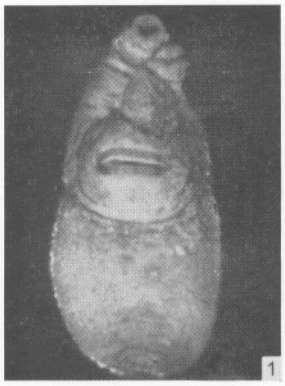
ODHNER, T., 1911. Zum natürlichen System der digenen Trematoden. III. Steringophoridae n. fam. *Zool. Anz.*, Bd. 38, pp. 97–117.

OZAKI, H. & OZAKI, Y., 1952. A new gasterostome trematode *Bucephalopsis tylosuris* n.sp. *J. Sci. Hiroshima Univ.*, Ser. B, Div. 1, Vol. 13, pp. 85–90.

PALOMBI, A., 1934. *Bacciger bacciger* (Rud.) trematode digenetico: fam. Steringophoridae Odhner. Anatomia, sistematica e biologia. *Pubbl. Staz. zool. Napoli*, Vol. 13, pp. 438–78.

PRYOR, M. G. M., 1955. Tanning of blowfly puparia. *Nature, Lond.*, Vol. 175, p. 600.

ROBERTSON, J. D., 1949. Ionic regulation in some marine invertebrates. *J. exp. Biol.*, Vol. 26, pp. 182–200.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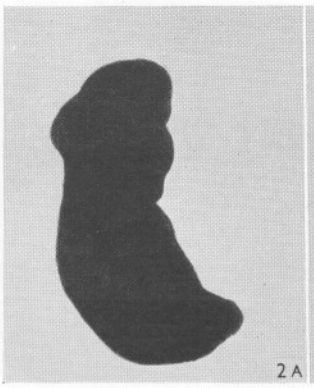
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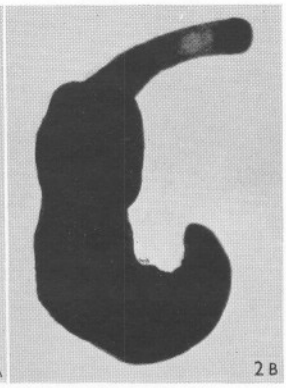
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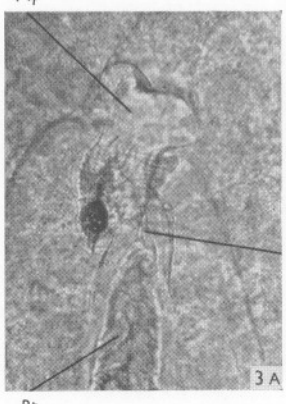
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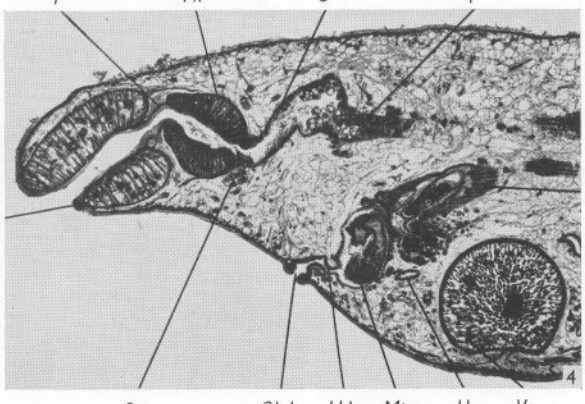
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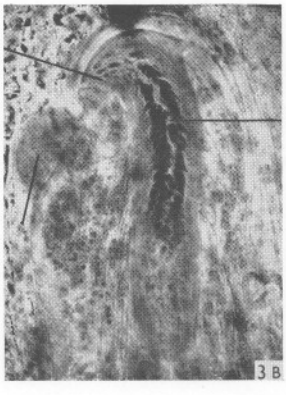
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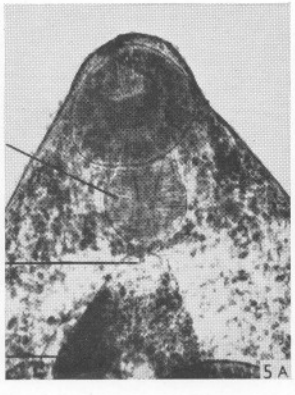
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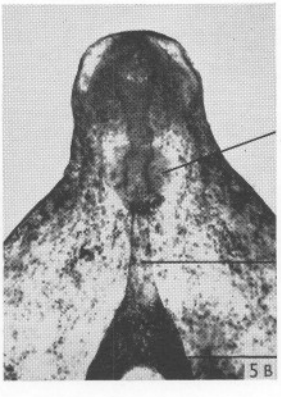
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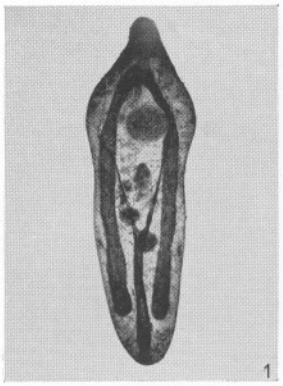
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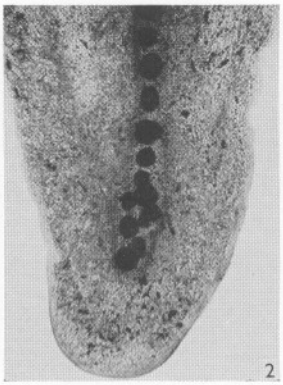
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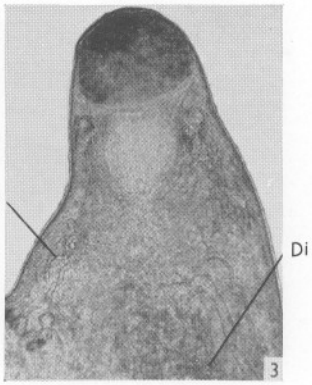
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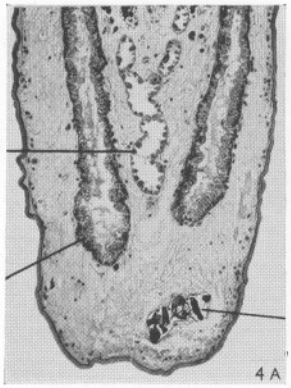
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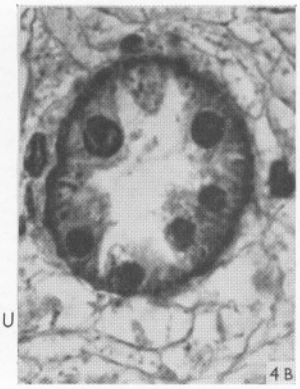
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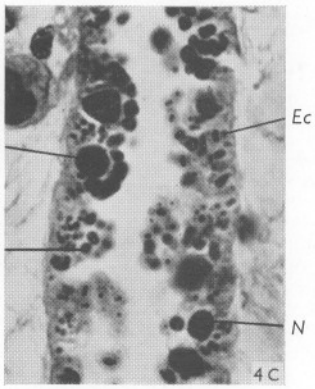
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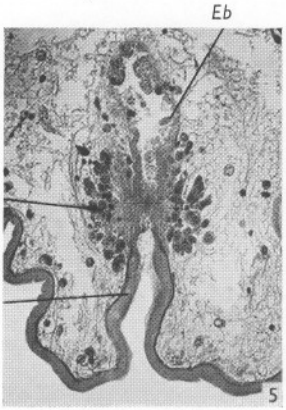
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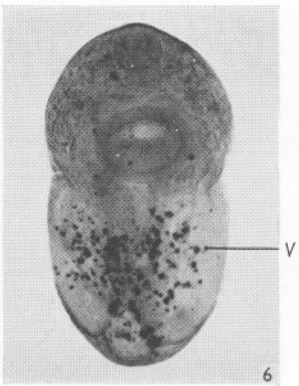
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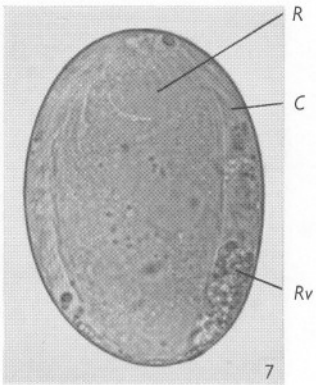
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SERKOVA, O. P. & BYCHOWSKY, B. E., 1940. *Asymphylodora progenetica* sp.n. i niekotoryie dannyie po ieie morfologii i razvitiu. *Mag. Parasit., Moscow*, Vol. 8, pp. 162–75.

SPOONER, G. M. & MOORE, H. B., 1940. The ecology of the Tamar estuary. VI. An account of the macrofauna of the intertidal muds. *J. mar. biol. Ass. U.K.*, Vol. 24, pp. 283–330.

STUNKARD, H. W., 1957. Intraspecific variation in parasitic flatworms. *Systematic Zoology*, Vol. 6, pp. 7–18.

ULLYOT, P. & BEAUCHAMP, R. S. A., 1931. Mechanisms for the prevention of self-fertilization in some species of fresh-water triclads. *Quart. J. micr. Sci.*, Vol. 74, pp. 477–89.

WINTER, H. A., 1954. *Proctoeces macrovitellus* nov.sp., de un pez embiotocido del Oceano Pacifico del norte (Tremat. Fellodistom.). *Ciencia, Méx.*, Vol. 14, pp. 140–2.

WLASSENKO, P., 1931. Zur Helminthofauna der Schwarzmeerfische. *Trav. Sta. Sci. nat. Karadagh*, Vol. 4, pp. 83–136.

WU, K., 1938. Progenesis of *Phyllodistomum lesteri* sp.nov. (Trematoda: Gorgoderidae) in fresh-water shrimps. *Parasitology*, Vol. 30, pp. 4–19.

YAMAGUTI, S., 1934. Studies on the helminth fauna of Japan. Part 2. Trematodes of fishes. I. *Jap. J. Zool.*, Vol. 5, pp. 249–541.

—— 1938. Studies on the helminth fauna of Japan, Part 21. Trematodes of fishes. IV. Published by the author, Kyôto, Japan. 139 pp.

—— 1953. *Systema Helminthum. Part I. Digenetic Trematodes of Fishes*. 405 pp. Tokyo.

EXPLANATION OF PLATES

Proctoeces subtenuis. Unless stated otherwise, all photomicrographs were made from fixed specimens mounted in Canada Balsam.

PLATE I

Fig. 1. Dead, 'petrified' specimen (unmounted). Fig. 2. (A) Contracted specimen (living). (B) Same specimen as (A), stretched. Fig. 3. Terminal part of cirrus, showing muscular papilla. (A) Living specimen. (B) Another specimen stained and mounted in Canada Balsam. Fig. 4. Vertical longitudinal section (slightly oblique) through anterior region. Fig. 5. Variation in length of the oesophagus in fixed preparations. (A) Contracted. (B) Stretched (different specimen from (A)).

PLATE II

Figs. 1–5. The excretory system

Fig. 1. The Y-shaped excretory bladder (from a preparation by Miss P. Milsom). Fig. 2. Concretions in the posterior region of the bladder (haematoxylin preparation). Fig. 3. Tributary canals in anterior region (living). Note also the droplets in the intestine. Fig. 4. The epithelial lining of the excretory bladder. (A) Horizontal section. (B) Transverse section. (C) Longitudinal section through epithelial cells charged with 'excretory corpuscles'. Fig. 5. Junction of epithelial-lined portion of bladder with terminal cuticular-lined portion, surrounded with gland cells (horizontal section). Fig. 6. The vitellaria. (Specimen treated with a diazo reagent to show phenolic substances.) Fig. 7. Egg capsule with ciliated embryo. (Living specimen, well pressed beneath cover-glass.)

Abbreviations

C, cilia; *Ced*, cuticular-lined terminal excretory duct; *De*, ductus ejaculatorius; *Di*, droplets in intestine; *Eb*, epithelial-lined excretory bladder; *Ec*, 'excretory corpuscles'; *G*, gland cells surrounding junction of epithelial- and cuticular-lined regions of excretory system; *Hd*, hermaphrodite duct; *I*, intestine; *Mcs*, muscular wall of cirrus sac; *Mp*, muscular papilla; *N*, nucleus; *O*, oesophagus; *Ohd*, opening of hermaphrodite duct; *Os*, oral sucker; *Pg*, pharyngeal glands; *Ph*, pharynx; *Pp*, pars prostatica; *Pph*, pre-pharynx; *R*, refractile body at anterior end of embryo; *Rv*, residual vitelline material; *Td*, tributary duct of excretory system; *U*, uterus; *V*, vitelline follicles; *Vs*, ventral sucker.