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Scanning electron microscopy of cercariae, metacercariae and adults of *Pygidiopsis ardeae* Køie, 1990 (Digenea, Heterophyidae)

Marianne Køie

Marine Biological Laboratory, University of Copenhagen, DK-3000 Helsingør, Denmark

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Abstract. The penetration apparatus of the cercaria of *Pygidiopsis ardeae* Køie, 1990 (Heterophyidae) is provided with five large preoral hooklets. Various types of presumably sensory structures surround the small oral aperture. Small, pointed spines protrude throughout the cercarial body. After parasite penetration and encystment in the fish intermediate host, the metacercarial tegument increases its absorptive area by developing irregular projections. Concurrently the pointed spines become scale-like and serrated. The tegumental outgrowths appear to have regressed in infective metacercariae. The external surface of mature worms removed from the intestine of domestic chickens does not differ from that of infective excysted metacercariae. Adults taken from experimentally infected chickens were identical to specimens obtained from naturally infected herons.

Adult members of the family Heterophyidae occur in the intestine of birds and of some mammals, including man. Species belonging to genera such as *Heterophyes, Haplorchis, Metagonimus, Pygidiopsis* and *Cryptocotyle* are potential pathogens to humans, dogs and other mammals. *P. ardeae* Køie, 1990 (Heterophyidae) matures in the intestine of the grey heron *Ardea cinerea* L. but may also develop in domestic chickens, pigeons and dogs (Køie 1990a, b). Metacercariae of *P. ardeae* are common in fishes in Danish inlets and the cercariae develop in snails of the genus *Hydrobia*.

The aim of the present study was to compare at the scanning electron microscopical (SEM) level the external surface of the cercarial, metacercarial and adult stages of *P. ardeae* in experimentally infected second intermediate and definitive hosts. SEM studies on the external surface of adult specimens of *P. ardeae* obtained from naturally infected grey herons have previously been carried out (Køie 1990a).

Materials and methods

Naturally released cercariae were obtained from naturally infected *Hydrobia ventrosa* (Montagu) (Prosobranchia, Hydrobiidae) dredged in the Isefjord, northern Zealand, Denmark. Metacercariae were obtained from experimentally infected three-pointed sticklebacks *Gasterosteus aculeatus* L. (Gasterosteidae). The fishes were held in seawater (2.0% S) at approx. 15° C. They were dissected at 1, 2, 3, 7, 8, 9, 11, 12 and 20 weeks postinfection. The metacercariae were excysted mechanically by means of fine needles. Adult specimens were obtained from the intestine of experimentally infected domestic chickens as described by Køie (1990b).

The specimens were fixed in 2.5% glutaraldehyde in cacodylate buffer (pH 7.4), postfixed in osmium tetroxide in the same buffer, dehydrated through ethanol and benzene and freeze-dried. The specimens were covered with gold and examined in a Jeol 840 scanning electron microscope.

Results

Cercariae

Cercariae fixed for SEM studies were dorso-ventrally flattened and demonstrated a pentagonal outline (Fig. 1A). Small, pointed spines protruded throughout the body, and long, cilium-like structures were commonly seen anteriorly, laterally and posteriorly. The ventral sucker was not visible. The penetration apparatus consisted of five (one cercaria had six; Fig. 1D) large, preoral hook-like spines or hooklets, the penetration glands and various types of structures presumed to be sensory (Fig. 1C–G). The openings of the penetration glands lay at the unspined anterior extremity. This part and the hooklets were occasionally withdrawn into the cercarial body (Fig. 1F). The hooklets of extended specimens were directed perpendicularly from the cercarial surface. The hooklets either were somewhat spread apart or lay closely together (Fig. 1C–E). The anterior row of body spines were slightly longer and stouter than the remaining body spines.

Both non-ciliate structures and short, cilium-like structures arising either at the general tegumental level

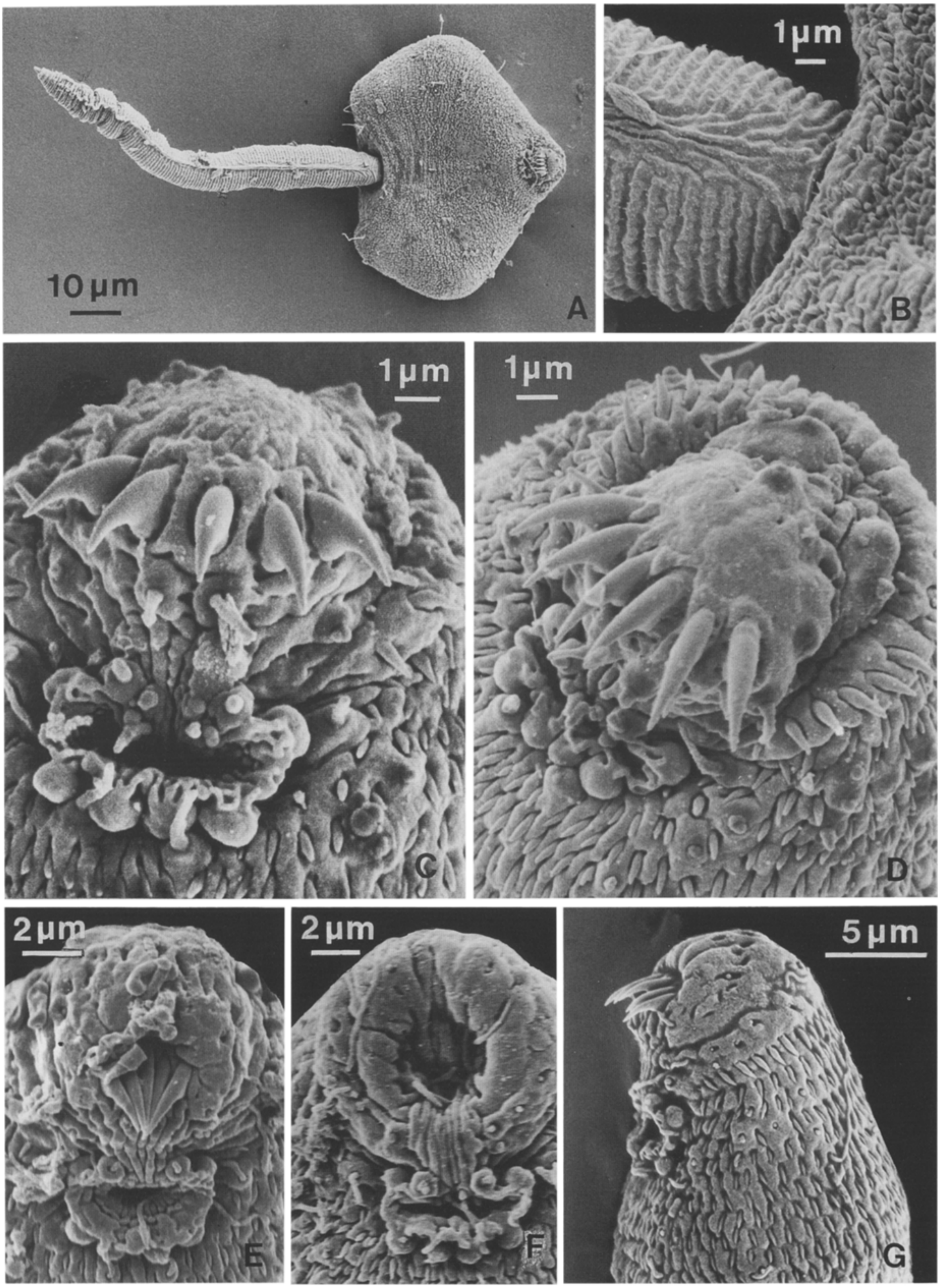

Fig. 1 A–G. Infective cercariae of *Pygidiopsis ardeae*. **A** Ventral view of a cercaria. **B** Posterior part of the cercarial body and the base of the tail. **C** Ventral view of the anterior end, showing the five preoral hooklets and the oral aperture surrounded by different types of sensory structures and small, pointed spines. **D** Anterolateral view of a cercaria with six hooklets. **E** Anterior end of a cercaria with the hooklets lying close together. The oval bodies on the apical spineless area probably represent secretory bodies released from the penetration glands. **F** Anterior end of a cercaria with completely retracted hooklets. Note the preoral flap that connects the retracted penetration apparatus with the oral aperture. **G** Lateral view of the anterior end of a cercaria with an extended penetration apparatus

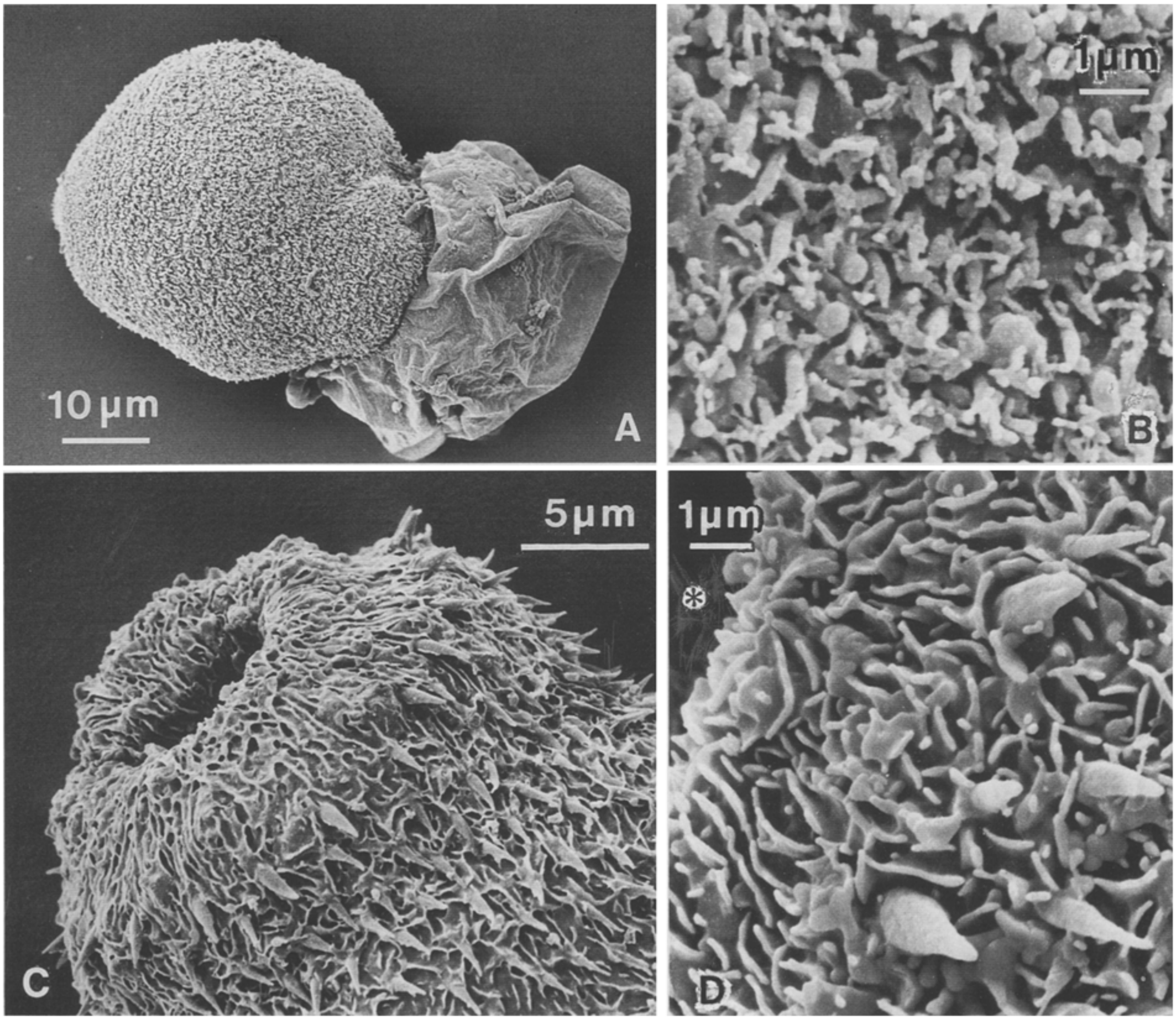

Fig. 2A–D. Excysted metacercariae of *Pygidiopsis ardeae* at 1 (**A**, **B**) and 7 weeks of age (**C**, **D**). **A** Dorsal view of a metacercaria partly embedded in its thin cyst. **B** Detail of the surface, showing the pointed spines and the irregular tegumental projections. The small granules on the tegument probably represent cystogenous material. **C** Anterior end with the oral aperture and the surrounded pointed spines. Note the tegumental folding throughout. **D** Detail of the area surrounding the oral aperture (*asterisk*), showing the highly folded tegument and the pointed spines

or on top of papillae of varying sizes and shapes were found around the oral aperture and at the spineless anterior end. The oral aperture appeared as a slit surrounded by an undulating membranous fold, which connected six smooth, hemispherical structures and, anteriorly, two papillae each with an apical cilium-like structure. Anteriorly, the membranous fold was interrupted medially; a preoral flap provided with longitudinal furrows was observed connecting the area just posterior to the hooklets with the oral aperture (Fig. 1E, F). The tegument of the tail was annulated and had a ventral and a dorsal low crest showing longitudinal folds.

Metacercariae

The external surface of 1-week-old metacercariae was increased by irregular tegumental projections (Fig. 2A, B). The hooklets were lost within the first 9 weeks (see Køie 1990b). The tegument of 9-week-old metacercariae continued to be increased by tegumental folds and irregular tegumental projections (Fig. 2C, D, Fig. 3). The spines grew in length and width but remained simply pointed in most 7- and 8-week-old metacercariae. The slightly longer and stouter spines of the anterior row appeared to have grown further and to have become flattened and multipointed (Fig. 3A). The body spines of some 8-week-old and most 9-week-old metacercariae appeared slightly shorter and exhibited multiple points (Fig. 3).

The tegumental projections were completely absent in most 11-week-old metacercariae (Fig. 4A). The 12-week-old metacercariae (Fig. 4B–D), which are infective when fed to chickens, were provided with scale-like, serrated spines that decreased in size posteriorly, except for two elongate, spineless areas located ventro-laterally

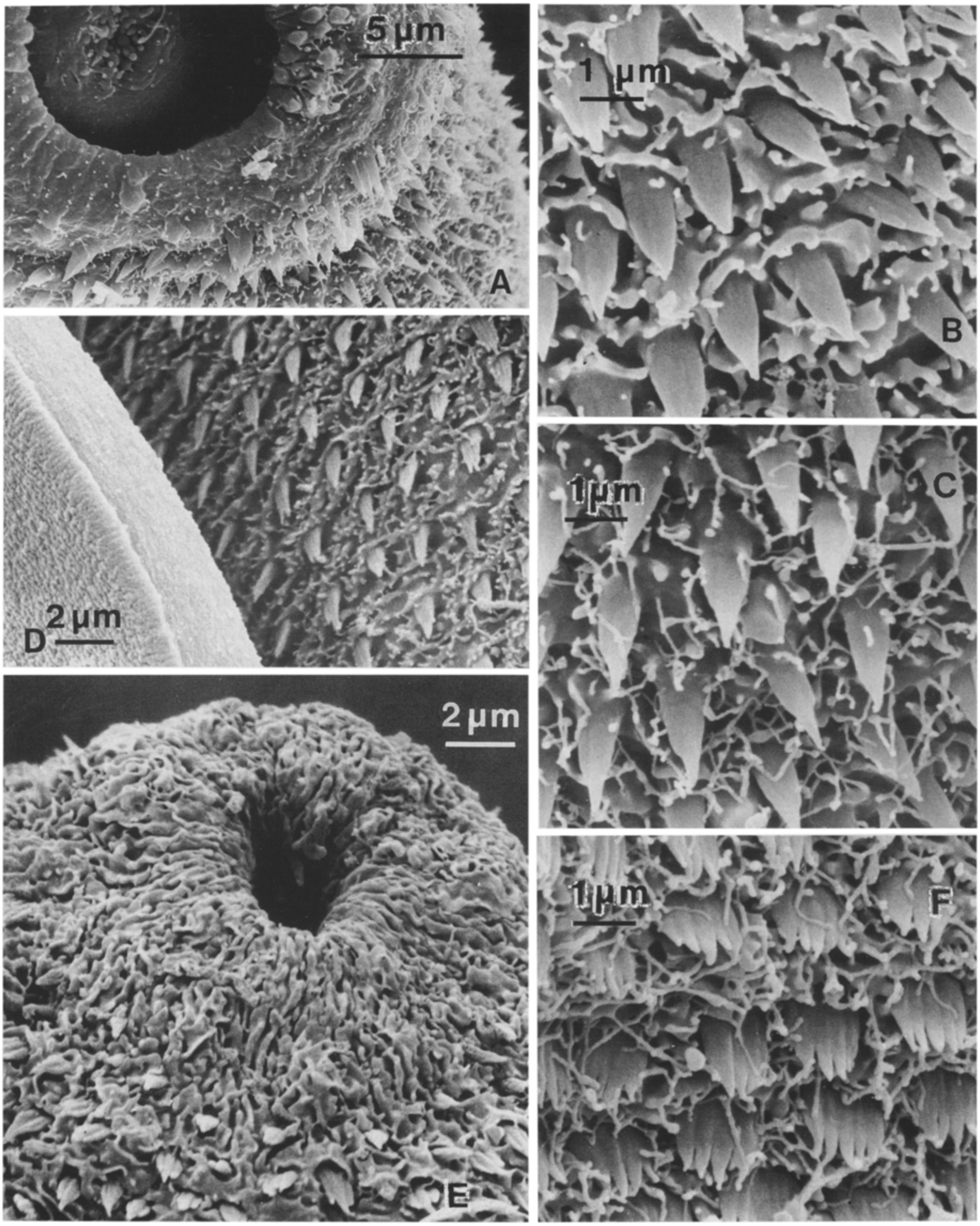

Fig. 3A–F. Details of 8-week-old (**A–C**) and 9-week-old (**D–F**) excysted metacercariae of *Pygidiopsis ardeae*. **A** Flattened, three-pointed spines are visible close to the oral aperture. The pharynx can be seen in the lumen of the oral sucker. **B** Surface of the forebody, showing the slightly flattened spines with up to three indistinct points, tegumental folds and short projections. **C** Surface of the forebody of another metacercaria with filamentous projections that probably represent cystogenous material. **D** Surface of the forebody of a metacercaria with serrated spines and irregular tegumental projections. Note the thick cyst wall. **E** Oral aperture and serrated body spination. **F** Serrated spines, with material presumed to be cystogenous lying between the spines

in the hindbody, a small preoral unspined area and a spineless area lying between the genital opening and the ventral sucker. The external surface of 20-week-old metacercariae was similar to that of 12-week-old specimens.

Adults

Maturity was attained within 4 days of infection, during which period the body developed a ventral cavity (Fig. 4E). The surface of mature specimens did not differ

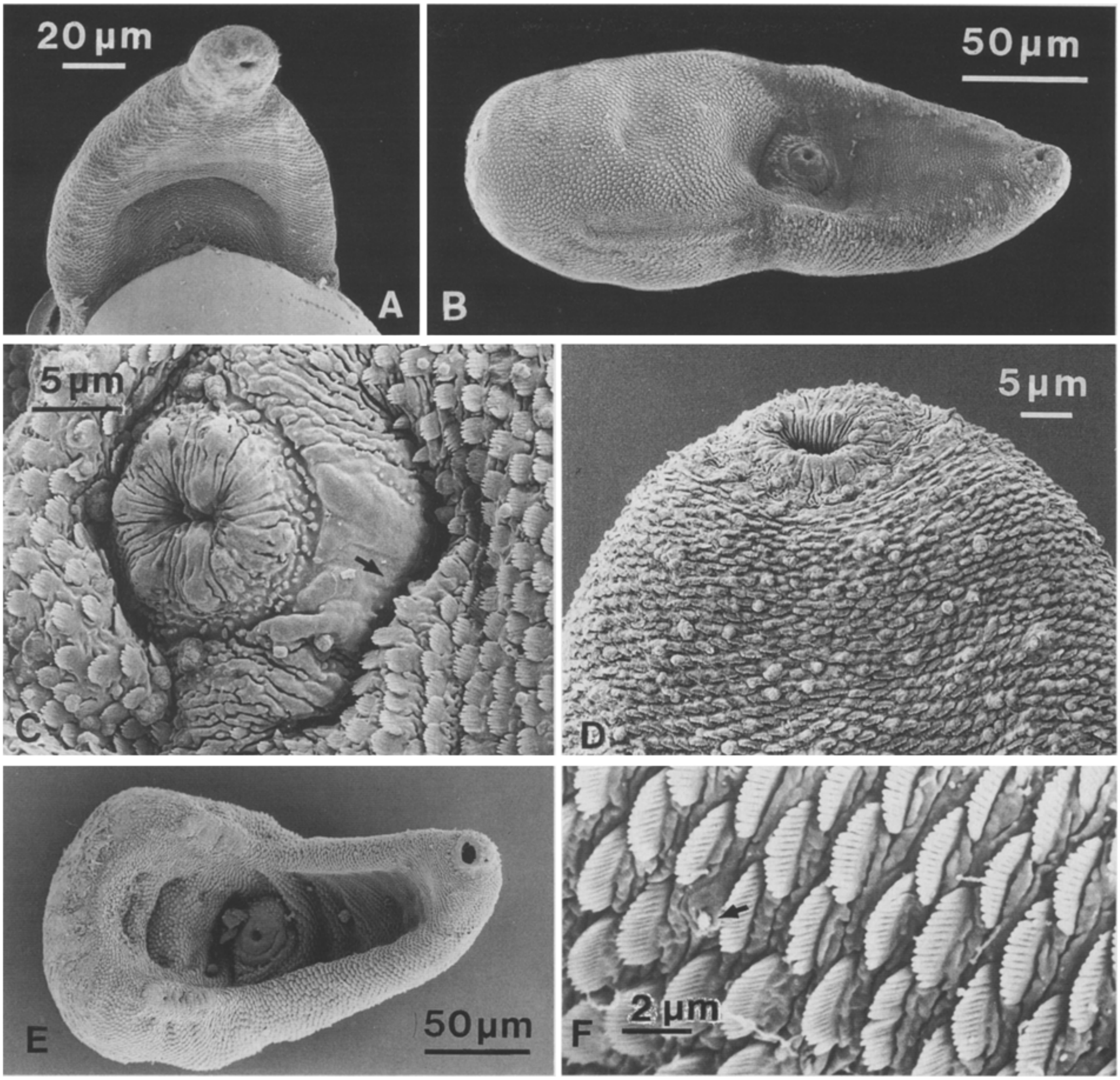

Fig. 4 A–F. Metacercariae aged 11 (**A**) and 12 weeks (**B–D**) and 4-day-old adults (**E, F**) of *Pygidiopsis ardeae* from experimentally infected chickens. **A** Anterior end of a partly excysted specimen. Note the thick cyst wall. **B** Ventro-lateral view of a whole excysted metacercaria. **C** Ventral sucker and genital opening (*arrow*). Note the flattened, serrated, backwardly directed spines. **D** Anterior end with flattened, serrated spines. **E** Ventral view of a whole specimen. **F** Surface of the forebody, showing flattened, serrated spines and a cilium (*arrow*) of a presumably sensory structure

from that of infective metacercariae. Short, cilium-like structures protruded between the spines (Fig. 4F). SEM views of eggs and of whole specimens of *Pygidiopsis ardeae* (with and without protruded gonotyl) obtained from naturally infected herons have been published elsewhere (Køie 1990a).

Discussion

SEM studies on heterophyid cercariae are scarce. Cercariae of *Metagonimus* spp. and *Cryptocotyle lingua* have been examined by Fujino et al. (1976) and Køie (1977), respectively. SEM studies of opisthorchiid cercariae have been carried out by Fujino et al. (1979). These cercariae encyst in the second intermediate fish host. They are provided with a penetration apparatus that includes modified spines; large, unicellular glands; and non-ciliate and ciliate structures presumed to be sensory.

The penetration apparatus, especially the preoral hooklets, of *Pygidiopsis ardeae* differs from that of the above-mentioned heterophyid and opisthorchiid cercariae in that it is extremely well developed. The cercarial body of *P. ardeae* is smaller and the tail is shorter, lacking the characteristic finfold of most heterophyids. The cercaria of *P. ardeae* is an active swimmer that does not assume a resting position with the tail inclined upwards. Most heterophyid cercariae penetrate the skin of the fish host, whereas cercariae of *P. ardeae* enter the buccal cavity with the respiratory water and penetrate the gill filaments (Køie 1990 b).

The extremely large, hook-like spines of the cercariae of *P. ardeae* may be important, since if initial attachment fails, the cercariae are lost through the host's gill opening, whereas skin-penetrating cercariae usually move about on the surface of the fish to locate a suitable place for penetration. The slightly larger spines of the anterior row of the body spination may act as a "rasp" to disintegrate the gill tissue and may, together with the remaining body spines, have an anchoring function during parasite migration through the host's blood vessels (Køie 1990b). The contents of the penetration glands are emptied during the penetration process, but their exact function is unknown.

The general penetration process of the cercaria of *P. ardeae* appears to be similar to that of the cercaria of *C. lingua* as suggested by Rees (1974) and Køie (1977). It is possible that the function of the preoral flap, a structure that was not seen in the cercaria of *C. lingua*, involves the transfer of excess penetration-gland secretion into the oral aperture, thus preventing it from coming into contact with the cercarial surface. As in *P. ardeae*, oral aperture of the cercaria of *C. lingua* is surrounded by a membranous fold and smooth, hemispherical structures (Køie 1977), but the functions of these structures are unknown.

Encysted metacercariae, which are not immediately infective to the next host in the life cycle but rather grow and mature before they obtain infectivity, usually increase their surface area by developing tegumental microvilli or folds. Several examples of such an increase in the absorptive surface of heterophyid and non-heterophyid metacercariae have been reported (see Køie 1977). Metacercariae that do not grow in their second intermediate hosts fail to develop tegumental projections during the encysted stage (Køie et al. 1977; Køie 1985, 1987). The digestive system of recently encysted metacercariae of *P. ardeae* is not fully developed; after it has completely developed, it acts as a reservoir for putative excretory products (Køie 1990b). Therefore, all of the nutrients required for growth of the metacercaria must enter the metacercarial body through the external surface.

Some of the tegumental outgrowths may contribute to the formation of the cyst, as the cyst wall increases in thickness concurrently with the growth of the metacercarial body. Simultaneously with the alteration in the tegumental surface, a change occurs in the morphology of the body spines. A change from the pointed cercarial spines to the scale-like, serrated spines of the infective metacercariae has been observed in other members of Heterophyidae (Køie 1977) and in non-heterophyid families (Køie 1985; Pekkarinen 1986, 1987). The alteration in spine morphology may occur in metacercariae that do not grow during the encysted stage; these metacercariae are not infective to the definitive host until the change in the spines has taken place (Køie 1985). The simple pointed spines occur in migratory developmental stages, whereas the serrated spines usually develop in more stationary stages, in which they may serve both an anchoring function and an abrasive function for parasite feeding on host tissues (see Køie 1985).

The external surface of adult specimens of *P. ardeae* taken from experimentally infected chickens does not differ from that of adult specimens obtained from the natural host *Ardea cinerea* (see Køie 1990 a). The general external surface of adult heterophyids is apparently always covered with scale-like, multipointed spines that decrease in point number and in size towards the posterior end, e.g. *C. lingua* (see Køie 1977), *Heterophyes* spp. (see Taraschweski 1984), *Haplorchis* spp. (see Fujino et al. 1989; Srisawangwonk et al. 1989), *Metagonimus* spp. (Fujino et al. 1989) and *Heterophyopsis continua* (see Hong et al. 1991). Different types of papillae with and without cilium-like structures have been observed anteriorly and between the spines (Køie 1977; Taraschewski 1984; Fujino et al. 1989; Srisawangwonk et al. 1989; Hong et al. 1991).

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