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Monorchiid and aporocotylid cercariae (Digenea) parasitising the purple clam *Amiantis purpurata* (Bivalvia, Veneridae) from the Southwestern Atlantic coast

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Abstract

Two cercariae, one of them ocellate and with well developed tail (Monorchiidae) and another apharyngeate brevifurcocercous (Aporocotylidae), parasite of *Amiantis purpurata* (Lamarck, 1818) (Bivalvia, Veneridae) from the Patagonian coast on the Southwestern Atlantic Ocean, are described. These reports comprise the second monorchiid intramolluscan infection reported for the Southern Hemisphere and first intramolluscan aporocotylid for the Southern Hemisphere. In addition, this constitutes the first report of aporocotylid intramolluscan stages (parthenita) occupying only the haemocoel of the gills of a marine molluscan host rather than the digestive gland and gonad, the usual site of infection.

Keywords

Digenea, Monorchiidae, Aporocotylidae (= Sanguinicolidae) cercariae, Bivalvia, Veneridae, molluscan-castration, Argentine Sea

Introduction

To date, sixteen monorchiid cercariae developing in sporocysts in marine bivalves have been described, most from the Northern Hemisphere. The presence of larval Monorchiidae Odhner, 1900 in the Southwestern Atlantic Ocean is only known for a microcercous cercaria parasitising the venerid clam *Amiantis purpurata* (Lamarck, 1818) (Cremonte *et al*. 2001). Marine members of the Aporocotylidae Odhner, 1912 develop in sporocysts or rediae in bivalves or polychaetes (Holliman 1961, Køie 1982, Lauckner 1983, Smith 1997, Cribb *et al*. 2003). Aporocotylidae Odhner, 1912 is a senior synonym of Sanguinicolidae Poche, 1926, as stated by Bullard *et al*. (2009). At present, there are six records of aporocotylid larvae in marine bivalves, all from the Northern Hemisphere (Linton 1915, Martin 1944, Holliman 1961, Fraser 1967, Wardle 1979, Stunkard 1983).

Amiantis purpurata (Lamarck, 1818) (Bivalvia, Veneridae) occurs from Rio de Janeiro, Brazil, to San Matías Gulf, Argentina. Towards the southern limit of its distribution it is commonly found in sandy substrates, from the intertidal region to 15 m depth (Castellanos 1967). This species is commonly exploited for local consumption (Lasta *et al*. 1998). The aim of this paper is to describe the morphology of two cercariae found in *A. purpurata* from the Patagonian coast, Southwestern Atlantic Ocean. In addition, the precise site of infection is established by histological procedures.

Materials and methods

With the objective of studying living larvae of a previously described microcercous monorchiid cercaria, which was first found by histology and later described by Cremonte *et al*. (2001), 690 clams from the intertidal of La Conchilla beach (40°49´S, 64°47´W) and from the shallow subtidal region of El Molino beach (40°48´S, 64°44´W), San Matías Gulf, were collected on three occasions. They were placed in individual flasks and examined for cercarial emergence twice every 24 hours; later the clams were dissected. In the first sampling occasion, August 1999 (n = 100), emergence was not observed but 3 clams parasitised by an apharyngeate-brevifurcate cercaria were found; in March 2000 (n = 150) the inspection resulted negative; in the third sampling occasion, June 2000 (n = 440), emergence was not observed but two parasitised clam were found, one infected by the microcercous monorchiid cercariae and another, with cercaria with well developed tail and ocellate cercarial body. Larvae were

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studied alive, stained with neutral red and Nile blue and unstained; later they were fixed in hot 4% seawater formaldehyde and stained with acetic carmine, dehydrated, cleared with methylsalicylate, mounted on glass-slide in Canada balsam and measured. The measurements were based on 10 specimens of each larval stage (means with the range in parentheses are given in micrometers) and drawings made with the aid of a camera lucida.

Soft tissues of parasitised clams were fixed in Bouin's fixative and stored in 70% ethanol. An oblique transverse 5 mm thick section of each clam, containing gill, digestive gland, intestine, mantle, foot, nephridia and gonad was cut and included in a histological cassette, later dehydrated, embedded and sectioned (5–7 µm) following standard histological procedures. Sections were stained with eosin and Harris haematoxylin (H & E).

Results

Two previously undescribed cercariae, one belonging to the family Monorchiidae and the other to Aporocotylidae, were found in the purple clam, *Amiantis purpurata*. Clams parasitised by the aporocotylid cercariae had the gills swollen and bright orange.

Monorchiid cercaria sp. (Figs 1–4)

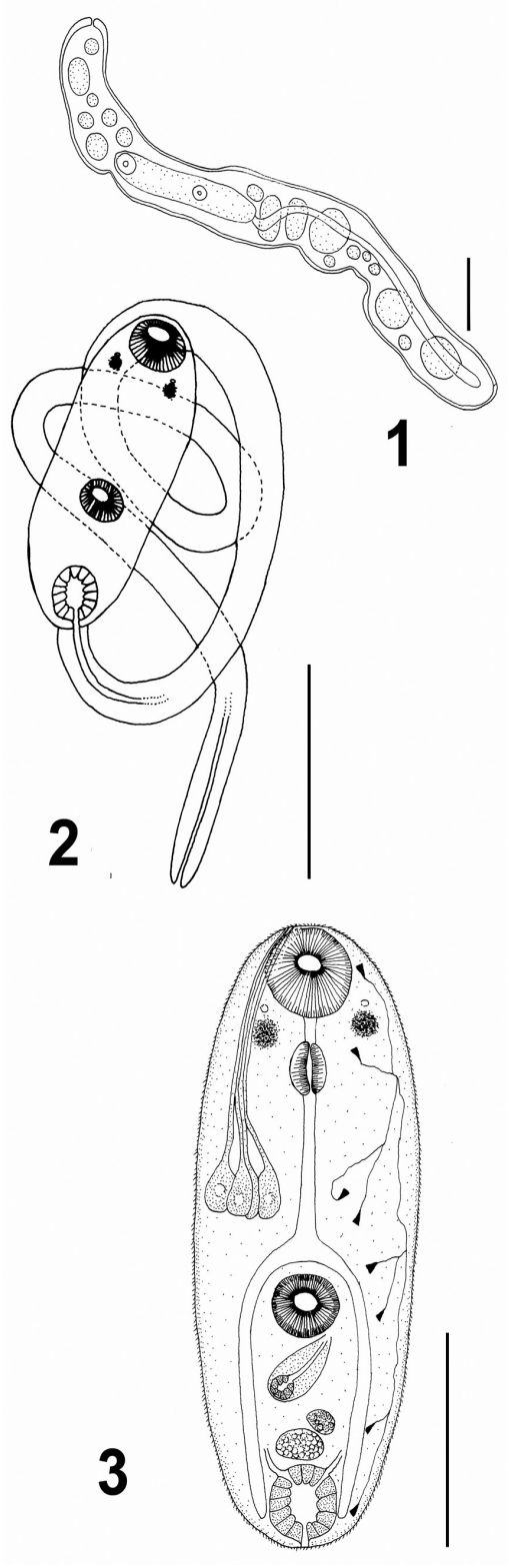
Sporocyst (Fig. 1): Yellowish, immotile, elongated, thick-walled, with a birth pore at one end, 811 (652–1,090) long by 112 (82–160) maximum wide. Thirteen to 27 cercariae with well developed tail and ocelli (mean 17) at different developmental stages in each sporocyst.

Cercaria (Figs 2 and 3) (not released, larger specimens measured): Body elongate, 253 (176–386) long by 59 (36–113) wide at acetabulum level. Tegumental spines in transverse rows in all body length. Four pairs of penetration glands located at oesophageal bifurcation. Oral sucker 40 (39–42) long by 48 (38–56) wide, opening subterminally. Ventral sucker 44 (43–45) long by 56 (54–57) wide. Two eye-spots at prepharynx level. Prepharynx short. Pharynx 26 (20–30) long by 25 (22–28) wide. Oesophagus 56 (48–67) long. Caeca ending at posterior end of body. Prominent cystogenous cells scattered throughout length of body. Genital primordium of ovary, testis and cirrus sac distinguishable. Excretory bladder sac-shaped with thick wall and narrow lumen. Flame cell formula: 2 [(2 + 2) + (2 + 2)] = 16. Tail about four times length of body, muscular and contractile, 1,012 (702–1,440).

Host: *Amiantis purpurata* (Lamarck, 1818) (Bivalvia, Veneridae).

Site of infection (Fig. 4): Mainly the gonad and digestive gland; also the mantle and the foot.

Locality and prevalence: La Conchilla beach (intertidal) (40º49´S, 64º47´W), San Matías Gulf (1 of 690, 0.14%).



Figs l-3. Monorchiid larvae from the venerid clam *Amiantis purpurata* from Argentinean waters. **1.** Sporocyst .Scale bar = 100 µm. **2.** Cercaria, ventral view. Scale bar = 200 µm. **3.** Detail of cercarial body, ventral view. Scale bar = 100 µm. Left penetration glands and right flam cells omitted

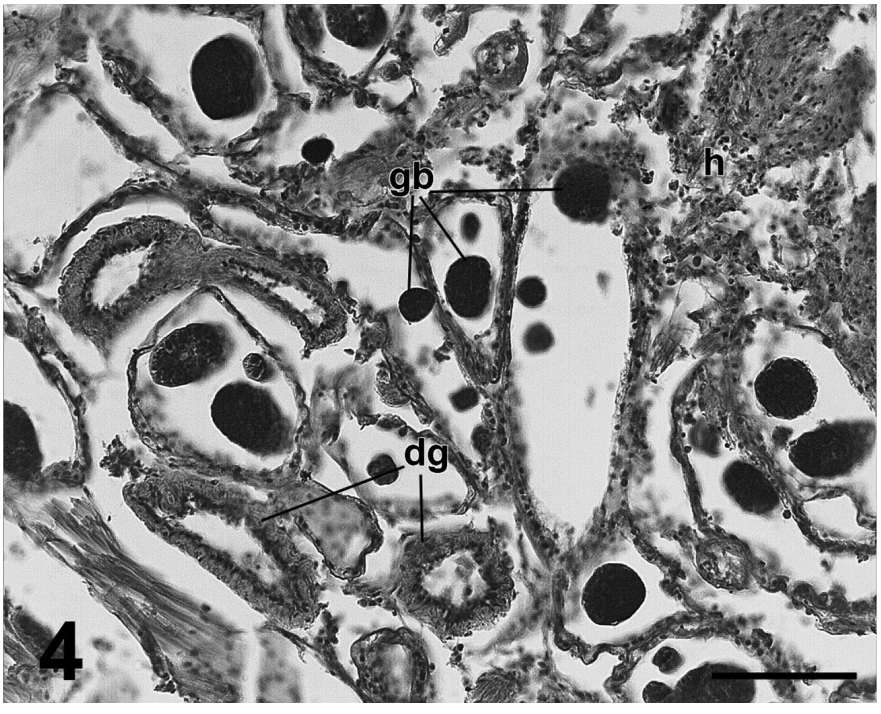


Fig. 4. Histological section (H&E) of the venerid clam *Amiantis purpurata* parasitised by monorchiid sporocysts. **Abbreviations:** dg – digestive gland, gb – germinal balls, h –haemocytic infiltration. Scale bar = 50 µm

Specimens deposited: Mounted larval stages and histological sections of parasitised clams at Helminthological Collection Museo de La Plata (MLP N° 6286, 6287), La Plata, and Parasitological Collection, Centro Nacional Patagónico (CNP-Par 29-30), Puerto Madryn, Argentina.

Taxonomic remarks

According to Cable (1956) and Cremonte *et al*. (2001) a monorchiid cercaria can be identified by the following characteristics: distome, pharyngeate larvae, tegument spinose, eyespots present or absent, stylet lacking, excretory vesicle not thin-walled, and a long and slender tail, shorter or collar like, tiny knob or brevifurcate tails.

Cremonte *et al*. (2001) established four groups of monorchiid cercariae according to their morphology. The first (1) include cercariae possessing a well-developed tail and usually ocellate; the second (2) cercariae with shorter or collar-like tails; the third group (3) comprise cercariae with a short furcae without tail stem; and those of the fourth group (4) have a tiny knob tail and are non ocellate. The cercaria described in the present paper belongs to the first group, which includes most of monorchiid cercariae.

The species here described is similar to *Cercaria caribbea LXIII* Cable, 1963 and *Cercaria longicaudata* Bartoli, 1966 described from *Tellina martinicensis* (Tellinidae) and *Venus fasciata* (Veneridae) respectively, differing from both in the number of penetration glands (Cable 1963, Bartoli 1966).

Aporocotylid cercaria sp. (Figs 5, 7)

Sporocyst (Fig. 5): Orange, immotile, spherical to oval with a birth pore at one end, thick-walled, 147 (116–176) long by 131 (102–156) maximum wide. Four to 15 brevifurcous cercariae (mean 8) at different developmental stages in each sporocyst.

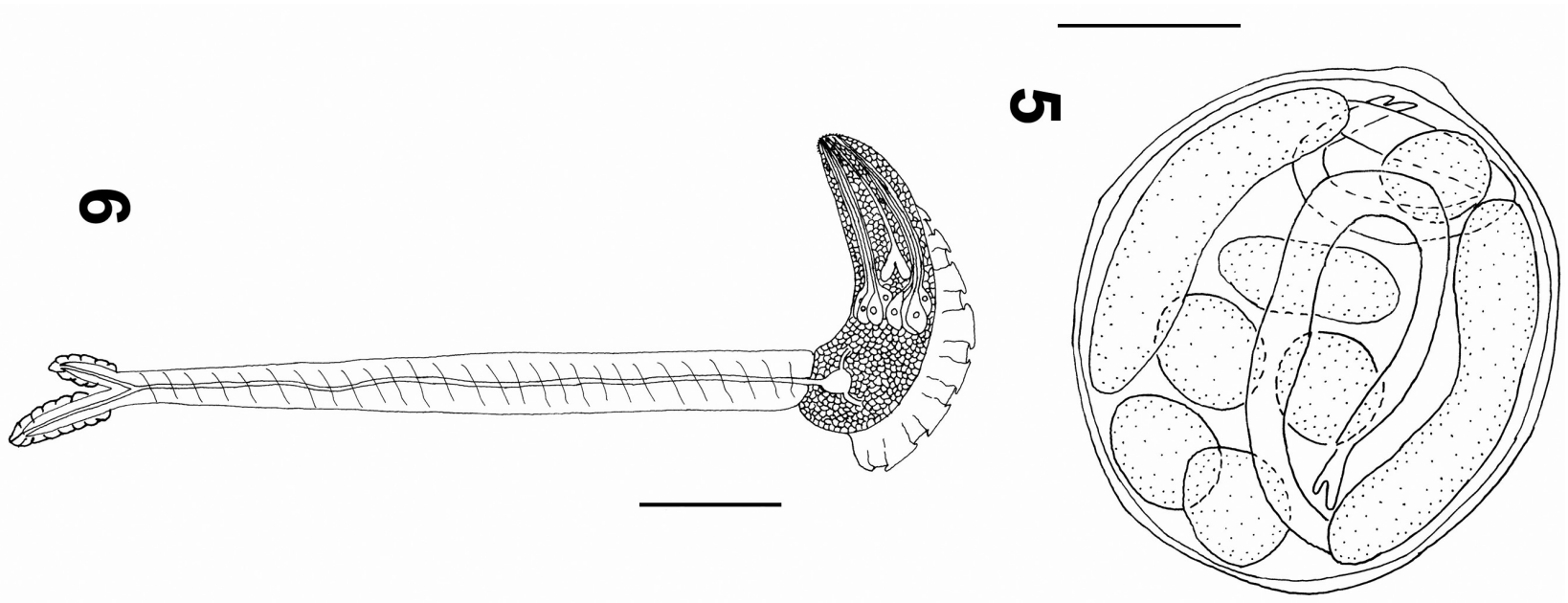
Cercaria (Fig. 6) (not released, larger specimens measured): Body smooth and elongate, 117 (83–148) long by 29.7 (26–31) maximum wide. Dorsal finfold of body plicate. Anterior tip of body protrusible, encircled with parallel rows of minute spines. Mouth terminal, oesophagus narrow, bifurcating into two short caeca. Penetration glands located posterior to caeca in four pairs. Cystogenous cells filling the parenchyma, making observation of excretory system and penetration glands difficult. Excretory bladder sac-shaped. Excretory formula not determined. Tail stem and furca with minute spines. Tail stem 308 (250–330) long; 21(15–23) width, asymmetrical furcae, right furca 53 (40–60) long and left furca 31 (24–35) long.

Host: *Amiantis purpurata* (Lamarck, 1818) (Bivalvia, Veneridae).

Site of infection (Fig. 7): Gills and also a little portion of the nephridia in one heavily infected clam.

Locality and prevalence: El Molino beach (subtidal) (40°48´S, 64°44´W), San Matías Gulf (3 of 690, 0.43%).

Specimens deposited: Mounted larval stages and histological sections of parasitised clams at Helminthological Collec-



Figs 5 and **6.** Aporocotylid larvae from the venerid clam *Amiantis purpurata* in Argentinean waters. **5.** Sporocyst. Scale bar = 50 µm. **6.** Cercaria. Scale bar = 50 µm

Table I. Records of sanguinicolid cercariae using marine bivalves as first intermediate hosts, indicating hosts, localities and prevalences (number of parasitised clams/number of examined clams)

Sanguinicolid species	Bivalve 1º intermediate host	Locality	Prevalence	Reference
Cercaria martini Stunkard, 1983	*Argopecten irradians* (Pectinidae)	Woods Hole, USA	0.27% (1/367)	Linton (1915a), Stunkard (1983)
unnamed	*Tagelus divisus* (Psammobiidae)	Florida, USA	12.7% (112/664)	Fraser (1967)
Cercaria asymmetrica Holliman, 1961	*Donax variabilis* (Donacidae)	Florida, USA	0.45% (8/1,763)	Holliman (1961)
Cercaria cristulata Holliman, 1961	*Chione cancellata* (Veneridae)	Florida, USA	0.83% (1/120)	Holliman (1961)
Cercaria mercenaria Wardle, 1979	*Mercenaria campechensis* (Veneridae)	Texas, USA	28.57% (8/28)	Wardle (1979)
Cercaria solemyae Martin, 1944	*Solemya velum* (Solemyidae)	Texas, USA	Not provided	Martin (1944)
unnamed	*Amiantis purpurata* (Veneridae)	San Matías Gulf, Argentina	0.45% (3/660)	present paper

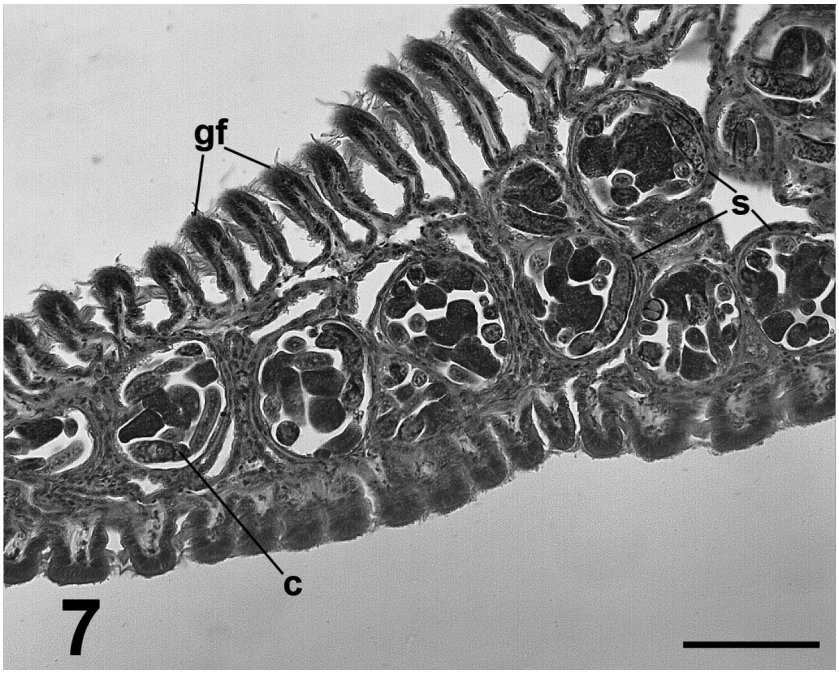


Fig. 7. Histological section (H&E) of the gill of the venerid clam *Amiantis purpurata* parasitised by aporocotylid sporocysts. **Abbrevations:** c – cercaria, gf – gill filaments sporocyst. Scale bar = 50 µm

tion, Museo de La Plata (MLP N° 6288, 6289), La Plata, and Parasitological Collection, Centro Nacional Patagónico (CNP-Par 30, 31), Puerto Madryn, Argentina.

Taxonomic remarks

According to Holliman (1961) an aporocotylid (= sanguinicolid) cercaria can be identified by the following characteristics: apharyngeate, non-ocellate, brevifurcate cercaria.

Previous records of larval aporocotylid from marine bivalves are listed in Table I. From these records, the cercaria described in the present paper resembles *C. asymmetrica* Holliman, 1961 described from *Donax variabilis* (Donacidae) in Florida, USA, because of the asymmetrical furcae, but it differs in having only one caecum (Holliman 1961).

Discussion

Present reports comprise the second monorchiid intramolluscan infection reported for the Southern Hemisphere and first intramolluscan aporocotylid for the Southern Hemisphere. The larvae described in the present paper may correspond to one of the adults reported by Kohn et al. (2007) from the Southwestern Atlantic Ocean or to a still undescribed species. Records of Aporocotylidae from marine Argentinean fishes are scarce. This circumstance is largely due to the fact that these peculiar digeneans (parasitic in the circulatory system of teleosts, elasmobranchs and holocephalans) are commonly overlooked by taxonomists who do not usually examine heart and blood vessels of the fish (Lauckner 1983).The only aporocotylid described from study area is *Aporocotyle argentiniensis* Smith, 1969 found in *Merluccius hubbsi* (Kohn *et al*. 2007).

Digeneans typically have a dramatic effect on the health of their first intermediate host. Intramolluscan stages live within the haemocoel of the gonad and to a lesser extent in the digestive gland, castrating the host (Lauckner 1983, 1987; Galaktionov and Dobrovolskij 2003, Cremonte in press). The phenomenon of parasitic castration is widespread. It is considered as a strategy of parasites, aimed at providing better conditions for their survival in the host and the completion of their life cycle (Kuris 1974, Baudoin 1975, Dobson 1988, Galaktionov and Dobrovolskij 2003). This effect does decrease or complete exclude the reproductive efforts of the host, thus releasing considerable energy resources which are used by the parasites (Galaktionov and Dobrovolskij 2003).

The case reported here, where the aporocotylid sporocysts are occupying only the haemocoel of gills, constitutes, as far as we know, the first report of intramolluscan marine larval stages parasitising tissues other than the digestive gland and gonad. The previous reported digeneans parasitising only the gills of their molluscan host are members of Gorgoderidae, parasitic in freshwater bivalves (Ginetsinskaya 1988). Curiously, the only gorgoderid reported in a marine bivalve is not parasitising the gills but the gonad; Bott and Cribb (2005) reported a single gorgoderid infection in a venerid clam, *Lioconcha castrensis*, in Australia.

Only a few oogonia and primary oocytes are attached to the inner acinar walls in two of the 3 clams parasitised by the aporocotylid parthenita; in the third, it was not possible to

identify the sex. The gametogenic cycle of *A. purpurata* in the study area is continuous, without a discrete gonadal arrest after the end of the reproductive season (Morsan and Kroeck 2005). However, because parasitised clams were collected in winter, where the early proliferative phase is occurring, a possible castrating effect could be overlooked. It needs to be confirmed if the presently reported aporocotylid is castrating the host through an unknown indirect mechanism, as the parthenitae do not parasitise the gonadal tissues. Parasitic castration of molluscan hosts is a frequent result of the development of trematode parthenitae in the gonad. It may be provoked by mechanical (rediae feeding on generative cells or tissue necrosis resulting from pressure of growing parthenitae) or chemical causes. In the latter case, parasites secrete certain substances which act negatively upon the reproductive function of the molluscan host, i.e., inhibiting spermatogenesis or oogenesis (Galaktionov and Dobrovolskij 2003 and references therein).

The apparent low prevalence of aporocotylid trematodes in molluscan hosts (Table I) is markedly contrasted by a general high prevalence of the adult worms in fishes (Lauckner 1983). It seems that transmission may be highly successful despite the low proportion of bivalves infected with cercariae. Low prevalences in the first intermediate host could be explained by the high pathogenicity of certain intramolluscan larval stages. Digeneans with sporocysts that are highly invasive (i.e., by occupying other organs besides the gonad) cause the weakness of the clams impairing their burrowing ability and hence, the clams are washed up on the beach after strong storms (e.g., Cremonte *et al*. 2001, Galaktionov and Dobrovolskij 2003).

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