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ON THE MORPHOLOGY AND LIFE-HISTORY OF *PODOCOTYLE REFLEXA* (CREPLIN, 1825) ODHNER, 1905, AND A COMPARISON OF ITS DEVELOPMENTAL STAGES WITH THOSE OF *P. ATOMON* (RUDOLPHI, 1802) ODHNER, 1905 (TREMATODA, OPECOELIDAE)

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ABSTRACT

The life-cycle of the digenetic trematode *Podocotyle reflexa* (Creplin, 1825) Odhner, 1905 (fam. Opecoelidae) is shown experimentally. The cotylomicrocercous cercaria of *P. reflexa, C. buccini* Lebour, 1911, develops in sporocysts in *Buccinum undatum* L. and *Neptunea antiqua* (L.) (Gastropoda, Prosobranchia). The cercariae encyst in muscles of various crustaceans. *Crangon crangon* (L.) was used as experimental intermediate host. The metacercariae developed into *P. reflexa* in cod, *Gadus morhua* L. given shrimps with two to four months old metacercariae.

The cercaria, metacercaria and adult of *P. reflexa* are redescribed and compared with similar developmental stages of *P. atomon* (Rudolphi, 1802) Odhner, 1905. *P. reflexa* has been considered a synonym of *P. atomon*. The tegument and suckers of the cercaria of *P. reflexa* were studied in the transmission electron microscope. All developmental stages of *P. reflexa* and *P. atomon* were studied in the stereoscan electron microscope. The surface of the cercaria of *P. reflexa* is smooth apart from a microvillous fringe which surrounds the suckers, whereas the whole surface of the cercaria of *P. atomon* is covered with microvilli. The microvilli of both cercarial species are shed a few hours after penetration into the crustacean host. A few days after encystment the metacercariae of both species develop short microvillus-like projections all over the body. These projections disappear about one month after encystment, and the metacercariae do not grow further.

INTRODUCTION

Podocotyle reflexa (Creplin, 1825) Odhner, 1905 (fam. Opecoelidae) has been recorded from fishes belonging to various families occurring in the arctic-boreal area. The cercaria of *P. reflexa, Cercaria buccini* Lebour, 1911, has been recorded from British waters, the Barents Sea, Danish waters and the German Bight (Lebour 1911, Chubrik 1966, Køie 1969, Lauckner 1980). The metacercaria has been recorded from the Barents Sea (Uspenskaya 1960, 1963).

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P. reflexa has been considered a synonym of *P. atomon* (Rudolphi, 1802) Odhner, 1905, but the present paper shows that both are valid species. The life-history of *P. atomon* was described by Hunninen & Cable (1943). Both species have the same geographical distribution, but *P. atomon* dominates in fishes living littorally, whereas *P. reflexa* mostly occurs in fishes which live sublittorally.

The interference contrast microscope used in this study was financed by the Danish Natural Science Research Council.

MATERIAL AND METHODS

The invertebrates and fishes studied were from the following three Danish localities: 1) The middle part of Øresund, 5-30 metres depth, and Nivå Bay, 0-1 m, throughout several years, 2) western Kattegat, off Frederikshavn, 0-30 m, and the islands of Hirsholmene, 0-½ m, August 1979, 1980, and 3) northern Bornholm, the Baltic, 0-10 m, October 1980.

The different developmental stages of the parasites were studied alive and after flattening, fixation and staining with carmalum. Material studied in the transmission and scanning electron microscopes was treated as described by Køie (1971a). All measurements are of slightly flattened living specimens.

All fishes used as experimental hosts were isolated and fed on frozen food for at least two months before infection.

RESULTS

Podocotyle reflexa

Natural infestation of the molluscan host

The natural infestation of *C. buccini* Lebour, 1911 in *Buccinum undatum* L. from Øresund (20-28 m) has been studied throughout one year (Køie 1969). The average incidence throughout the year was 0.9 % (1375 *B. undatum* studied). The same incidence was found at Kristineberg, the Gullmar Fjord, western Sweden. 325 *B. undatum* from a few metres depth were studied (Køie 1969). 10 % of *Neptunea antiqua* (L.) (>50 mm in shell length) from the middle part of Øresund, 25-28 m, were infested with *C. buccini* (100 snails studied). Fully developed cercariae were found in the snails all the year round.

Most cercariae were released within the first day after the snails were brought to the laboratory. Later on the number of shed cercariae showed great variations.

Most infested *B. undatum* were seriously affected by the parasite and some infested snails died within a few months after they were brought to the laboratory. The foot appeared thin, dehydrated and more red than that of uninfested snails. *N. antiqua* appeared to be less affected by the parasite. Infested *N. antiqua* were kept in aquaria for one year.

The daughter sporocyst

The daughter sporocyst of *C. buccini* and its effect on the host tissue of *B. undatum* have been studied in the light microscope, transmission and scanning electron microscopes (Køie 1971b).

The cercaria

C. buccini is a typical cotylomicrocercous cercaria (Figs 1, 2A, 4A). Naturally released living flattened cercariae including tail are 290-370 μm (mean of 10 specimens: 330 μm) long and 70-100 μm (mean: 86 μm) wide at the level of the

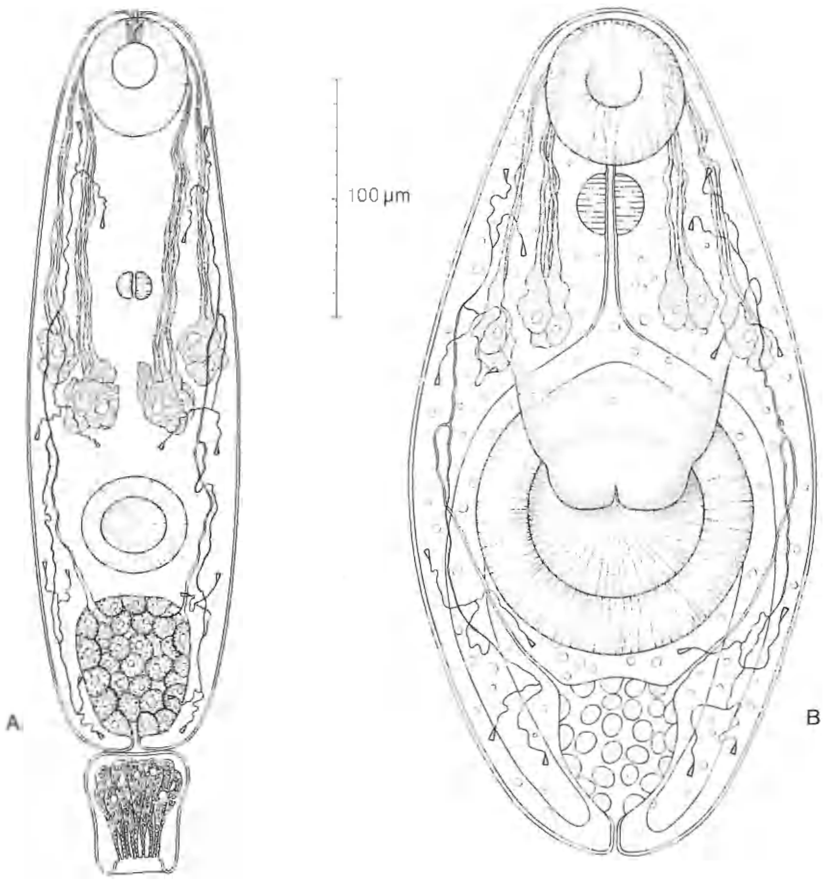

FIG. 1. A, cercaria and B, two-month old metacercaria of *Podocotyle reflexa*. Ventral views of living flattened specimens.

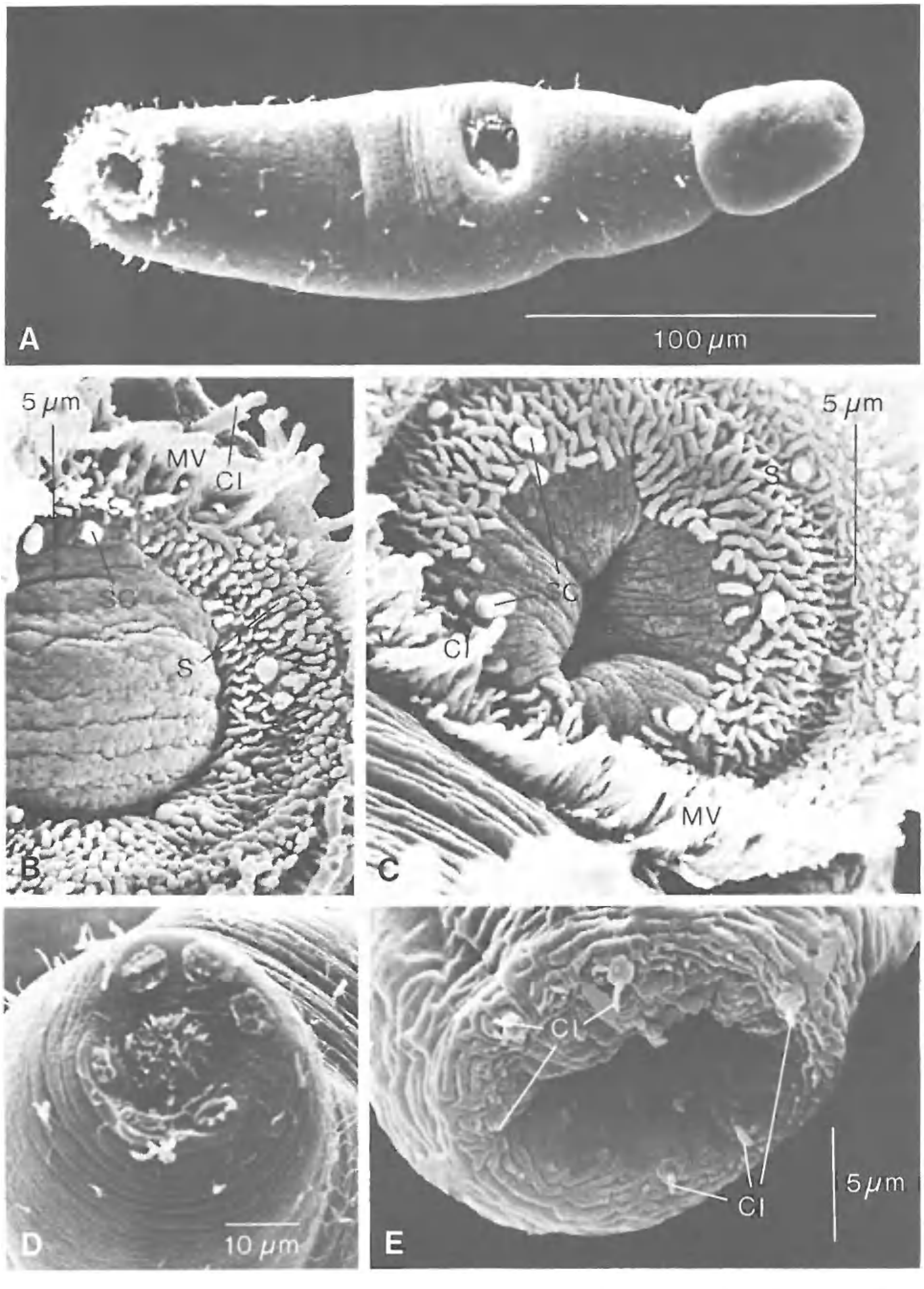

FIG. 2. Scanning electron micrographs of naturally released cercariae of *Podocotyle reflexa*. A, ventral view of cercaria. B, part of the oral sucker showing spines (S), cilia surrounded by tegument (SC), microvilli (MV) and cilia (CI). C, the ventral sucker. D, the posterior end of a tail-less cercaria with the six attachment areas of the tail and, in the middle, the opening of the excretory vesicle. E, the distal end of the tail with six sensory cilia.

ventral sucker. The living unpressed cercariae are able to elongate and contract the body and tail enormously; the length of the cercariae then ranges from 200 to 400 μm.

The oval oral sucker of flattened specimens is 40-43 μm (mean: 41 μm) wide and 42-60 μm (mean: 51 μm) long. The stylet is 14 μm long and 8 μm wide. It is found in the stylet pocket, and only the distal ends of the two points protrude above the surface. The double-pointed central part of the stylet is surrounded by a collar. For this reason the stylet may appear four-pointed with two small lateral points. The prepharynx is 50-80 μm long and the pharynx 12-16 μm (mean: 14 μm) in diameter. The oesophagus and caeca could not be seen. The ventral sucker is 38-42 μm (mean: 40 μm) in diameter. There appeared to be five pairs of cephalic glands in the preacetabular region. The three ducts of the median glands open dorsally at the stylet, the two lateral ducts open more laterally and more ventrally.

The wall of the oval excretory vesicle is composed of rounded cells with granular contents. The flame cell formula is $2[(2 + 2) + (2 + 2)] = 16$.

The tail is attached to the body at six points (Fig. 2D). The tail is 45-65 μm (mean: 55 μm) long and 30-40 μm (mean: 36 μm) wide. It is filled with 20-30 unicellular glands containing small spindle-shaped bodies. The gland ducts open into a sucker-like concavity posteriorly. Six papillae each with an about 1.5 μm long apical cilium occur on the distal edge of the tail (Fig. 2E).

The body surface is slightly irregular with circumferential furrows. The spineless tegument is about 1 μm thick (Fig. 3C). Mitochondria, membrane-bound irregular vesicles and two kinds of electron-dense bodies are sparsely distributed. The elongated dark bodies occur close to and perpendicular to the external membrane, whereas the remaining bodies are found more basally. The internal tegumentary membrane forms tubular invaginations into the tegument.

Four types of structures presumed to be sensory receptors protrude above the surface. Type 1 consists of a short cilium surrounded by a tegumentary collar. This type is especially common anteriorly, but is also found on the tail (Fig. 2E). Type 2 consists of an up to 8 μm long cilium without a surrounding collar. Type 3 consists of a pit with a tuft of up to 12 2-8 μm long cilia. The two latter types are regularly distributed all over the body, but are especially common anteriorly. Type 4 is composed of a cilium completely surrounded by tegument (Fig. 3A). The embedded cilia are up to 2 μm long.

The two suckers are essentially identical (Fig. 2B, C). The central spineless tegument is only about 0.5 μm thick (Fig. 3A). Crypts and short channels into the tegument increase the external surface. Medially and anteriorly in the oral sucker occur two of the above mentioned sensory structures with cilia covered by tegument (type 4). Similar structures are found in the spineless area of the ventral sucker and in the spined area of both suckers. The radially arranged flattened spines often have an apical thickening which in sections may appear as knobs (Fig. 3B). Anterior to the two anterior sensory structures in the oral sucker is the spined area replaced by microvilli (Fig. 2B). The peripheral spines in both suckers have three points (Fig. 3A). On the rim of the suckers are found about 5 μm long microvilli and more peripherally occur about 8 μm long cilia (type 2).

The cercariae crawl with the aid of the two suckers. Sometimes they stop crawling and attached to the substratum with their glandular tail wag their elongated body back and forth. If contact with a suitable host is not established they continue alternately to crawl and wag. If they get contact with a suitable host they attach themselves to it by the suckers.

The cercariae may survive for about one week at temperatures below 10 °C. At higher temperatures the cercariae become inactive and die within about 24 hours.

Experimental infection of the crustacean host

Various crustaceans were placed together with hundreds of *C. buccini* from crushed *B. undatum* or *N. antiqua* for about 20 hours. Apart from *Haploops* sp. and *Macropipus depurator* (L.) the crustaceans used were from a few metres depth, where *B. undatum* and *N. antiqua* never occur. Opecoelid metacercariae from natural infestations were found, but these metacercariae were rare and easely distinguished from those from the experimental infections.

To observe whether cercariae in the daughter sporocysts were fully developed, sporocysts were isolated and teased apart. The freed cercariae penetrated and encysted at the same rate as migrating cercariae from the tissue of the same snail host.

Crangon crangon (L.) (Decapoda), *Haploops* sp. (Amphipoda) removed from their mud tubes and unidentified mysids (Mysidacea) were easily infected. Small *M. depurator*, *Carcinus maenas* (L.) (Decapoda) and *Gammarus* spp. (Amphipoda) were only infected with a few metacercariae although they were repeatedly exposed to numerous cercariae. The cercariae did not attempt to penetrate *Pagurus bernhardus* (L.), *Palaemon* sp. (Decapoda) and *Idothea* sp. (Isopoda).

In *C. crangon* most cercariae encysted anteriorly and dorsal to the intestine, but metacercariae were found in the muscles throughout the body. The intensity of metacercariae in experimentally infected *C. crangon* varied enormously. Sometimes none or only a few cercariae penetrated into the shrimps although they were exposed to apparently fully developed cercariae. In other cases each shrimp was penetrated by hundreds of cercariae; this always resulted in the death of the host. Infections with up to about 30 metacercariae did not harm large shrimps, and several shrimps with about this number of metacercariae survived for more than one year.

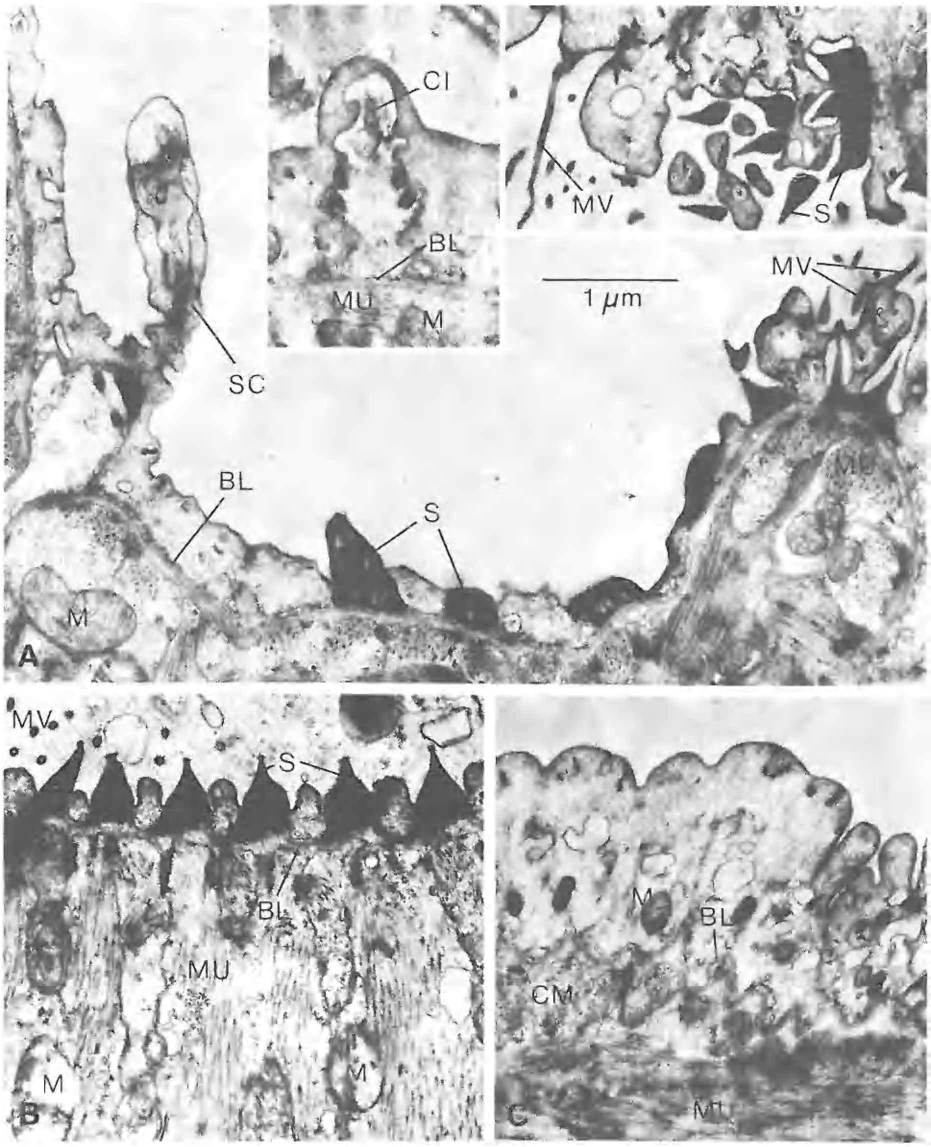

FIG. 3. Transmission electron micrographs of the ventral sucker and surrounding tegument of the cercaria of *Podocotyle reflexa*. All figures to same scale. A, radial section through the ventral sucker. The peripheral irregular spines are seen to the right, and a laterally sectioned sensory structure (SC) is seen to the left. Below the thin tegument are seen the basal lamina (BL) and muscle fibres (MU) with large mitochondria (M). Inset, middle: sensory structure composed of a short cilium surrounded by tegument. Inset, right: tripointed peripheral spines and thin microvilli. B, section perpendicular to the flattened spines. C, longitudinal section through area near the ventral sucker showing the relatively thick tegument and subjacent circular muscles (CM). Mitochondria and various more or less electron-dense vesicles are seen in the tegument.

(Fig. 4D). The cyst wall was thick and multi-layered. The ventral sucker had developed an anterior two-divided projection, and the sucker ratio was 1 : 1.9 to 1 : 2.4 averaging 1 : 2.2.

Two months after encystment the microvilli had disappeared (Fig. 5D, E). Otherwise the two-month old metacercariae appeared identical with the one-month old specimens. Short isolated cilia were found anterior to the mouth, and ventrally and posteriorly to the mouth opening occurred low papillae with up to six short cilia. No dead metacercariae were found. Most cysts were surrounded by a thin more or less brown capsule of host cells.

The cyst of two-month old metacercariae measured 230-320 × 210-270 μm (mean of 10 cysts: 270 × 230 μm). Excysted two-month old metacercariae were 270-350 μm (mean: 325 μm) long and 160-190 μm (mean: 170 μm) wide (Fig. 1B). The oral sucker was 50-60 μm (mean: 54 μm) and the ventral sucker 110-140 μm (mean: 120 μm) in diameter. The ventral sucker had a prominent anterior two-divided projection. The diameter of the pharynx was 26-32 μm (mean: 30 μm). The cephalic glands and ducts were still present. There was a small prepharynx and an about 30 μm long oesophagus. The caeca extended to the posterior end of the body. Small lipid droplets were scattered throughout the body. The excretory vesicle was spherical to triangular depending on the state of contraction. The number and shape of the cells lining the excretory vesicle were as in the cercaria. The flame cell formula was as in the cercaria. A weak outline of the testes was seen; otherwise the reproductive system was undeveloped.

The six-month old metacercariae did not differ from the two-month old specimens. A few per cent of the metacercariae were dead.

Only about 15 % of the metacercariae were still alive one year after infection. They differed from the six-month old metacercariae by being more shrunken and in having more distinct cephalic glands apparently filled with a homogeneous mass. Most empty cysts or cysts with remnants of a metacercaria were smaller than cysts containing living metacercariae; they were often more or less collapsed. The cyst wall was opaque and dark brown, whereas the cyst wall of living metacercariae was translucent and colourless. The cysts of living or recently dead metacercariae were surrounded by a thick brown capsule of host cells.

The metacercariae from naturally infested *C. allmani* were slightly larger than those from the experimental infections. Ten randomly selected cysts measured 300-400 × 250-350 μm. Excysted metacercariae were 320-430 μm long. The oral sucker was 50-60 μm and the ventral sucker was 120-160 μm in diameter. Otherwise they were identical with the two-month old metacercariae. The greater size and the fact that no dead metacercariae or encapsulated cysts were found indicate that *C. allmani* may be a more suitable host than *C. crangon*.

Metacercariae of *P. reflexa* have previously only been recorded by Uspenskaya

(1960, 1963). She found the metacercariae in six species belonging to the genera *Hetairus*, *Eualus*, *Spirontocaris* and *Pandalus* (Decapoda, Natantia) from the Barents Sea. Excysted metacercariae were 325-525 μm long. The oral sucker was 50 × 60-50 × 75 μm. The ventral sucker which measured 110 × 105-185 × 196 μm protruded enormously from the surface of the body. The sucker ratio was 1 : 2-3. The reproductive system was poorly developed. Although shrimps were studied all the year round no mature specimens were found. She found one adult *P. reflexa* with five eggs three days after the metacercariae from naturally infested shrimps were given to small butterfish *Pholis gunnellus* (L.).

Experimental infection of the fish host

It was first suggested that *C. buccini* would develop into a species belonging to the genus *Plagioporus*. Therefore some of the fishes which according to Dawes (1947) are hosts for this genus were used as experimental hosts. Two 0-group and two adult plaice *Pleuronectes platessa* L., two 0-group and two adult flounders *Platichthys flesus* (L.), one adult spotted dragonet *Callionymus maculatus* Rafinesque-Schmaltz, in addition to two adult dabs *Limanda limanda* (L.) and two goldsinnies *Ctenolabrus rupestris* (L.) were given *C. crangon* with two to four months old metacercariae. Each fish was fed on 2-4 infected shrimps once a week for four weeks in addition to frozen shrimps and mussels. When dissected one week after the last ingestion of infected shrimps none were infected with *Plagioporus* or related genera. Two large eelpouts *Zoarches viviparus* (L.) and two cods *Gadus morhua* L. (25-30 cm long) were also given infected shrimps. When dissected only *G. morhua* was infected with opecoelid trematodes. Between 30 and 50 specimens were found in the pyloric caeca, and they were identified as *Podocotyle reflexa*. Nearly all specimens contained eggs. The experimental infection was repeated with three cods (25-30 cm), which were fed only once with infected shrimps. They were dissected three, five and seven days after ingestion of the infected shrimps and found to contain between 30 and 60 *P. reflexa*.

Natural infestation of the fish host

About 30 % of *G. morhua* from Øresund (28 m) and western Kattegat (10-30 m) were infested with *P. reflexa*. Small and mature specimens were found throughout the year. The intensity was usually 1-4, but up to 40 were occasionally found. Four of nine four-bearded rocklings *Rhinonemus cimbrius* (L.) from western Kattegat (30 m) were also infested with *P. reflexa*. The intensity was 1-20. Four of ten lumpsuckers *Cyclopterus lumpus* L. from Øresund (Feb. 1981) contained 1-4 mature *P. reflexa*.

Podocotyle reflexa from the fish host

Three days after infection *P. reflexa* taken from the pyloric caeca of cod were 0.7-0.8 mm long (Fig. 6A). Compared with the developed metacercariae the area posterior to the ventral sucker and the testes had enlarged enormously.

The five-day old specimens were up to 1.0 mm long. The hindbody had become more elongated (Fig. 7A).

The seven-day old specimens were 1.2-1.4 mm long and contained up to three eggs (Fig. 6B). The testes, which were nearly as wide as the hindbody, occurred close together with no vitellaria in between. The cirrus sac extended to or even behind the middle of the distance between the posterior edge of the ventral sucker and the ovary. The ventral sucker had changed into a complicated structure with lappets antero-laterally and small pockets postero-laterally (Fig. 7B). Hemispherical structures, probably sensory, were regularly arranged on the muscular edge of the sucker, short cilia occurred more peripherally. The spineless surface was corrugated with circumferential folds. The metacercarial sensory structures which occurred anteriorly and ventrally still existed. Compared with the metacercariae the number of cilia anterior to the mouth opening had increased.

The largest specimens from the first experimental infection, which may be up to five weeks old, were nearly 3 mm long. They had up to 40 eggs (Fig. 6C). They were identical with *P. reflexa* found in naturally infested *G. morhua*, although these specimens were up to 4.5 mm long. *P. reflexa* from *C. lumpus* were 4-6 mm long. Flattened elongated specimens were often recurved, a character which is alluded to in the name of the species.

Mature *P. reflexa* has been adequately described previously (e.g. by Odhner 1905, Manter 1926 (described as *P. olssoni* Odhner, 1905), Miller 1941 and Brinkmann 1975). Brinkmann (1975) gives a list of synonyms of *P. reflexa*.

Podocotyle atomon

The life-cycle of *P. atomon* was elucidated by Hunninen & Cable (1943), and the different developmental stages have been described repeatedly. Therefore, in the following, only new observations and observations necessary for a comparison between *P. atomon* and *P. reflexa* are mentioned.

The cercaria of P. atomon compared with that of P. reflexa

The cercaria of *P. atomon* has been recorded from *Littorina* spp. and *Lacuna* spp. from arctic-boreal areas. The most common hosts are *L. saxatilis* (Olivi) and *L. rudis* (Maton). The cercaria has been described several times (Hunninen & Cable 1943, Uspenskaya 1963, Chubrik 1966, James 1969, Reimer 1970, and Szuks 1975).

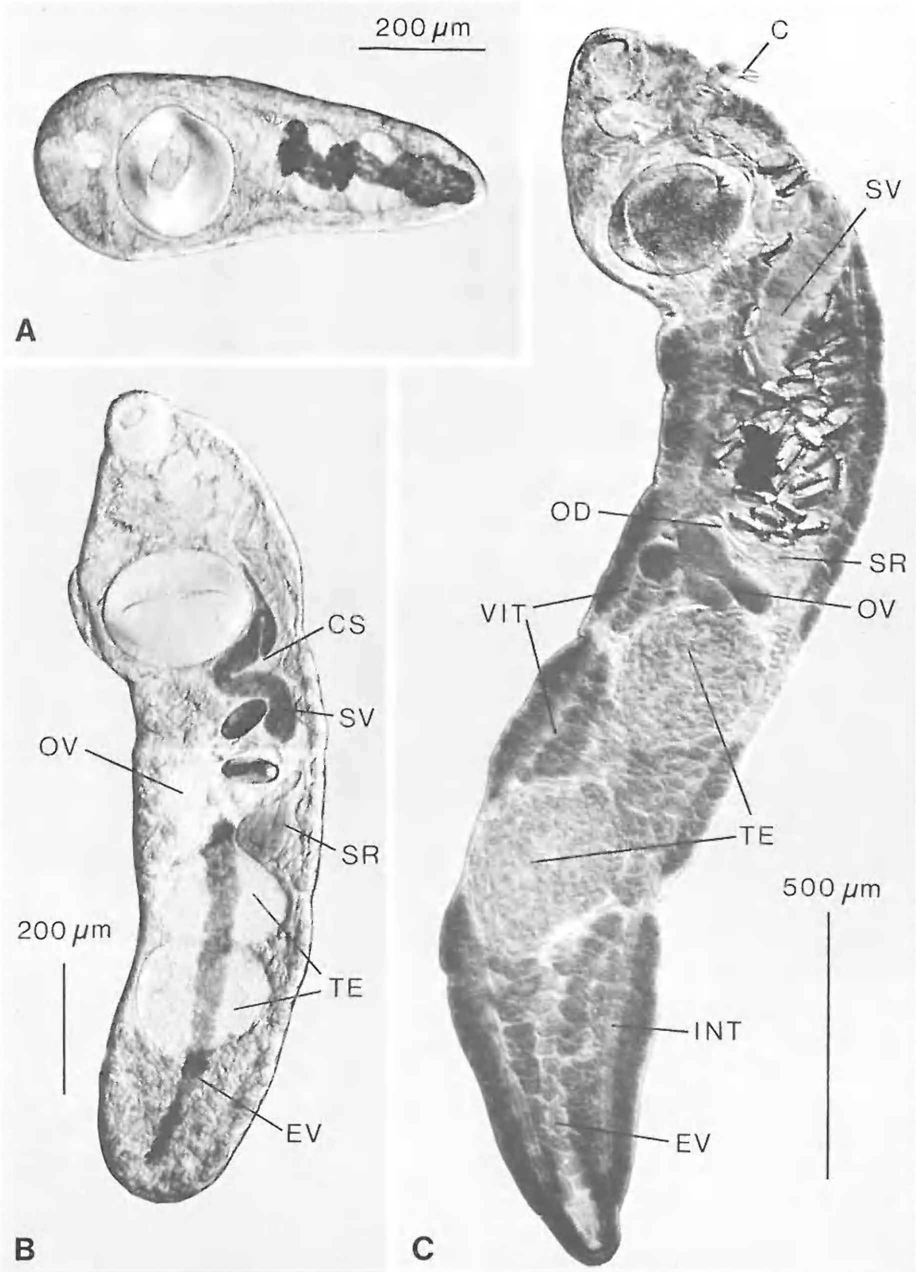

FIG. 6. *Podocotyle reflexa* from experimentally infected cod, *Gadus morhua*. A and B, living flattened specimens. A, a three-day old specimen. B, a seven-day old specimen. C, stained flattened specimen of unknown age, but less than five weeks old. C: cirrus, CS: cirrus sac, INT: intestinal caecum, OD: oviduct, OV: ovary, SR: seminal receptacle, SV: seminal vesicle, TE: testis, VIT: vitellaria.

In Danish waters *L. saxatilis* from the islands of Hirsholmene in the western Kattegat off Frederikshavn were not infested with *P. atomon*. About 1 % of *L. saxatilis* collected in Nivå Bay, Øresund, and 10 % of *L. saxatilis* from northern Bornholm, the Baltic, were infested with *P. atomon*. About 300 snails were

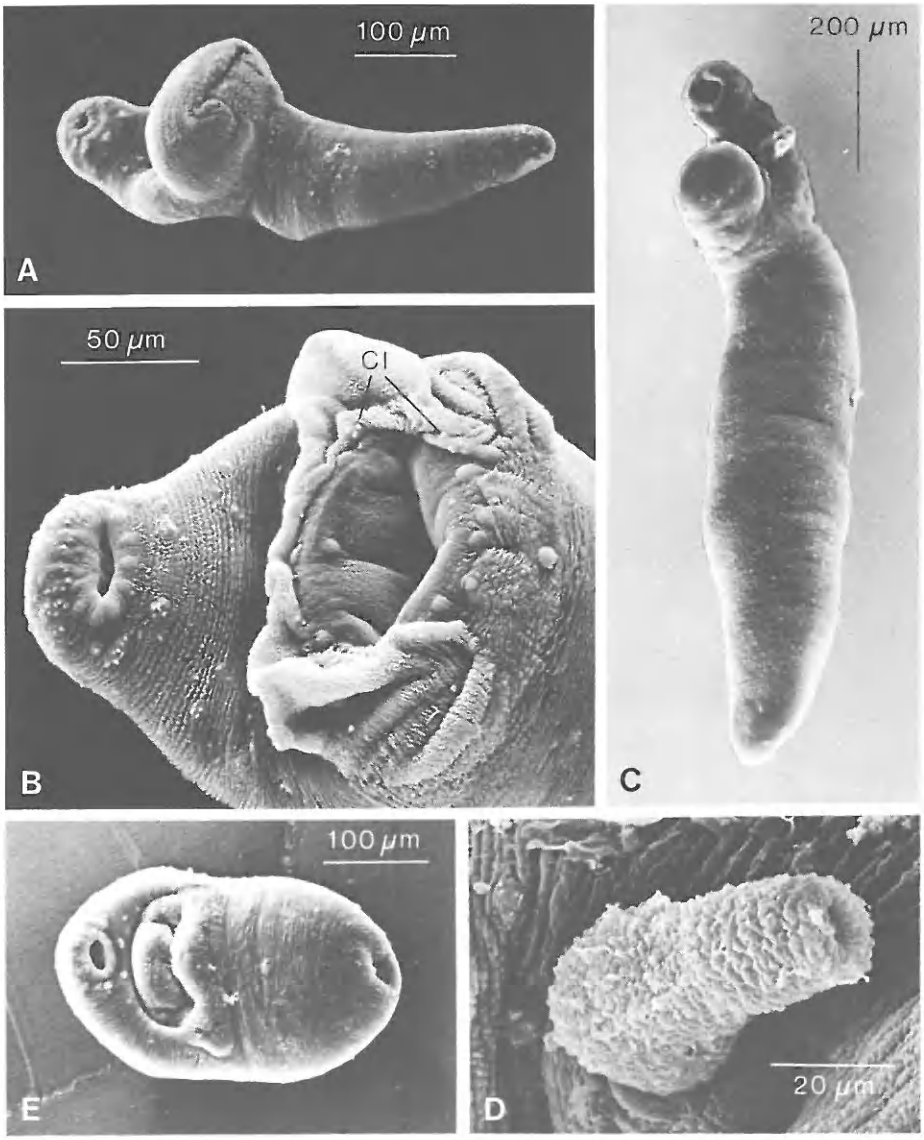

FIG. 7. *Podocotyle reflexa* from cod (A-D) and four-bearded rockling, *Rhinonemus cimbrius* (E). A, a five-day old specimen with everted ventral sucker. B, a seven-day old specimen with partly everted ventral sucker. C, a specimen with protruded cirrus from a naturally infested cod. D, partly protruded cirrus of another specimen. E, immature specimen with retracted ventral sucker.

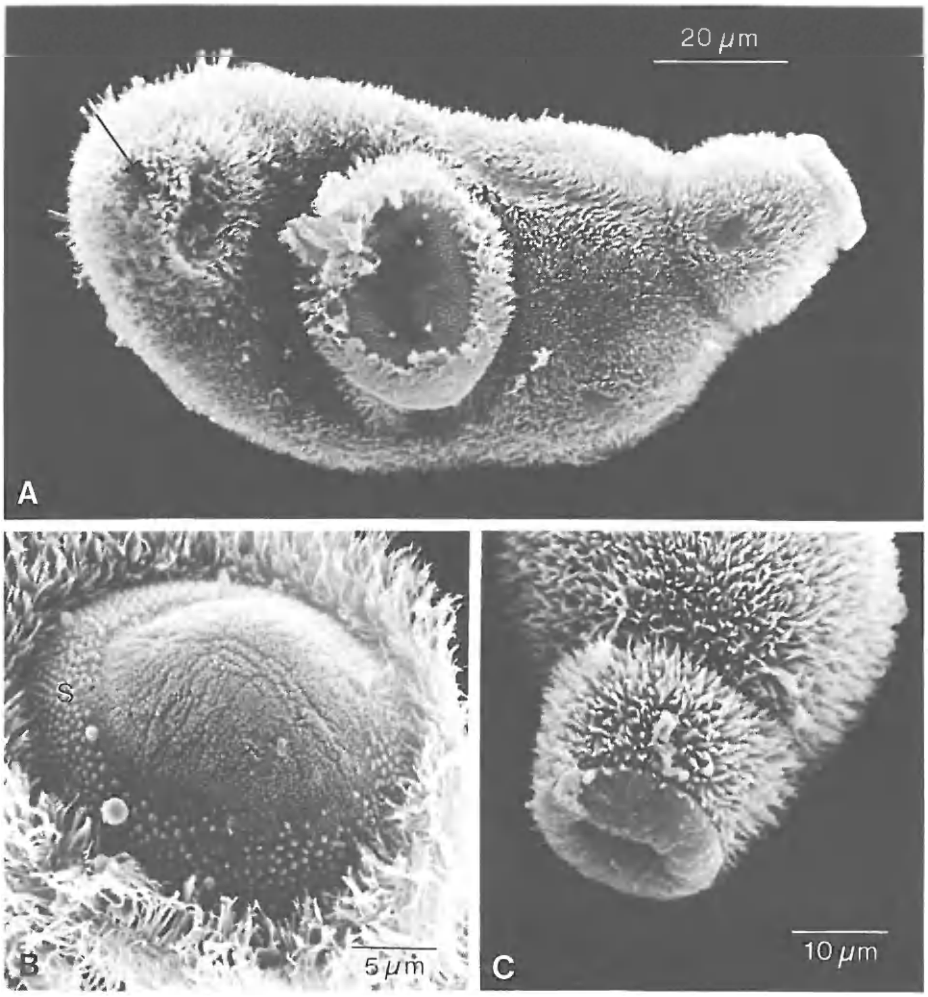

FIG. 8. Naturally released cercariae of *Podocotyle atomon*. A, ventral view. Arrow shows opening of stylet pocket. B, everted ventral sucker showing microvilli and spines. C, posterior part of cercarial body and tail.

studied from each locality. From Kattegat and Øresund only snails with a shell longer than 8 mm were studied; in the Baltic only snails with a shell longer than 5 mm were studied.

The main differences between the cercariae of the two *Podocotyle* species are that the double-pointed stylet of *P. atomon* (Fig. 9A) is more narrow than the stylet of *P. reflexa*, and that *P. atomon* only has three pairs of cephalic glands, whereas *P. reflexa* has at least five pairs. In addition, the cercaria of *P. atomon* has fewer tail glands which, furthermore, differ in shape from those of *P. reflexa*.

However, the greatest difference between the two cercarial species pertains to the external surface. That of *P. reflexa* is nearly smooth, whereas that of *P. atomon* is provided with about 5 μm long thin microvilli (Fig. 8). The only areas not covered with long microvilli are the distal edge of the tail and the central area of the suckers. The peripheral spines in the suckers are smaller than the similar spines of *P. reflexa*. Two sensory structures occur anterior in the oral sucker, and other sensory structures are found in the spined area of the suckers. The types of these structures could not be identified.

The metacercaria of P. atomon compared with that of P. reflexa

Metacercariae of *P. atomon* have previously been found in various amphipods (Levinsen 1881, Hunninen & Cable 1943, Uspenskaya 1960, 1963, James 1969, Shotter 1969, Reimer 1970, Szuks 1975, Valter 1977), in isopods (Uspenskaya 1960, 1963, Reimer 1970, Szuks 1975) and in mysids (Shotter 1969, Reimer 1970, Szuks 1975). The metacercaria has been described by Hunninen & Cable (1943), Uspenskaya (1963), James (1969), Reimer (1970) and Szuks (1975).

About 10 % of the *Gammarus* spp. living in the vicinity of the infested *L. saxatilis* in Nivå Bay, Øresund, and about 80 % of *G. duebenii* Lilljeborg from

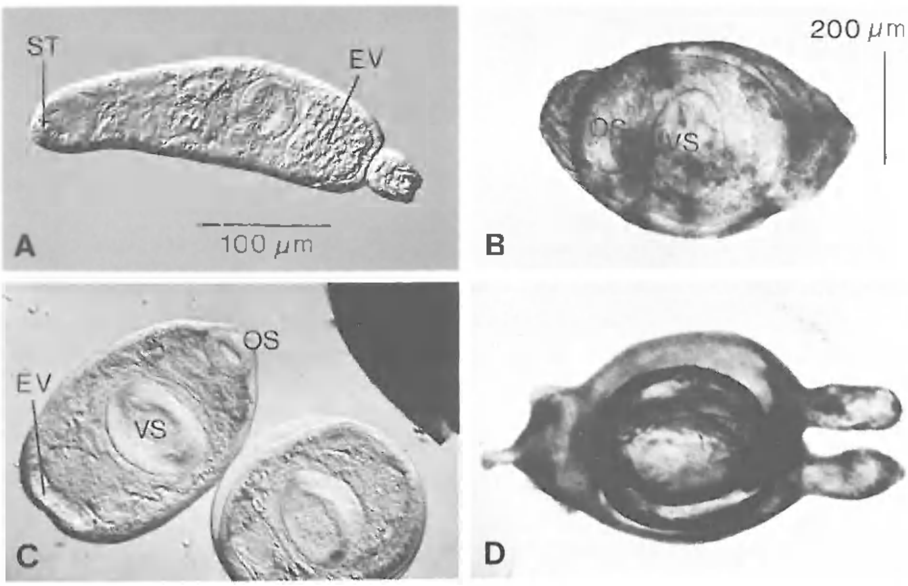

FIG. 9. Cercaria (A) and metacercariae (B-D) of *Podocotyle atomon*. B-D to same scale. A, a mature cercaria. B, a five-day old metacercaria in transformed muscle of *Gammarus*. C, one-month old metacercariae. The metacercaria to the left is excysted, the other is surrounded by the thin cyst wall. Part of the outer 'cyst' is seen upper right. D, an apparently empty outer 'cyst' from a naturally infested *Gammarus*.

the rocky shores of northern Bornholm, were infested with metacercariae of *P. atomon*. The intensities were 1-2 and 1-12, respectively. A few *Idothea* sp. from Nivå Bay and unidentified mysids from shallow water in Øresund were also infested with *P. atomon*. No metacercariae were found in western Kattegat.

Only *Gammarus* sp. and unidentified mysids were used as experimental hosts. Both were easily infected after exposure to cercariae for 20 hours. *C. crangon* were repeatedly exposed to cercariae of *P. atomon*, but with negative results. The growth of the metacercariae varied enormously, not only according to the kind of crustacean, but also in the same host specimen. The metacercariae were found in all parts of the body of the mysids, whereas most metacercariae in *Gammarus* were found in the muscles dorsal to the intestine, similar to metacercariae in naturally infested *Gammarus*. The development in mysids were more slowly than in *Gammarus* and was only followed for one week. No brown outer 'cyst' was developed within this time.

All metacercariae which occurred dorsal to the intestine in *Gammarus* were surrounded by an elongated, spindle-shaped or three-pointed outer 'cyst' which with increasing age became darker and harder (Figs 9B-D, 11B, C). The cercariae which had encysted in other parts of *Gammarus* did not develop this outer 'cyst'; they stopped development and died within one or two weeks. The brown 'cysts' were visible through the host cuticula. Up to three encysted metacercariae were found in the same 'cyst'.

Study of experimentally infected *Gammarus* showed that the outer 'cyst' is a transformed muscle which surround the smooth metacercarial cyst (Fig. 11B). A few days after encystment inside the muscle, the muscle is detached and dies within about one week. Nuclei may still be identified in stained preparations up to two weeks after infection. This outer 'cyst' did not appear to harm the metacercaria, as empty 'cysts' rarely were found.

James (1969) found 'an extremely hard fibrous substance which is probably secreted by the host' in experimentally infected amphipods. Uspenskaya (1963) who studied *G. locusta* (L.) found that the host reaction resulted in formation of the external cyst which was described as a layer of brown pigment with an irregular outline. Cross-sections of the compact pigment structure did not show lamellar cell formations. Szuks (1975) found that the metacercariae in gammaridean amphipods had 'eine unförmige rötliche Hülle' which was absent in infested *Idothea*.

In the present material no progenetic metacercariae were found. Progenetic metacercariae of *P. atomon* were found by Hunninen & Cable (1943), James (1969), Reimer (1970), and Szuks (1975). According to Szuks (l.c.) progenetic metacercariae do not develop in *Gammarus* due to the hard outer cover, whereas they frequently occurred in *Idothea*.

Gammarus dissected 24 hours after exposure to the cercariae released tailless but unencysted cercariae. Most cercariae had lost all the microvilli (Fig. 10A).

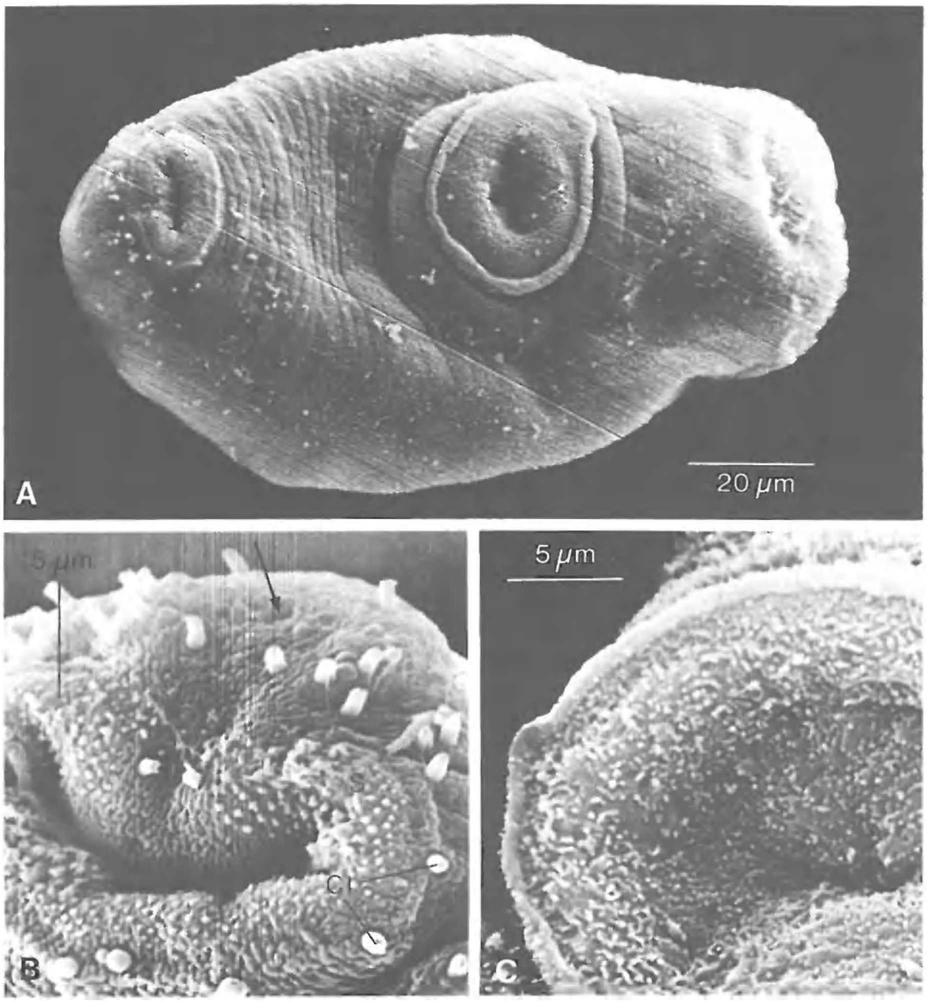

FIG. 10. Metacercariae of *Podocotyle atomon*. A, unencysted tailless specimen removed from tissue of *Gammarus* 24 hours after exposure. All microvilli are lost. B, the oral sucker of a specimen as that in A. Arrow shows opening of stylet pocket. C, the ventral sucker of a four-day old metacercaria.

Free microvilli indicate that the microvilli are shed and not absorbed. Sensory structures are revealed lateral and anterior to the mouth opening (Fig. 10A, B). Each structure consists apparently of a cilium surrounded by a tube-like tegumentary collar.

48-hour old excysted metacercariae appeared more shrunken, probably due to emptying of the cystogenous glands.

All four-day old metacercariae had lost the spines, and small microvillus-like projections covered the whole surface (Fig. 11C).

The surface of one and two weeks old metacercariae were covered with small microvillus-like projections. Semispherical structures had developed on the ventral sucker (Fig. 11A). The sensory structures found in the cercaria and young metacercariae had disappeared.

The microvillus-like projections had disappeared four weeks after infection, and these metacercariae were morphologically undistinguishable from the smallest specimens found in fishes.

Apart from the different choices of hosts, the most important differences between the metacercariae of *P. reflexa* and *P. atomon* are, as also pointed out by Uspenskaya (1963), the size and the different degree of development of the reproductive system. Excysted metacercariae of *P. reflexa* rarely exceed 0.5 mm in length, whereas those of *P. atomon* may be more than 1.0 mm long. The reproductive system of the metacercariae of *P. reflexa* is always undeveloped, whereas metacercariae of *P. atomon* may contain several eggs which also may

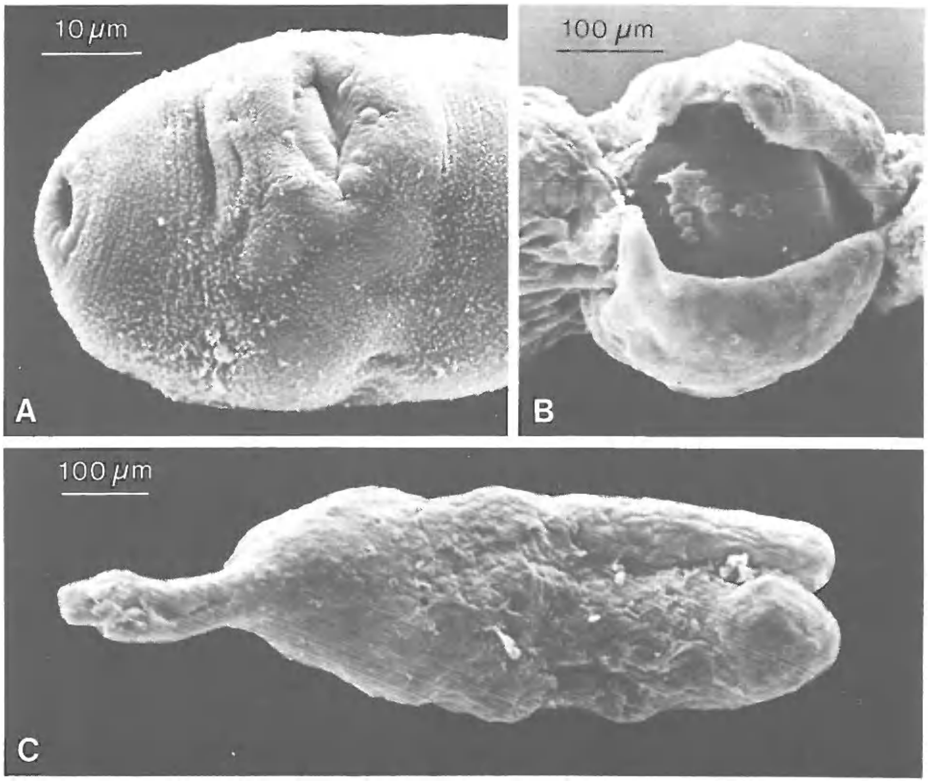

FIG. 11. Metacercariae of *Podocotyle atomon*. A, the anterior end of a two-week old excysted metacercaria. B, part of an opened outer 'cyst' from a naturally infested *Gammarus* showing the smooth metacercarial cyst. C, an outer 'cyst' from a naturally infested *Gammarus*. The metacercarial cyst is not seen, but occurs inside the transformed muscle.

occur free in the cyst. Uspenskaya (1963) also pointed out that the sucker ratio of the metacercaria of *P. reflexa* is 1 : 2-3, whereas it is 1 : 1.5-2 in *P. atomon*. The cephalic glands of the metacercaria of *P. reflexa* remain, whereas they are replaced by numerous smaller unicellular glands in *P. atomon*. In addition, the prominent two-divided projection anterior in the ventral sucker of *P. reflexa* is absent in the metacercaria of *P. atomon*.

The adult P. atomon compared with adult P. reflexa

Mature *P. atomon* has been described by e.g. Odhner (1905), Lebour (1908), Hunninen & Cable (1943), Sculman-Alboa (1952), Polyanski (1955), and Szuks (1975).

Cods and flounders from Øresund (28 m) are rarely infested with *P. atomon*, and the intensity is always less than 10 for flounder and only 1-2 for cod. Two cods (25-30 cm) and two flounders (25-30 cm) from Øresund (28 m) were used as experimental hosts. They were offered large numbers of *Gammarus* infected with four to six weeks old metacercariae of *P. atomon* for a few hours. One cod and one flounder were dissected five days after infection, the remaining two fish ten days after infection. The fishes contained between 20 and 50 *P. atomon*. In flounders they were found in the intestine. In cods two thirds occurred in the pyloric caeca and the remaining in the anterior part of the intestine. No differences in size and degree of development were observed in the two host species. The smallest specimens were of the same length as most metacercariae, i.e. about 0.5 mm long (Fig. 12A). Such small specimens occurred in both the five and ten days old infections. In worms without eggs or with a few eggs the testes were nearly as wide as the hindbody with no vitellaria in between (Fig. 12B). The largest worms in the ten-day old infections were 1.4 mm long and contained up to ten eggs. The specimens from the experimental infections did not differ from worms from naturally infested fishes, although these were up to 3.5 mm in length. Mature worms showed great morphological variations even in the same host species (Fig. 12 C-E).

The external surface of *P. atomon* has circumferential furrows (Fig. 13A, C). The same types of sensory structures as found in *P. reflexa* occur near the mouth opening (Fig. 13C). The long thin cirrus is covered with small knobs (Fig. 13B).

As seen from the descriptions of *P. reflexa* and *P. atomon* it may be difficult or impossible to distinguish between the two species if the worms are immature or have a few eggs and are less than about 1 mm long (Fig. 6A and Fig. 12B).

Brinkmann (1975) listed the main differences between the two species as follows: 1) The body of *P. atomon* is flattened, whereas that of *P. reflexa* is cylindrical. 2) The distance between the oral and ventral suckers is about 1/4 of body length in *P. atomon*, whereas it is about 1/7 in *P. reflexa*. 3) The testes of *P.*

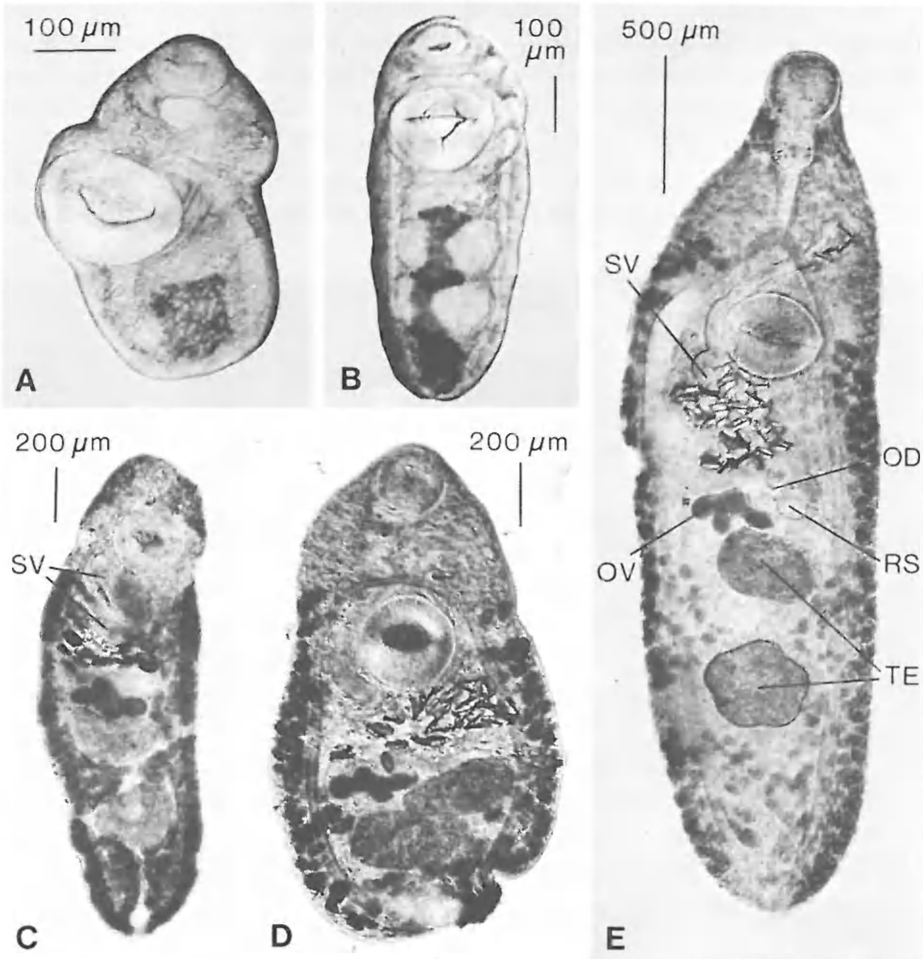

FIG. 12. *Podocotyle atomon* from cod (A-C) and flounder (D-E). A & B, living flattened specimens from experimentally infected cods. A, a five-day old specimen. B, a ten-day old specimen. C-E, stained flattened specimens from naturally infested fishes from Bornholm, the Baltic. For abbreviations see Fig. 6.

atomon are relatively small, not occupying more than half of the cross-section of the body, whereas those of *P. reflexa* are large, occupying the greater part of the body in cross-section. 4) The vitellaria of *P. atomon* are unbroken (covering the intestinal caeca) and do not come together between the testes. Body not constricted at level with the testes. The vitellaria of *P. reflexa* are broken lateral to each testis (uncovering the intestinal caeca) and come together between the testes. Body commonly constricted at level with the testes.

According to my observations the characters 1-4 may only be used for large mature specimens, and even then they are not always valid. The hindbody of living *P. reflexa* is extremely variable, changing from being short, wide and flattened, and to being cylindrical, long and thin. In some of the large *P. atomon* from cods from Bornholm the distance between the suckers was $1/5$ of the body length, and in flattened specimens the testes were nearly as wide as the hindbody. No. 4 is not always valid, as the vitellaria often are broken lateral to the testes (Fig. 12E) and often occur between the testes (Fig. 12C). Heller (1949) described how a slight pressure caused the yolk follicles to be pushed into the

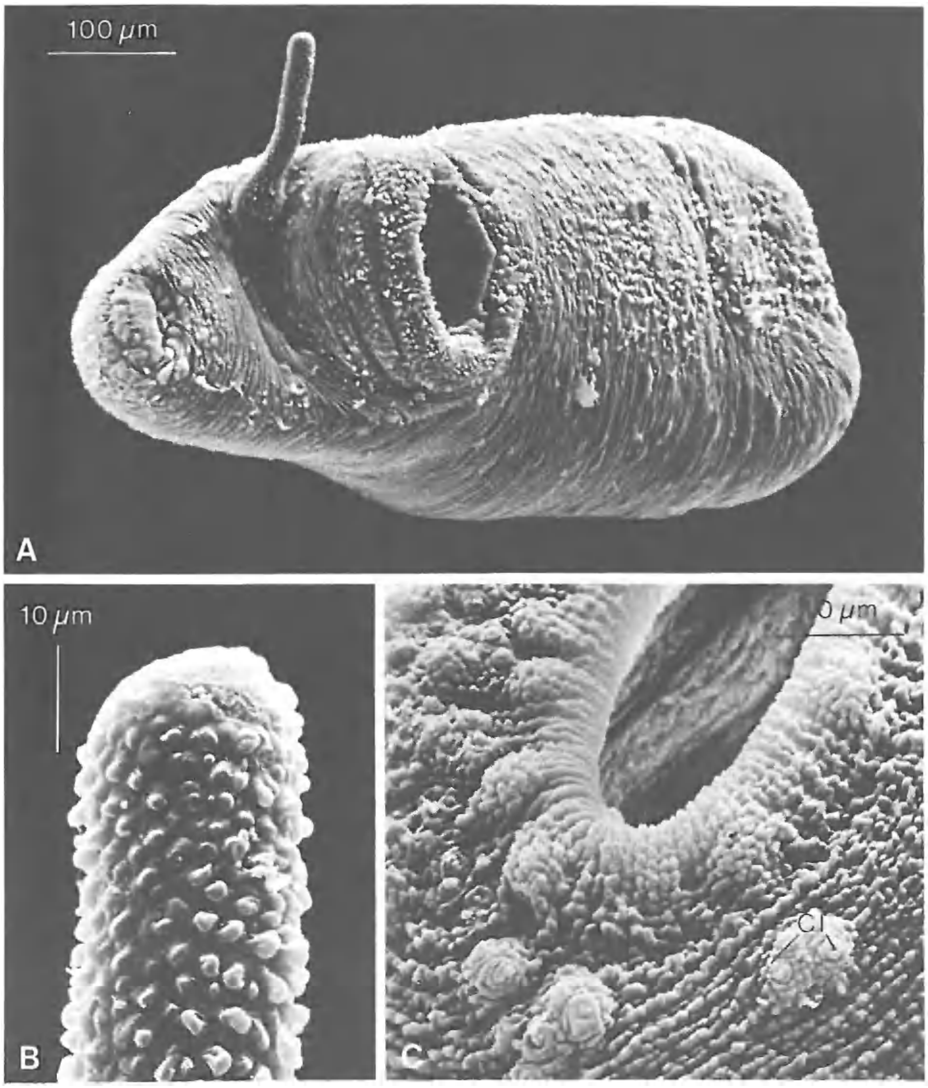

FIG. 13. Mature *Podocotyle atomon* from naturally infested fishes. A, a specimen with protruded cirrus from a cod. B, detail of the cirrus. C, detail of oral sucker of a specimen from a flounder.

intertesticular space of *P. atomon*. Nicoll (1909) wrote that the vitellaria are extremely variable and may be entirely absent between the testes or may fill up a considerable part of the intertesticular space, and that they are fairly often discontinuous laterally at the level of the intertesticular space or the posterior testis. In addition, Nicoll (1909) described how killing in fresh water changed *P. atomon* so the condition of the parasite agreed much more closely with Odhner's description of *P. reflexa* than with his description of *P. atomon*.

The life-cycles

In Øresund *B. undatum* and *N. antiqua* only live at depths greater than about 15 metres. *L. saxatilis*, on the other hand, lives in the littoral zone, i.e. in Øresund the cercariae of *P. reflexa* and *P. atomon* are separated vertically. A certain scattering by the crustacean hosts may not be excluded, but seems to play a minor role as crustaceans caught in deep water (28-30 m) have not been found infested with *P. atomon*, and crustaceans caught in shallow water were never found infested with *P. reflexa*. A vertical and horizontal scattering of both species by mysids is, however, a possibility.

Both species have been recorded from a wide variety of hosts with apparently no preference for certain families. Several fish species harbour both *Podocotyle* species. However, the unsuccessful experimental infections with *P. reflexa* indicate that the presence of the parasite species may not only depend on the food of the fish or the distribution of the fish.

The fish or the parasite may behave differently in different areas. Polyanski (1955) found *P. reflexa* in eelpout, whereas I did not succeed in infecting this fish.

Fishes living in brackish shallow water, e.g. eelpout, flounder and sea-snails, *Liparis* spp., become infested with *P. atomon* only. Large cods caught in deep water (28-30 m) in western Kattegat harbour *P. reflexa* only, whereas small cods living in shallow water in Øresund may contain *P. atomon* only. However, some cods caught in Øresund are infested with both species. In the Baltic near Bornholm neither *B. undatum* nor *N. antiqua* occur, and cods from this area harbour *P. atomon* only.

It has been pointed out that *P. atomon* in arctic areas is characteristic of littoral fishes, whereas *P. reflexa* mostly is found sublittorally in the open sea (Polyanski 1955, Zhukov 1960, Strelkov 1960, Uspenskaya 1963).

DISCUSSION

The cercaria of *P. reflexa* was described as *Cercaria buccini* by Lebour (1911). She found it in four of 80 *B. undatum* from the English coast. Rees (1935) suggested that a cotylomicrocercous cercaria found in *Littorina littorea* (L.)

was identical with *C. buccini* Lebour, 1911, but a later comparison with living cercariae from *B. undatum* revealed some differences (Rees 1936). The cercaria from *L. littorea* was named *C. littorinae*. In a survey of cercariae from Littorinidae James (1968) shows drawings of *C. littorinae* Rees, 1936 and another cotylomicrocercous cercaria which he mentions as *C. buccini* Lebour, 1911. This cercaria is, however, not identical with *C. buccini* from *B. undatum*.

In *B. undatum* from Egedesminde, western Greenland, Levinsen (1881) once found a cercaria which differed in small details from *C. cotylura* Pagenstecher, 1862 - a cotylomicrocercous cercaria very similar to *C. buccini*. He did not describe or figure the cercaria, which probably is identical with *C. buccini* as no other cotylomicrocercous cercaria is recorded from this snail. In addition, the adult *P. reflexa* is recorded from the same area (Brinkmann 1975).

C. buccini has only been recorded from members of the family Buccinidae. Chubrik (1966) found one of 35 *Neptunea despecta* (L.) from the Barents Sea infested with *C. buccini*. In addition, Lauckner (1980) states that 0.55 % of 910 *B. undatum* dredged off Helgoland, the North Sea, were infested with *C. buccini*.

Gibson (1974) and Lo *et al.* (1975) studied the ultrastructure of other opecoelid cercariae. These cercariae had complicated suckers like the cercariae of *P. reflexa* and *P. atomon*. Lo *et al.* suggested that the suckers may be concerned with host finding, attachment or penetration. Gibson suggested that the suckers may function as organs of nutrition as well as organs of attachment. It is obvious that the suckers of the cercariae of *P. reflexa* and *P. atomon* also are fitted for these uses.

The presence of a fringe of microvilli around one or both suckers has been observed in cercariae belonging to other families than Opecoelidae (Køie 1971 c, 1976, 1978, Ždárská 1977). The microvilli are lost – either shed or resorbed – during the stay in the second intermediate host, and in the case of *P. atomon* already before encystment. This indicates that their function may be associated with absorption of nutriments during the migration in the snail tissue, or that they may facilitate migration in the molluscan or second intermediate hosts. The microvilli which cover the surface of the cercaria of *P. atomon* may have the same functions. No cercaria has previously been described with long tegumentary microvilli.

Sensory structures composed of an isolated cilium or composed of a pit with several cilia are common in trematodes. The type with a cilium completely covered by tegument has apparently previously only been described from adult *Schistosoma mansoni* (see Hockley 1973).

A similar increase in the tegumentary surface as found for young metacercariae of *P. reflexa* and *P. atomon* has previously been described for other opecoelid metacercariae (Lo *et al.* 1975, Popiel 1976), but also for metacercariae belonging to other families (see Køie 1977). All these metacercariae have the common feature that they grow inside the intermediate host, and the fact that the microvilli or other kinds of tegumentary processes disappear when the growth is completed suggests that the increase of the surface area is associated with absorption of nutriments.

Due to the great similarity between mature *P. atomon* and *P. reflexa* the latter has by e.g. Reimer (1970) and Szuks (1975) been considered a synonym of *P. atomon*. Dawes (1947) regarded *P. reflexa* as a somewhat dubious species.

The type-host of *P. reflexa* is the lumpsucker, *Cyclopterus lumpus* L., and the type-locality is the middle part of the Baltic off the coast of East Germany. Neither *B. undatum* nor *N. antiqua* live in this part of the Baltic showing that *C. lumpus* must have been infested in an area with more saline water.

In Danish waters *P. atomon* and *P. reflexa* are the only *Podocotyle* species identified.

Pritchard (1966) listed 17 *Podocotyle* species from fishes from the colder waters of the Pacific and Atlantic Oceans and adjacent cold-water areas. Some of these species have already been listed as synonyms, and the number will undoubtedly be further reduced in the future.

Elucidation of life-cycles and comparison of the larval stages may apparently be the only methods by which it is possible to separate such morphologically variable species as those of *Podocotyle*. Ching (1979) worked out experimentally the life-cycle of *P. endophrysi* Park, 1937 from Vancouver, the Pacific coast of Canada. The larval species were described as similar to those of *P. atomon*, and this species has been found in fishes from the same area. The first host of *P. endophrysi* was the snail *Lacuna marmorata* Dall. An amphipod, *Hyale plumulosa* Stimpson, was used as second intermediate host. Cercariae of *P. atomon* has been recorded from two *Lacuna* species from the Barents Sea (Chubrik 1966), and the amphipod *H. nilssoni* Rathke was found naturally infested with *P. atomon* in Wales, Great Britain (James 1969). According to Park (1937) *P. endophrysi* differs from *P. atomon* in the following characters: 1) Seminal vesicle sinuous instead of being coiled, 2) anterior part of cirrus sac protruded instead of unprotruded, 3) testes larger, close together instead of being smaller and separated by a considerable distance or by vitellaria. However, I found that these characters also are valid for *P. atomon*. The protruded part of the cirrus sac of *P. endophrysi* is covered with minute spinules (Park 1937). These spines could not be found on a paratype specimen of *P. endophrysi* studied by Pritchard (1966). The knobs found on the protruded cirrus of *P. atomon* in my material may, using light microscopy, be interpreted as small spines. The morphology of the mature *P. endophrysi* figured by Park (1937) and Ching (1979) falls within the range of variations found in *P. atomon*.

MacKenzie & Gibson (1970) found *Podocotyle* sp. and *P. atomon* in flounders from Scotland. *Podocotyle* sp. differed from *P. atomon* in the length to breadth ratio, size of testes and ovary, length of cirrus sac, ratio of oral to ventral sucker and other unmentioned characters. However, the drawings of the two species show that both fall within the range of variations found in *P. atomon*. Although no experimental infections apparently were carried out the above mentioned *Podocotyle* sp. was later (Gibson 1974) named *P. staffordi* Miller, 1941. The cercaria suggested to develop into *P. staffordi* was found in *L. saxatilis*, but differed from the cercaria of *P. atomon*. *P. staffordi* has been considered a synonym of *P. atomon* (see Heller 1949, Pritchard 1966).

REFERENCES

BRINKMANN, A. JR., 1975. Trematodes from Greenland. – Meddr Grønland 205(2): 1-88.

CHING, H.L., 1979. The life cycle of *Podocotyle enophrysi* Park, 1937 (Trematoda: Opecoelidae). – Can. J. Zool. 57: 1341-1344.

CHUBRIK, G.K., 1966. Fauna and ecology of trematode larvae from molluscs in the Barents and White Seas. – Trudy murmansk. biol. Inst. 10(14): 78-166. (In Russian).

DAWES, B., 1947. The Trematoda of British Fishes. – Ray Society, London, 364 pp.

GIBSON, D.I., 1974. Aspects of the ultrastructure of the daughter-sporocyst and cercaria of *Podocotyle staffordi* Miller, 1941 (Digenea: Opecoelidae). – Norw. J. Zool. 22: 237-252.

HELLER, A.F., 1949. Parasites of cod and other marine fish from the Baie de Chaleur region. – Can. J. Res. 27: 243-264.

HOCKLEY, D.J., 1973. Ultrastructure of the tegument of *Schistosoma*. – Adv. Parasitol. 11: 233-305.

HUNNINEN, A.V. & R.M. CABLE, 1943. The life history of *Podocotyle atomon* (Rudolphi) (Trematoda: Opecoelidae). – Trans. Am. microsc. Soc. 62: 57-68.

JAMES, B.L., 1968. The distribution and keys of species in the family Littorinidae and of their digenean parasites, in the region of Dale, Pembrokeshire. – Fld Stud. 2: 615-650.

JAMES, B.L., 1969. The Digenea of the intertidal prosobranch, *Littorina saxatilis* (Olivi). – Z. zool. Syst. EvolForsch. 7: 273-316.

KØIE, M., 1969. On the endoparasites of *Buccinum undatum* L. with special reference to the trematodes. – Ophelia 6: 251-279.

KØIE, M., 1971a. On the histochemistry and ultrastructure of the redia of *Neophasis lageniformis* (Lebour, 1910) (Trematoda, Acanthocolpidae). – Ophelia 9: 113-143.

KØIE, M., 1971b. On the histochemistry and ultrastructure of the daughter sporocyst of *Cercaria buccini* Lebour, 1911. – Ophelia 9: 145-163.

KØIE, M., 1971c. On the histochemistry and ultrastructure of the tegument and associated structures of *Zoogonoides viviparus* in the first intermediate host. – Ophelia 9: 165-206.

KØIE, M., 1976. On the morphology and life-history of *Zoogonoides viviparus* (Olsson, 1868) Odhner, 1902 (Trematoda, Zoogonidae). – Ophelia 15: 1-14.

KØIE, M., 1977. Stereoscan studies of cercariae, metacercariae, and adults of *Cryptocotyle lingua* (Creplin 1825) Fischoeder 1903 (Trematoda: Heterophyidae). – J. Parasit. 63: 835-839.

KØIE, M., 1978. On the morphology and life-history of *Stephanostomum caducum* (Looss 1901) Manter 1934 (Trematoda, Acanthocolpidae). – Ophelia 17: 121-133.

LAUCKNER, G., 1980. Diseases of molluscs: Gastropoda. Kap. 12: 311-424. – *In* O. Kinne (ed.): Diseases of marine animals. Vol. 1: General aspects. Protozoa to Gastropoda. Wiley (U.K.). 484 pp.

LEBOUR, M., 1908. Fish trematodes of the Northumberland coast. – Rep. scient. Invest. Northumb. Sea Fish Comm. (1907): 23-67.

LEBOUR, M., 1911. A review of the British marine cercariae. – Parasitology 4: 416-456.

LEVINSEN, G.M.R., 1881. Bidrag til Kundskab om Grønlands Trematodfauna. – Overs. K. danske Vidensk. Selsk. Forh. (1) **23**: 52-84.

LO, S.-J., J.E. HALL, P.A. ALLENDER & A.S. KLAINER, 1975. Scanning electron microscopy of an opecoelid cercaria and its encystment and encapsulation in an insect host. – J. Parasit. **61**: 413-417.

MACKENZIE, K. & D.I. GIBSON, 1970. Ecological studies of some parasites of plaice *Pleuronectes platessa* L. and flounder *Platichthys flesus* (L.). – Symp. Br. Soc. Parasit. **8**: 1-42.

MANTER, H.W., 1926. Some North American fish trematodes. – Illinois biol. Monogr. **10**: 1-138.

MILLER, M.J., 1941. A critical study of Stafford's report on 'Trematodes of Canadian Fishes' based on his trematode collection. – Can. J. Res., Ser. D, **19**: 28-52.

NICOLL, W., 1909. A contribution towards a knowledge of the Entozoa of British marine fishes. Part 2. – Ann. Mag. nat. Hist., Ser. 8, **4**: 1-25.

ODHNER, T., 1905. Die Trematoden des arktischen Gebietes. – Fauna arct. 4: 289-372.

PARK, J.T., 1937. A revision of the genus *Podocotyle* (Allocreadiinae), with a description of eight new species from tide pool fishes from Dillon's Beach, California. – J. Parasit. 23: 405-422.

POLYANSKI, Y.I., 1955. Parasites of the fish of the Barents Sea. – Trudy zool. Inst. Leningr. **19**: 5-170. (English translation: I.P.S.T. 1966, 158 pp.).

POPIEL, I., 1976. The ultrastructure of the metacercaria of *Cercaria stunkardi* Palombi, 1934 (Digenea: Opecoelidae) in an experimental intermediate host, *Amphithöe rubricata*. – Norw. J. Zool. **24**: 353-364.

PRITCHARD, M.H., 1966. A revision of the genus *Podocotyle* (Trematoda: Opecoelidae). – Zool. Jb. Syst. **93**: 158-172.

REES, W.J., 1935. The anatomy of *Cercaria buccini* Lebour, 1911. – Proc. zool. Soc. Lond. **2**: 309-312.

REES, W.J., 1936. The effect of parasitism by larval trematodes on the tissue of *Littorina littorea* (Linné). – Proc. zool. Soc. Lond. 2: 357-368.

REIMER, L.W., 1970. Digene Trematoden und Cestoden der Ostseefische als natürliche Fischmarken. – Parasit. SchrReihe **20**: 144 pp.

SHOTTER, R.A., 1969. A new second intermediate host, *Schistomysis spiritus* (Sars), for the digenean, *Podocotyle atomon* (Rudolphi). – Rep. mar. biol. Stn Port Erin **82**: 35-37.

SHULMAN-ALBOVA, R.E., 1952. On the question of variability in the digenetic fish trematode *Podocotyle atomon* (Rud.) Odhner, 1905. – Uchen. Zap. leningr. gos. Univ. No. 141, Ser. biol., Nauk No. 28: 110-126. (In Russian). Transl. Ser. Fish. Res. Bd Can., No. 831 (1966).

STRELKOV, J.A., 1960. Endoparasitic worms of marine fishes of East Kamchatka. – Trudy zool. Inst. Leningr. **28**: 147-196. (In Russian).

SZUKS, H., 1975. Zum Artsproblem bei digenen Trematoden, dargestellt am Beispiel der Gattung *Podocotyle* (Dujardin, 1845) Odhner, 1905. – Wiss. Z. Pädagog. Hochsch. 'Liselotte Herrmann' Güstrow **2**: 259-282.

USPENSKAYA, A.V., 1960. Parasitofaune des crustacés benthiques de la mer de Barents. – Annls Parasit. hum. comp. **35**: 221-242.

USPENSKAYA, A.V., 1963. Parasitic fauna of benthic crustaceans from the Barents Sea. – Acad. Nauk SSSR, Kolskij filial. Moskva, Leningrad, 128 pp. (In Russian).

VALTER, E.D., 1977. Study of the biology of trematodes of fish in the White Sea. – Mater. nauch. konferent. vses. Obshch. Gelmint. **29**: 8-11. (In Russian).

ŽĎÁRSKÁ, Z., 1977. Scanning electron microscopy of the cercaria of *Dicrocoelium lanceatum*. – Folia Parasit. **24**: 265-267.

ZHUKOV, E.V., 1960. Endoparasitic worms of the fishes in the Sea of Japan and South-Kuril shallow waters. – Trudy zool. Inst. Leningr. **28**: 3-146. (In Russian).