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On the morphology and life history of *Lecithaster gibbosus* (Rudolphi, 1802) Lühe, 1901 (Digenea, Hemiuroidea)

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Abstract. A previously undescribed cystophorous cercaria, which develops in the pyramidellid opisthobranch *Odostomia eulimoides* Hanley, was experimentally shown to be the cercaria of *Lecithaster gibbosus* (Rudolphi, 1802) Lühe, 1901, a common digenean in the intestine of marine teleosts in the North Atlantic Ocean and adjacent seas. The immotile cercariae are ingested by calanoid copepods. Pressure by the mouth parts triggers delivery tube eversion, with consequent injection of the cercarial body into the haemocoel. *Acartia* sp., *Pseudocalanus elongatus* and *Centropages hamatus* were experimentally infected, but the metacercariae became infective only in *Acartia* sp. The stickleback, *Gasterosteus aculeatus*, was the experimental final host. The infective cercaria of *L. gibbosus* differs from that of *L. confusus* Odhner, 1905, which develops in *O. trifida* (Totten), in that the former has a double-layered, swollen caudal cyst and a longer delivery tube. The cercaria, metacercaria and adults of *L. gibbosus* are described by means of interference contrast and scanning electron microscopy (SEM).

Lecithaster gibbosus (Rudolphi, 1802), Lühe, 1901 (Lecithasteridae) is one of the most widely distributed digeneans in the North Atlantic Ocean and adjacent seas. It has been recorded in the intestine of most teleost families, including the Clupeidae, Salmonidae, Gadidae and Pleuronectidae. No larval developmental stages have been found and nothing is known about its life cycle. Very little is known about the life cycle of marine hemiuroids and the only two developmental cycles that have to date been experimentally elucidated are those of *L. confusus* Odhner, 1905 by Hunninen and Cable (1943) and *Derogenes varicus* (Müller, 1784) Looss, 1901 by Køie (1979).

Materials and methods

Specimens of *Odostomia eulimoides* Hanley (Gastropoda, Opisthobranchia, Pyramidellidae) were dredged from the western Kattegat off Frederikshavn, Denmark, at a depth of 18–24 m (March, June, July) using a detritus sledge furnished with a 265-µm mesh bag. In this area *O. eulimoides* parasitises the prosobranch snail *Turritella communis* Risso. Other specimens of *O. eulimoides* were obtained throughout the year from the northern Øresund at a depth of 22–25 m; *T. communis* is extremely rare here, and *O. eulimoides* parasitises various species of bivalves.

Immediately after their capture, calanoid copepods from the northern Øresund were transferred to cylindrical cages about 10 cm in diameter made of 250-µm plankton net. The cages were placed in aquaria supplied with running seawater from the recirculating system (30‰ salinity, 10° C). The copepods were fed for 1 h five times a week with a mixture of *Rhodomonas* sp., *Dunaliella tertiolecta, Isochrysis galbana* and *Heterocapsa* sp. Several hundred copepods from each sample were examined for natural infections with metacercariae, but no naturally infected copepods were found.

Copepods thus isolated for a minimal period of 1 week were placed in 250-ml glass jars without running water but with an air supply. They were exposed to cystophorous cercariae from crushed, naturally infected *O. eulimoides* for 24 h (10° C). After exposure the copepods were transferred to the cylindrical cages and fed as described above. Copepods harbouring 2-, 3- and 5-week-old metacercariae were exposed to sticklebacks, *Gasterosteus aculeatus* L., which had been kept in aquaria for 1 year, during which they fed on frozen food only. Ten control specimens of these *G. aculeatus* did not harbour any natural infection.

The material for scanning electron microscope (SEM) studies was fixed for 2–3 h in 2.5% glutaraldehyde buffered with cacodylate (pH 7.4), washed in buffer and post-fixed for 1–2 h in cacodylate-buffered OsO_4, dehydrated in ethanol and benzene, and freeze-dried. The mounted specimens were coated with gold and examined in a Jeol 840 SEM. All measurements are based on live specimens.

Results

Natural infection of the molluscan host

Of more than 200 *O. eulimoides* examined, 5 specimens were infected: 3 came from the western Kattegat (March, June, July) and the other 2 from

the northern Øresund (August and November). Several hundred specimens of other species of *Odostomia* were not infected. All five infected snails harboured fully developed infective cercariae. Each infected snail contained fewer than ten germinal sacs up to 2 mm long that were provided with irregularly arranged constrictions. It was impossible to distinguish a pharynx, caecum or birth pore. The germinal sacs occurred intertwined in the digestive gland and were difficult to separate from the host tissue. The gonad appeared only slightly affected; during the summer, infected snails contained both eggs and spermatozoa, although in smaller amounts than uninfected snails.

Experimental infection of the second intermediate hosts

Metacercariae were found in the haemocoel of *Acartia* sp. (Fig. 4A), *Pseudocalanus elongatus* Boeck and *Centropages hamatus* (Lilljeborg), indicating that these copepods had ingested the cercariae. Most specimens of these species, as well as of other copepods belonging to several other genera, were not infected. In infected *Acartia* sp. the metacercariae, which always occurred singly, prevented the normal development of the gonad.

Experimental infection of the final host

The 2-week-old metacercariae were not infective. However, 3-week-old metacercariae in *Acartia* sp. were infective (Fig. 4D), whereas the metacercariae in *P. elongatus* and *C. hamatus* developed more slowly and even the 5-week-old metacercariae in these hosts did not survive in the sticklebacks.

The cercaria

Young cercariae in the germinal sac had a club-shaped delivery tube (Fig. 2A), whereas the delivery tubes of slightly older specimens were withdrawn into the caudal cyst. An appendage with an apical spherical dilation was attached to the external wall of the caudal cyst of young specimens (Fig. 2A, B). The appendage of older specimens lost its apical dilation, appearing as a filament about 150 µm long (Figs. 1A, 2C).

Shortly after emergence from the snail, the double-layered wall of the caudal cyst took up water, resulting in more than a doubling of the diameter of the cyst (Fig. 2D). The swollen caudal cyst was slightly flattened, with a central depression on each side (Fig. 3A); the swelling of its wall usually took

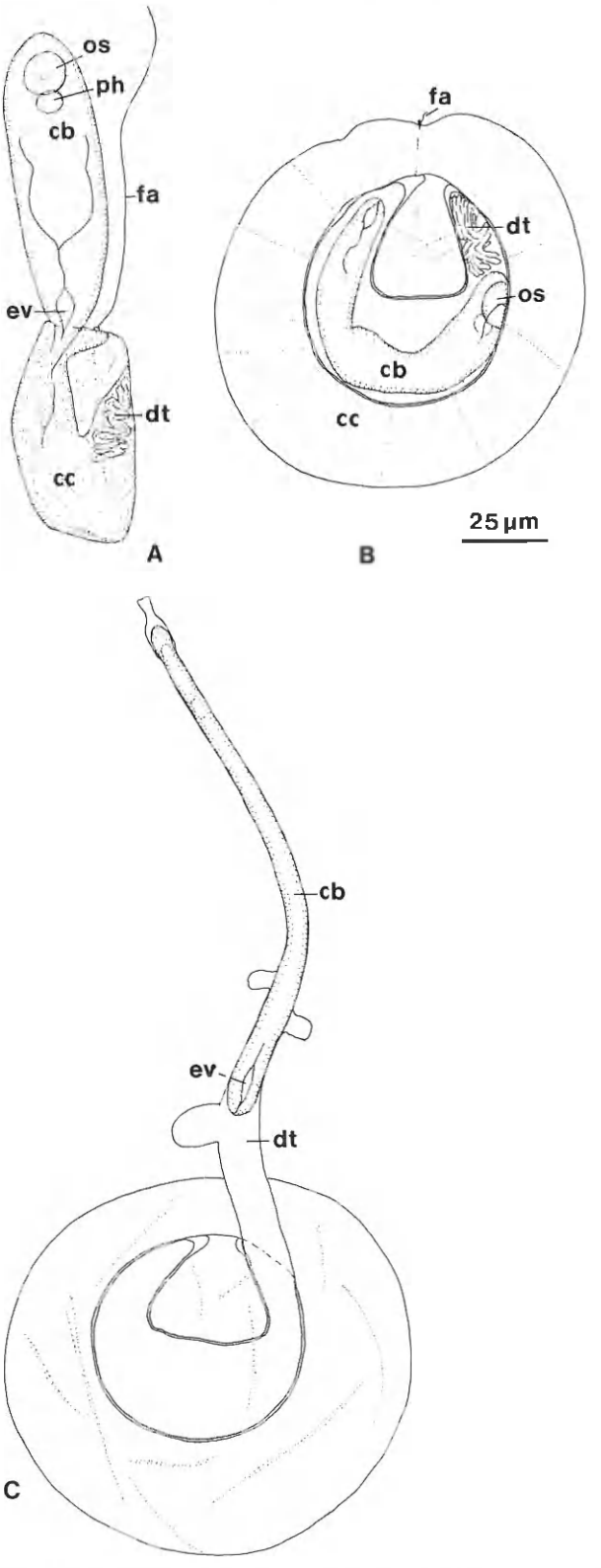

Fig. 1A–C. Different developmental stages of the cercaria of *L. gibbosus*. **A** Cercaria immediately after release from the snail host. **B** Infective cercaria. **C** Cercaria with everted delivery tube containing the cercarial body. *cb*, cercarial body; *cc*, caudal cyst; *dt*, delivery tube; *ev*, excretory vesicle; *fa*, filamentous appendage; *os*, oral sucker; *ph*, pharynx

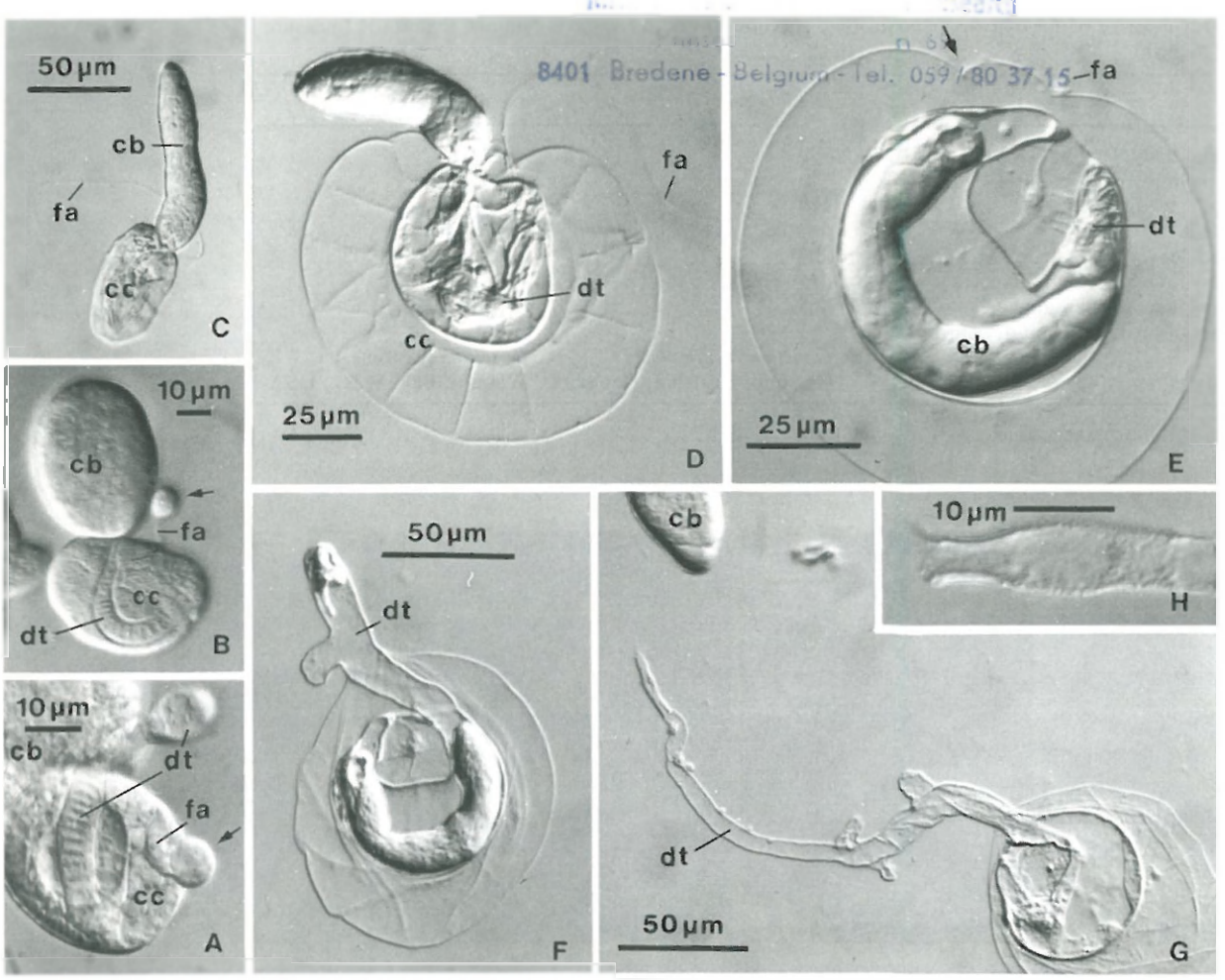

Fig. 2A–G. Interference contrast micrographs of differental developmental stages of the cercaria of *L. gibbosus*. **A** Young intramolluscan stage, showing the spherical dilation on the filamentous appendage (*arrow*). **B** Slightly older intramolluscan stage. The protruding delivery tube is not seen. The *arrow* shows the spherical dilation on the filamentous appendage. **C** Cercaria immediately after release from the snail. **D** Cercaria with swollen caudal cyst. **E** Infective cercaria showing the remains of the filamentous appendage and the mark left by the withdrawn cercarial body (*arrow*). **F** Coverglass pressure has caused the partial eversion of the delivery tube. **G** Complete eversion of the delivery tube and ejection of the cercarial body. **H** End piece of the delivery tube. *cb*, cercarial body; *cc*, caudal cyst; *dt*, delivery tube; *fa*, filamentous appendage

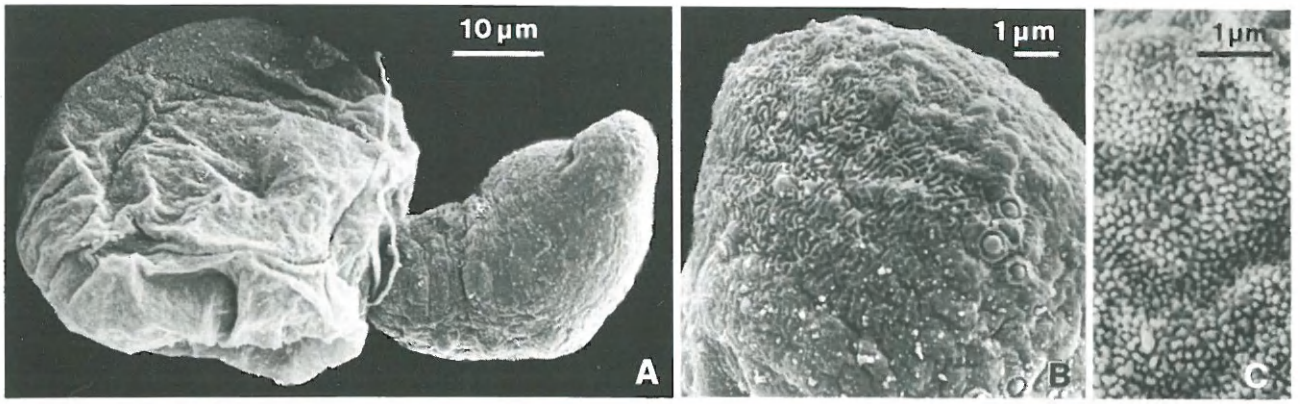

Fig. 3A–C. SEM micrographs of a cercaria of *L. gibbosus*. **A** Ventrolateral view showing the swollen caudal cyst. **B** Detail of **A**. Anterolateral view of the cercarial body, showing the folded tegument and presumed sensory structures. **C** Surface of the swollen caudal cyst

Table 1. Cercariae of *Lecithaster gibbosus* (live specimens) and *L. confusus* (stained material)[a] (measurements in µm)

	L. gibbosus	*L. confusus*
Cercarial body, length	90	85
Cercarial body, width	25	20
Caudal cyst, diameter	110	50
Cyst wall	double-layered, swollen	non-swollen
Everted delivery tube, length	200	150
Delivery tube, proximal diameter	15	10
Delivery tube, distal diameter	5	7
Number of projections on delivery tube	3	2
Host	*Odostomia eulimoides*	*O. trifida* (Totten)
Locality	Øresund, Kattegat, Denmark	Waquoit Bay, Mass., USA

[a] From Hunninen and Cable (1943)

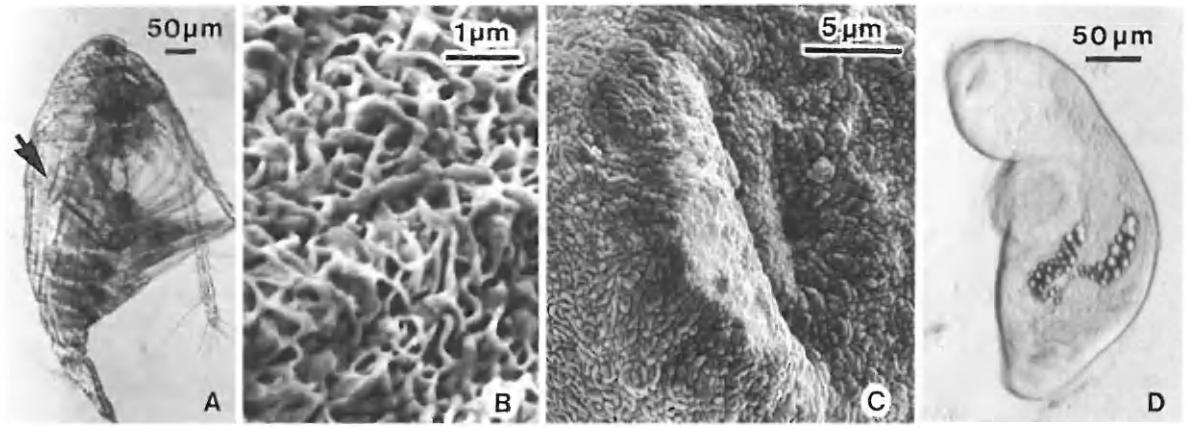

Fig. 4A–D. Metacercariae of *L. gibbosus* from experimentally infected copepods. **A** Live *Acartia* sp. with a less than 1-day-old metacercaria in the haemocoel (*arrow*). **B** SEM micrograph showing the tegumental surface of a 10-day-old specimen. **C** SEM micrograph of a 17-day-old metacercaria seen from the front. **D** Slightly flattened, live 21-day-old metacercaria with lipid droplets in the intestinal caeca

a few minutes. At the beginning, compartments connected the two layers and the filamentous appendage could still be seen (Fig. 2D). Shortly after completion of the swelling the cercarial body retracted into the caudal cyst, where the detached body became elongate and slender, sometimes lying with the anterior and sometimes with the posterior end adjacent to the delivery tube. Concurrently, the filamentous appendage disappeared. The surface of the swollen caudal cyst was covered with small microvilli (Fig. 3C).

Measurements and some morphological details of the infective cystophorous cercaria are shown in Table 1. Two indentations close to each other show the attachment of the filamentous appendage and the opening through which the cercarial body withdrew into the caudal cyst (Figs. 1B, 2E). The delivery tube was coiled up at one end of the crescent-shaped cyst cavity. Light coverglass pressure resulted in immediate delivery tube eversion and the simultaneous extrusion of the cercarial body into the proximal part of the delivery tube (Fig. 2F). The complete eversion of the delivery tube and passage of the cercarial body to the exterior usually first occurred after additional pressure on the caudal cyst (Figs. 1C, 2G, H).

The oral sucker and pharynx of infective cercariae were poorly developed and the ventral sucker was not discernible; however, a pre-oral lobe could be seen. The excretory vesicle continued into a Y-shaped excretory duct whose arms extended a little more than halfway to the anterior end of the body. Spines, stylet and penetration glands were absent. The external surface of the fully developed cercaria was slightly annulated. The tegument of the oral lobe had anastomosing

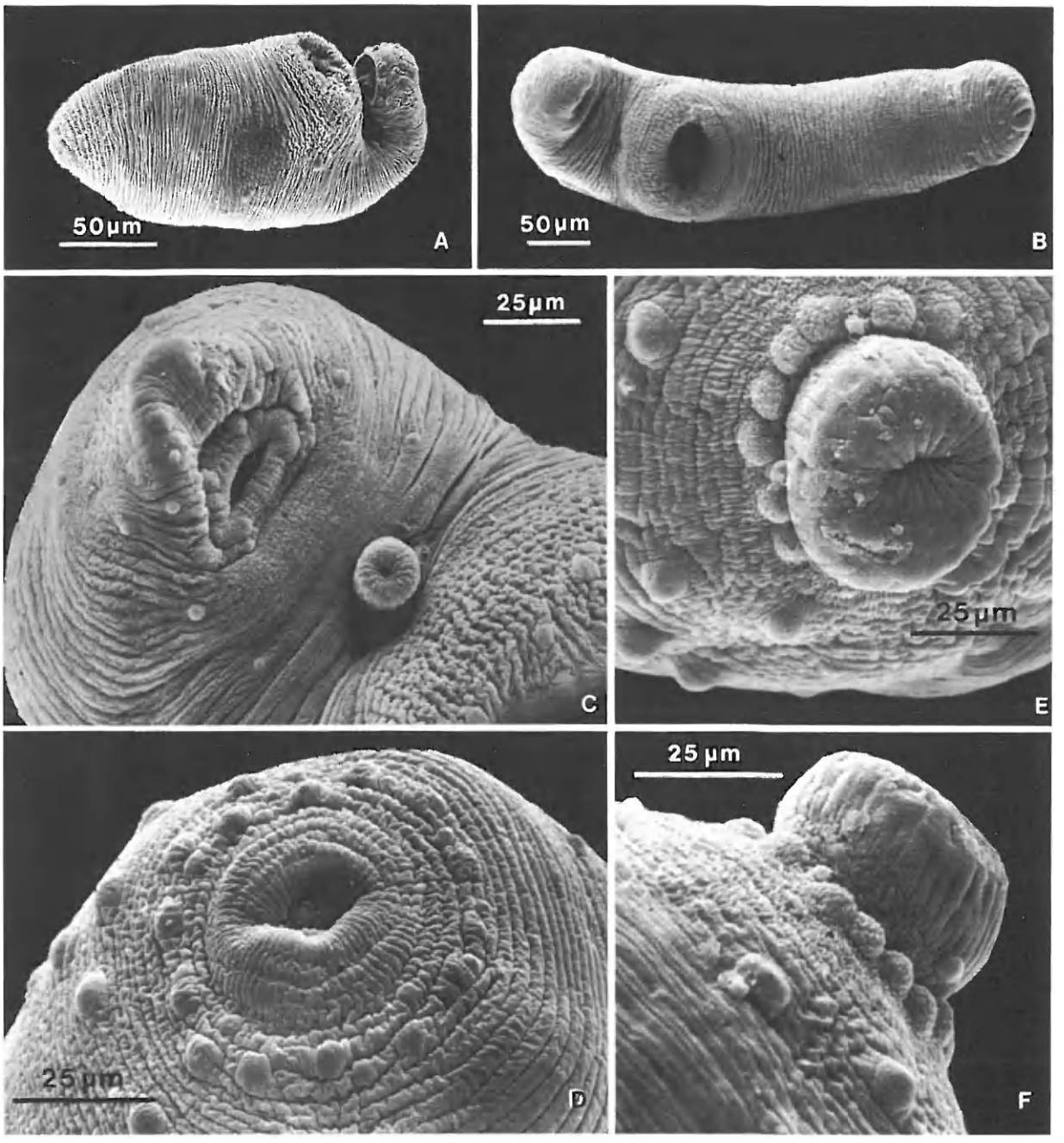

Fig. 5A–F. SEM micrographs of *L. gibbosus* from experimentally infected stickleback (**A**) and naturally infected herring from the Øresund (**B–F**). **A** Lateral view of a 1-week-old specimen. **B** Ventral view of a specimen from a herring. **C** Anterior end with everted sinus organ. **D** Posterior end of a specimen with the posterior extremity withdrawn. The ventral surface is towards the *left*. **E** Specimen with extruded posterior extremity. Note the papillae surrounding the posterior extremity, being most numerous ventrally (*upper left*). **F** Ventral view of the specimen shown in **E**

ridges, and small papillae, probably sensory, occurred laterally (Fig. 3B).

The metacercaria

The external surface of the unencysted 10-day-old metacercariae from *Acartia* sp. was increased enormously by numerous deep, irregular ridges or folds (Fig. 4B). The 17-day-old metacercariae from the same copepod host had an irregularly folded tegument (Fig. 4C); the presumed sensory structures of the cercaria were not observed. The 3-week-old infective metacercariae were 350–390 µm (mean, 380 µm) long and 140–150 µm (mean, 145 µm)

wide (measurements of four specimens). The diameter of the oral and ventral suckers were 45–60 µm (mean, 50 µm) and 75–100 µm (mean, 80 µm), respectively. These metacercariae had small lipid droplets in the caeca (Fig. 4D), whereas younger developmental stages had an apparently empty, non-functioning digestive system.

The adult

The small specimens of *L. gibbosus* found in the intestine of sticklebacks 1 week after the ingestion of infective metacercariae (Fig. 5A) were indistinguishable from those in naturally infected herring from the northern Øresund. These herrings harboured both small, immature specimens and larger, mature *L. gibbosus*. Both immature and mature *L. gibbosus* were provided with papillae of different sizes and surface structures (Fig. 5). The large papillae were especially common around the mouth, on and inside the ventral sucker and ventrally surrounding the posterior extremity. The extreme posterior end may protude, forming a "tail" (Fig. 5E, F); however, this should not be confused with the ecsoma of some members of the family Hemiuridae.

Discussion

L. gibbosus is the only species of *Lecithaster* in Danish waters; it has adequately been described by authors such as Odhner (1905) and Lebour (1908). *L. gibbosus* is common in the intestine of planktophagous marine fishes such as sprat, herring and sand eel but also occurs in large benthophagous and piscivorous fishes such as gurnards, flatfishes, gadoids and salmonids (Dawes 1947). The predatory fishes probably become infected after preying on planktophagous fishes, suggesting that *L. gibbosus*, like many stomach hemiuroids, may be transferred from one fish to another.

The cystophorous cercaria of *L. gibbosus* differs from other known cystophorous cercariae, including that of *L. confusus* (Table 1), in its swollen, double-layered cyst wall. Although the former cannot swim, its relatively low density enables it to remain suspended in the water, where it may be seized by copepods. The inoculative mechanism whereby the cercaria infects the copepod host is similar to that of other cystophorous cercariae, described by authors such as Hunninen and Cable (1943), Køie (1979) and Matthews (1981, 1982).

The increased surface area of the small, non-infective metacercariae of *L. gibbosus* suggests that the external surface plays a role in the absorption of nutrients when the digestive system is not yet functioning. Older metacercariae can apparently ingest host tissue, including lipid droplets.

Ciliated and non-ciliated papillae are commonly found in adult digeneans, but *L. gibbosus* is provided with more large, non-ciliated papillae than are most species. Hunninen and Cable (1943) emphasized the contractile papillae in the suckers and on the body of *L. confusus*, which had previously been overlooked. The papillae of adult *L. gibbosus* are not contractile and are differently distributed on the body. They are probably contact receptors and have features in common with the contact receptors and button papillae described at the TEM and SEM levels by Bennett (1975) and Hoole and Mitchell (1981), respectively.

Boyce (1969) found that *Centropages abdominalis* Sato and *Pseudocalanus minutus* (Krøyer) from the Pacific Ocean off British Columbia were naturally infected with metacercariae of *Lecithaster* – according to the author, presumably *L. gibbosus*. He also found the cercariae of what he believed to be *L. gibbosus* in prosobranch snails of the genus *Thais*. The cercariae, which were not described, were ingested by planktonic copepods, in which they developed to infective metacercariae in about 3 weeks. The life span of *L. gibbosus* in Pacific salmon, *Oncorhynchus* spp., has experimentally been determined to be less than 9 months (Margolis and Boyce 1969).

As the present study shows that the cercaria of *L. gibbosus* uses opisthobranch snails of the genus *Odostomia* as its first intermediate host, it is unlikely that the *Lecithaster* in the Pacific salmon studied by Boyce (1969) and Margolis and Boyce (1969) would also be identical with this species. Ching (1960) suggested that two different cystophorous cercariae in different species of *Thais* developed into *L. salmonis* Yamaguti, 1934 and *L. confusus*. Margolis and Boyce (1969) considered *L. salmonis* to be a synonym of *L. gibbosus*. Ching (1960) concluded that it was possible that the cercaria described by Hunninen and Cable (1943) was not the larva of *L. confusus*. Because many species of *Lecithaster* are difficult to distinguish, it is likely that one or more of the above-mentioned species have been incorrectly identified.

From the above, it appears that two species of *Lecithaster* in the Atlantic Ocean use species of *Odostomia* as first intermediate hosts and that two species of *Lecithaster* in the Pacific Ocean use species of *Thais* as first intermediate hosts (see also Køie 1983). Cystophorous cercariae have not been found in *Thais* sp. in the Atlantic Ocean. *Odosto-*

mia spp. occur in the Pacific Ocean, but it is unknown whether they are infected there.

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Possible roles of cAMP and Ca^{2+} in the regulation of miracidial transformation in *Schistosoma mansoni*

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Abstract. The triggering action of physiological saline in the miracidial transformation of *Schistosoma mansoni* was analyzed using various agents affecting cAMP- and Ca^{2+}-dependent pathways. Potent activators of adenylate cyclase, such as forskolin and serotonin, strongly inhibited the transformation provoked by saline in RPMI-1640. These inhibitory actions were diminished by the combined administration of phosphodiesterase activators such as ammonium salts or imidazole. Furthermore, the exposure of miracidia to ammonium salts or imidazole in dechlorinated tap water "mimicked" the transformation, i.e., the cessation, of swimming and then shedding of epithelial plates. This mimic transformation was also inhibited by serotonin or forskolin. In contrast, treatment of miracidia with Ca^{2+} antagonists such as TMB-8 (an inhibitor of Ca^{2+} release), nicardipine (a Ca^{2+} channel blocker), or W-7 (a calmodulin inhibitor) in tap water produced severe vesiculation on their body surfaces and resulted in death. However, these toxic effects were abolished by a combined administration of these Ca^{2+} antagonists with saline or NH_4Cl, and the transformation was reestablished except with W-7 treatment. W-7 strongly inhibited the triggering action of saline and NH_4Cl and the worms swam slowly, whereas W-5, an inactive analogue of W-7, had no inhibitory effect on the transformation. These results suggest that the initiation of micracidial transformation to young sporocysts may be synergistically regulated by cAMP and Ca^{2+} and that a decrease in cAMP levels and an increase in Ca^{2+} mobilization may be provoked in worms transformed by saline, ammonium salts, or imidazole.

Schistosome miracidia transform to young sporocysts by shedding their epithelial plates after penetrating snails or in in vitro cultures containing saline (Voge and Seidel 1972; Samuelson et al. 1984). Therefore, saline is believed to act as a trigger in miracidial transformation (Samuelson and Caulfield 1985); however, little is known about the mechanisms by which transformation is elicited (Voge and Seidel 1972).

It is well known that cellular responses to extracellular signals such as hormones and growth factors are mediated by signal transduction systems of cyclic adenosine 3′,5′-monophosphate (cAMP)- and/or Ca^{2+}-dependent pathways (Nishizuka 1984, 1986). In this regard, there is little information on the roles of these systems in schistosomal development in either snails or final hosts. In *S. mansoni*, cAMP has been reported to act as a second messenger for the regulation of carbohydrate metabolism, and serotonin is reportedly involved in the regulation of cAMP levels by activating adenylate cyclase (Mansour 1984; Estey and Mansour 1987). On the other hand, serotonin has been reported to inhibit *Schistosoma mansoni* miracidial transformation to young sporocysts in in vitro cultures (Samuelson et al. 1984). Although there has been no analytical data on the mode of the inhibitory action by serotonin in this parasite, these findings seem to suggest that an increase in cAMP levels induced by serotonin may prevent the transformation. Estey and Mansour (1987) recently reported that a potent activator of adenylate cyclase,

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Abbreviations: Nicardipine, 2-(*N*-bentyl-*N*-methylamino)ethyl-methyl-2,6-dimethyl-4-(m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate; TMB-8, 8-(*N*,*N*-diethyl amino)-octyl-3,4,5-trimethoxybenzoate; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; W-7, *N*-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide