

# The life cycle and geographical distribution of the monorchiid *Proctotrema bartolii* (Digenea) in the clam *Darina solenoides* from the Patagonian coast, Argentina

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## Abstract

The life cycle of *Proctotrema bartolii* Carballo, Laurenti & Cremonte 2011 (Digenea: Monorchiidae) at Fracasso Beach (the type locality) (42°25'S, 64°07'W), Península Valdés, Argentina, was investigated. This digenean uses the clam *Darina solenoides* (Mactridae) as both the first and second intermediate hosts in the natural environment. The metacercariae were located mainly at the tip of the incurrent siphon, with an infection prevalence of 100%. Experimental infections in other macroinvertebrates, such as the clam *Tellina petitiana* and the polychaete *Glycera americana*, were successful, but these and other invertebrates are not naturally infected. Silversides *Odontesthes smitti* and *Odontesthes nigricans* (Pisces: Atherinopsidae) and the mullet *Eleginops maclovinus* (Eleginopidae) act as the definitive hosts of both experimentally and naturally obtained adults. Fish acquire infection by eating either the siphon or the entire clam. *Proctotrema bartolii* seems to be endemic to the Magellan Region and is distributed where its intermediate clam host is present, from the San José Gulf in Península Valdés to the southern tip of South America.

## Introduction

Monorchiid trematodes are fish parasites that normally develop into sporocysts in marine bivalves (Lauckner, 1983; Cremonte *et al.*, 2001), and members of the genus *Asymphyllodora* Looss, 1890 (subfamily Asymphyllodorinae Szidat, 1943) have rediae that develop in gastropods (Lauckner, 1983; Našincová & Scholz, 1994). The Asymphyllodorinae have now been transferred to the Lissorchiidae (Madhavi, 2008). To date, 18 monorchiid cercariae that develop in sporocysts in bivalves have been described, and only two of them are from the southern hemisphere (Cremonte *et al.*, 2001; Gilardoni *et al.*, 2011), whereas the life cycles of seven species are known, all from the northern hemisphere (Martin, 1940; Young, 1953; DeMartini & Pratt, 1964; Bartoli, 1965; Maillard, 1975;

Stunkard, 1981a, b; Bartoli *et al.*, 2000). The metacercariae occur in marine environments, either in the same species of bivalve or in different bivalves, and the adults live in the intestines of teleost fish (see table 1 in Cremonte *et al.*, 2001).

The presence of adult monorchiids in the south-western Atlantic Ocean has been described in teleost fish from Brazil (Amato, 1982; Kohn *et al.*, 2007), the Patagonian coast of Argentina, and Antarctic sub-Antarctic waters (Szidat, 1950; Gosztonyi, 1979; Zdzitowiecki, 1997; MacKenzie, 2003; Carballo *et al.*, 2011). *Proctotrema bartolii* Carballo, Laurenti & Cremonte 2011 (Monorchiidae) was described in natural and experimental adults obtained from the silversides *Odontesthes smitti* and *Odontesthes nigricans* (Pisces: Atherinopsidae) and the mullet *Eleginops maclovinus* (Eleginopidae) at the San José Gulf and Nuevo Gulf on the Patagonian coast.

The clam *Darina solenoides* (King & Broderip, 1832) (Mactridae) was reported as suspected to act as the first

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Table 1. The prevalence (%) and intensity ( $\pm$  standard deviation) of metacercariae of *Proctotrema bartolii* from the incumbent siphon of the clam *Darina solenoides* collected along the Patagonian coast, Argentina; *N* = number of clams examined, with shell length in millimetres given as  $\pm$  standard deviation and the numbers in brackets representing ranges.

Site	Date	<i>N</i>	Clam size (mm)	Intensity	Prevalence (%)
Fracasso Beach (42°25'S, 64°07'W)	March 2000	20	10 $\pm$ 1 (8–12)	3 $\pm$ 1 (1–6)	75 (65–85)
Fracasso Beach (42°25'S, 64°07'W)	March 2000	20	14 $\pm$ 1 (13–16)	10 $\pm$ 9 (1–32)	95 (94–96)
Fracasso Beach (42°25'S, 64°07'W)	March 2000	20	21 $\pm$ 1 (19–22)	12 $\pm$ 7 (3–25)	100
Fracasso Beach (42°25'S, 64°07'W)	July 2000	20	17 $\pm$ 2 (14–21)	14 $\pm$ 9 (5–34)	100
Colombo Beach (42°36'S, 64°14'W)	April 2002	15	23 $\pm$ 3 (18–27)	6 $\pm$ 3 (2–12)	100
Punta Medanosa (48°06'S, 65°54'W)	March 1998	15	32 $\pm$ 4 (23–37)	127 $\pm$ 133 (32–505)	100
Punta Loyola (51°38'S, 68°58'W)	March 1998	15	35 $\pm$ 7 (16–43)	167 $\pm$ 123 (26–537)	100

and second intermediate hosts of *P. bartolii* (Carballo *et al.*, 2011). Indeed, one of the most fundamental characteristics of a parasite is the spectrum of host species used at each stage in its life cycle; however, this information is not available for many parasite species (Combes, 2001). Certain species show a marked specialization for one host or very few hosts, particularly if the ecosystem in which they live offers numerous taxonomically related species to exploit (Rhode, 1993). The aims of the present work were to elucidate the life cycle of *P. bartolii* at San José Gulf (type locality) on the Patagonian coast and to identify the spectrum of hosts that could be involved. The geographic distribution of *P. bartolii* is also provided.

## Materials and methods

### Study site and geographical distribution

Fracasso Beach (42°25'S, 64°07'W) is located on the south-east coast of the San José Gulf, Península Valdés, Chubut Province, Argentina. Península Valdés is a Protected Provincial Area and was declared a Natural Human Patrimony site (UNESCO). *Darina solenoides* is the dominant invertebrate of the intertidal zone at Fracasso Beach (Hernández *et al.*, 2008) and is distributed in the south-western Atlantic Ocean from the San Matías Gulf throughout the Magellan Region (Carcelles, 1950). Other significant components of the invertebrate assemblages are the clam *Tellina petitiana* d'Orbigny, 1845 (Tellinidae), which inhabits the lower intertidal level, the gastropod *Buccinanops globulosus* (Kiener, 1834) (Nassariidae), and the polychaetes *Glycera americana* Leidy, 1855 (Glyceridae), *Travisia* sp. (Opheliidae) and *Laonereis acuta* (Treadwell, 1923) (Nereidae) (Zaixso *et al.*, 1998; Hernández *et al.*, 2008). Silversides are present along the coast throughout the year and the mullet mainly in the autumn, when they are caught by artisanal fishermen using coastal nets.

To determine the geographic range of *P. bartolii*, samples of *D. solenoides* were collected at the following locations: Colombo Beach (42°36'S, 64°14'W), Nuevo Gulf; Punta Medanosa (48°06'S, 65°54'W), and Punta Loyola (51°38'S, 68°58'W) in Santa Cruz Province (fig. 1). These sites encompass almost the entire distribution area of *D. solenoides*. The clams were immediately fixed in 10% seawater formalin and examined using a stereomicroscope. The number of metacercariae was recorded. The maximum shell lengths of clams were measured with a

calliper and the prevalence and the intensity of infection (only in the incumbent siphon) were calculated for each locality. The intensity of infection between localities was compared with a Kruskal–Wallis test and pairs comparison. In all cases, the parasitological indices of Bush *et al.* (1997) were used.

### Study of intramolluscan stages

The specimens of *D. solenoides* were collected in August 1999, March 2000, July 2000, and monthly from November 2001 to November 2002 (except May). About 200 clams were collected on each occasion. The cercariae were studied from emitted specimens. The larvae (sporocysts, cercariae and metacercariae) were studied *in vivo* using both neutral red and Nile blue stained and unstained specimens. The larvae were killed with heated seawater

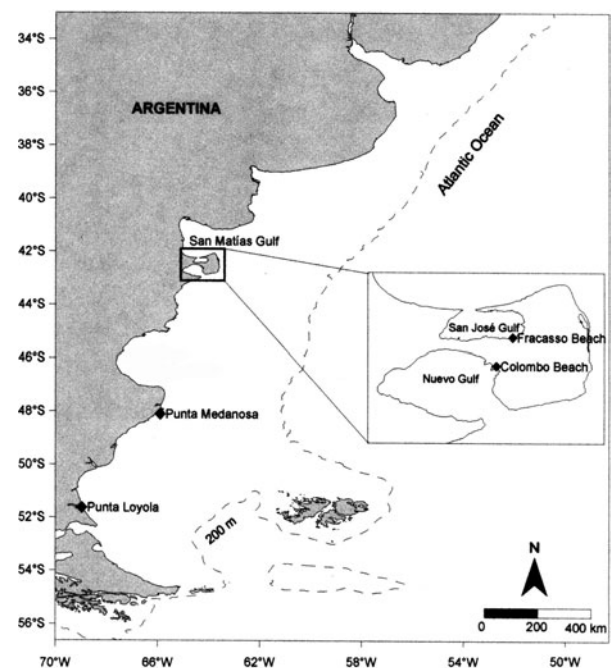


Fig. 1. Map showing the distribution of *Proctotrema bartolii* (Digenea: Monorchiiidae) along the Patagonian coast, Argentina; the area in the rectangle (Península Valdés) is enlarged on the right.

and were immediately fixed with 10% formalin, preserved in 70% ethanol and stained with Semichons's acetocarmine. After being cleared with creosote, the larvae were mounted in Canada balsam and measured. Drawings were made with the aid of a camera lucida, and the measurements are given in micrometres ( $\mu\text{m}$ ), followed by the range in parentheses. Some of the fixed metacercariae were dehydrated and dried by rinsing for a few minutes in hexamethyldisilazane. The metacercariae were then gold-coated for observation; the samples were photographed using a Jeol JSM-6460LV scanning electron microscope (SEM).

The sporocysts, cercariae and metacercariae of *P. bartolii* and whole, parasitized *D. solenoides* from the sampled localities were deposited in the Parasitological Collection, Centro Nacional Patagónico (CNP-Par 02-05), Puerto Madryn, Argentina.

#### *Prevalence, behaviour and site of infection of intramolluscan stages*

For each clam collection, approximately 200 clams were maintained in individual flasks for the observation of cercarial emission. Those clams emitting cercariae were used as the sources of the infective material. The clams were exposed to light to stimulate the emission of cercariae, and the penetration behaviour of cercariae was observed using a stereomicroscope.

The prevalence of *P. bartolii* sporocysts and metacercariae was calculated for 60 specimens of *D. solenoides*, and the samples were sorted according to three size classes of shell length (8–12, 13–16 and 17–23 mm). Several specimens were dissected under a stereomicroscope to establish the precise location of the sporocysts and metacercariae in the host. The soft parts of five whole infected clams were fixed in Davidson's fixative, stored in 70% ethanol, and later dehydrated, embedded and sectioned (thickness 5–7  $\mu\text{m}$ ) following standard histological protocols. The sections were stained with Harris's haematoxylin–eosin. To establish the precise location of the metacercariae, a total of 60 clams were dissected, and the metacercariae were counted in each affected organ. The intensity of metacercarial infection in the incurrent siphon among three size classes of shell length (8–12, 13–16 and 17–23 mm) was compared with a Kruskal–Wallis test and pairs comparison.

Six uninfected clams were selected for experimental infection in July 2000 (the clams that did not emit cercariae) and were exposed to a great quantity of emitted cercariae; the clams were dissected after 48 h for the metacercariae, which were then measured and counted. Because the prevalence of metacercariae was 100%, it was not possible to obtain uninfected individuals. Therefore, the intensity of infection in 20 naturally infected clams of the same size (average of 17 mm, range of 14–21 mm) and date of collection (July 2000) was calculated and then compared with the intensity of those experimentally obtained, using a Kruskal–Wallis test.

#### *Macroinvertebrates as the second intermediate hosts*

To establish the existence of other macroinvertebrates acting as the second intermediate host for *P. bartolii*,

samples of *T. petitiiana* ( $n = 167$ ; August 1999, March 2000 and December 2001), *B. globulosus* ( $n = 61$ ; April 2001), *G. americana* ( $n = 56$ ; August 1999, April 2001 and December 2001), *Travisia* sp. ( $n = 86$ ; August 1999, April 2001 and December 2001) and *L. acuta* ( $n = 17$ ; April, 2002) were collected. The specimens were immediately fixed in 10% seawater formalin, stored in 70% alcohol and examined for parasites. Moreover, an experimental infection was performed by placing a large number of cercariae in a small flask with one specimen of each species (five replicates) and then dissecting after 48 h to identify the metacercariae. To determine the location and the effects of the metacercariae, three individuals of *G. americana* were dissected using a stereomicroscope. The soft parts were fixed in Davidson's fixative, stored in 70% ethanol, and later dehydrated, embedded and sectioned (thickness 5–7  $\mu\text{m}$ ) following standard histological protocols. The sections were stained with Harris's haematoxylin–eosin.

## Results

### *Study area and geographical distribution*

*Proctotrema bartolii* was present in all of the sites sampled, which extend through almost the entire distribution range of *D. solenoides* (fig. 1). Table 1 shows the prevalence and intensity of infection at the different localities. The intensity of metacercarial infection and the clam size were higher in the southern localities (Punta Medanososa and Punta Loyola) than in the type locality, Fracasso Beach, which is located at the northern limit of the distribution ( $H = 48.76$ ;  $P < 0.0001$ ). The clams from Colombo Beach had the lowest intensity of metacercarial infection, but did not present significant differences with Fracasso Beach ( $P = 0.23$ ). The differences between the two southern localities (Punta Medanososa and Punta Loyola) were not significant ( $P = 0.47$ ).

### *Description of intramolluscan stages*

Measurements are given in table 2.

#### *Sporocysts*

Microcercous cercariae develop in colourless, immobile, thick-walled sporocysts (fig. 2a). Eight to 14 cercariae (average: 10) at different developmental stages were found in each sporocyst.

#### *Cercariae*

Elongated body (fig. 2b). Tegumental spines in transverse rows, same sizes for all body lengths. Seven pairs of penetration glands. Oral sucker opening subterminally. Ventral sucker equatorial. Sucker ratio 1:1. Caeca reaching almost to the end of body. Prominent cystogenous cells scattered through body parenchyma. Genital primordia present, located between ventral sucker and end of excretory vesicle. Testis ovoid, located anteriorly to excretory vesicle. Ovary pretesticular. Excretory bladder I-shaped, with thick wall and narrow lumen, open dorsally, subterminally at end. Flame cell formula:  $2 [(2 + 2) + (2 + 2)] = 16$ . Tiny knob tail, spiny.

Table 2. Mean measurements ( $\mu\text{m}$ ) of cercariae and metacercariae of *Proctotrema bartolii*, with the range of measurements given in brackets;  $n$  = number of larval stages measured.

Morphometrics		Mean (range)		
		Cercaria ( $n = 20$ )	Metacercaria ( $n = 11$ )	Metacercaria from experiments ( $n = 13$ )
Body	Length	478 (368–559)	–	–
	Width	91 (74–110)	–	–
	Forebody	199 (147–264)	–	–
Oral sucker	Length	52 (48–61)	50 (46–56)	47 (41–60)
	Width	51 (48–58)	60 (43–72)	57 (48–66)
Ventral sucker	Length	50 (48–53)	46 (34–70)	45 (39–50)
	Width	50 (47–57)	51 (49–54)	55 (50–63)
Prepharynx	Length	13 (3–26)	–	–
	Width	–	–	–
Pharynx	Length	24 (21–31)	29 (22–38)	29 (27–31)
	Width	22 (20–28)	23 (19–32)	21 (10–28)
Oesophagus	Length	78 (50–98)	–	–
	Width	–	–	–
Excretory bladder	Length	166 (133–218)	–	–
	Width	–	–	–
Tail	Length	23 (17–28)	–	–
	Width	19 (17–21)	–	–
Cyst	Diameter	–	167 (152–188)	171 (161–183)

Measurements of sporocysts ( $n = 10$ ): body length, 1551 (760–7300); body width, 235 (180–290). Forebody is the distance from the anterior body end to the anterior edge of the ventral sucker.

#### Metacercariae

Cyst (fig. 3a) rounded, translucent and membranous. The metacercariae excyst (fig. 3b) when applying pressure to the coverslip. Tegumental spines in transverse rows, same sizes for all body lengths. Inner circle of approximately ten papillae in oral sucker and approximately six papillae in ventral sucker (only observed by SEM; fig. 3b). Three longitudinal rows of papillae distributed ventrally from anterior end to ventral sucker level. Genital primordia as in the cercariae.

#### Prevalence, behaviour and site of infection of intramolluscan stages

*Proctotrema bartolii* utilizes only the clam *D. solenoides* as both the first and second intermediate hosts in the natural environment. The overall prevalence of sporocysts was 5% (5% in clams 8–12 mm long, 0% in clams 13–16 mm long and 10% in clams 17–23 mm long). The sporocysts with cercariae were located mainly in the gonad and also occupied the digestive gland and gills at high intensities (fig. 4a).

The cercariae were expelled in a contracted form by the excurrent siphon one by one or in groups of two or more (up to 10). The cercariae then attached to the bottom of the flask via their minute tails, mainly to a particle or by adhering to each other, forming groups; the larvae moved by adhering their tails and their oral suckers. Some cercariae display a parabolic trajectory from the excurrent siphon, arriving directly at the incurrent siphon. Once they arrived at the exposed part of the mantle, the foot or the siphons, the cercariae adhered by their tails and then remained for a few minutes with the oral sucker on the tissue until they penetrated. They did not exhibit photo-orientation.

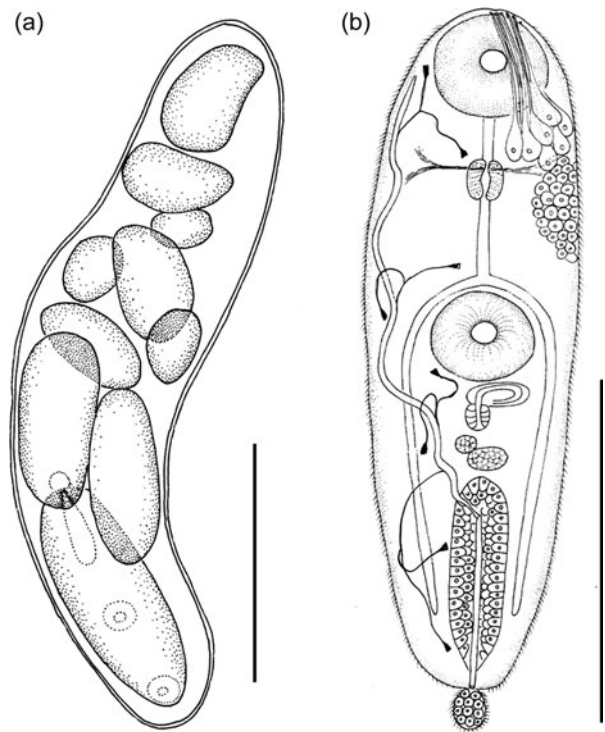


Fig. 2. Line drawings of the larval stages of *Proctotrema bartolii* (Digenea: Monorchhiidae) that are parasitic in the clam *Darina solenoides* from the Patagonian coast: (a) sporocyst; (b) cercaria, ventral view, the flame cells of the left side are omitted, the penetration glands of the right side are omitted. Scale bars: (a) 300  $\mu\text{m}$ ; (b) 200  $\mu\text{m}$ .



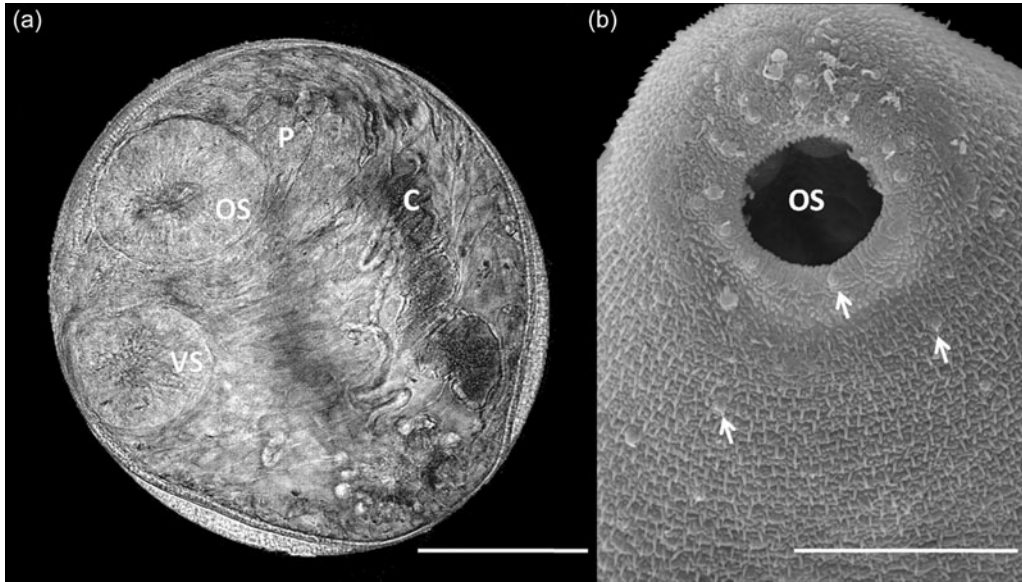


Fig. 3. Metacercaria of *Proctotrema bartolii* (Digenea: Monorchhiidae) that are parasitic in the clam *Darina solenoides* from the Patagonian coast: (a) encysted metacercaria *in vivo*; (b) excysted metacercaria by SEM. Abbreviations: C, caeca, OS, oral sucker; P, pharynx; VS, ventral sucker; the white arrows indicate papillae. Scale bars: (a) 100  $\mu\text{m}$ ; (b) 20  $\mu\text{m}$ .

The prevalence of metacercariae increased with the size ( $H = 16.24$ ;  $P < 0.0003$ ) of the clams (table 1) and mainly affected the incurrent siphon (table 3). The intensity of infection in the incurrent siphon of the two bigger size classes (13–16 and 17–23 mm) did not present significant differences ( $P = 0.83$ ), but these two presented significant differences with the small size class (8–12 mm) ( $P < 0.0001$ ). The metacercariae were located to a lesser degree in the excurrent siphon, mantle, orbicular muscle, adductor muscles, gills and siphon retractor muscles (table 3, figs 4a and b).

The experimental infections of *D. solenoides* exposed to the emitted cercariae resulted in a higher intensity and a different location of the metacercariae in comparison with the naturally infected clams. The intensity of infection in the incurrent siphon from experimentally infected clams presented significant differences from that of naturally infected clams ( $H = 13.33$ ;  $P < 0.0003$ ). After 48 h of infection, the mean intensity and range of the experimentally infected clams was  $185 \pm 61$  (31–123), whereas the mean intensity and range from the naturally infected clams collected on the same day was  $14 \pm 9$  (5–34). In the

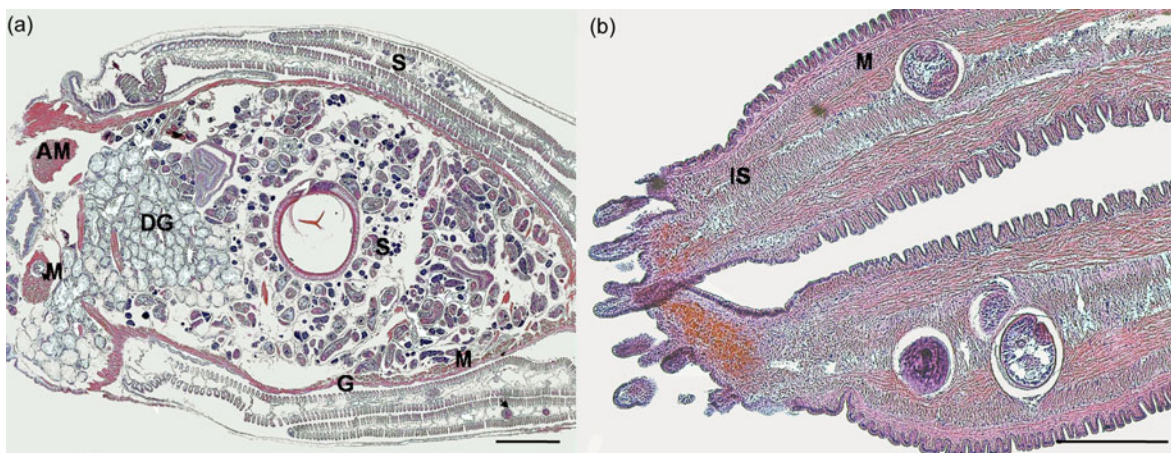


Fig. 4. (colour online) Histological sections (haematoxylin and eosin stain) of the clam *Darina solenoides* from the Patagonian coast. (a) Sporocysts of *Proctotrema bartolii* in the gonad and gills, and metacercariae in the adductor muscle and gills. (b) Metacercariae of *P. bartolii* in the siphons. Abbreviations: AM, adductor muscle; DG, digestive gland; G, gill; IS, incurrent siphon; M, metacercaria; S, sporocyst; the black arrow shows a metacercaria in the gills. Scale bars: (a) 500  $\mu\text{m}$ ; (b) 100  $\mu\text{m}$ .

Table 3. The prevalence (%) of *Proctotrema bartolii* metacercariae per organ in three sizes of clams, *Darina solenoides*, at Fracasso Beach.

Clam size (mm)	Organ affected						
	Incurrent siphon	Excurrent siphon	Mantle	Orbicular muscle	Adductor muscles	Gills	Retractor siphon
8–12	75	5	5	0	0	0	0
13–16	95	30	5	15	0	0	5
17–23	100	40	45	20	5	5	15

experimentally infected clams, 32% of the metacercariae were located in the incurrent siphon, 32% in the foot, 22% in the orbicular muscle, 10% in the excurrent siphon, 1.4% in retractor muscle of the siphon, 0.18% in the border mantle muscle and 0.6% in adductor muscle. In contrast, 78% of the metacercariae were in the incurrent siphon, 13% in the foot, 3% in orbicular muscle, 5% in the excurrent siphon, 0.7% in the border mantle muscle and 0.35% in retractor muscle of the siphon in the naturally infected clams.

#### *Macroinvertebrates as second intermediate hosts*

All of the examinations for the natural infections and the attempts to obtain experimental infections in the gastropod *B. globulosus* and the polychaetes *Travisia* sp. and *L. acuta* were negative. In the case of the clam *T. petitiiana*, seven out of nine individuals exposed to emitted cercariae became infected: metacercariae were found in the incurrent and excurrent siphons, mantle and foot. In the case of the polychaete *G. americana*, three individuals from the five exposed to the emitted cercariae became infected. In the histological sections, the metacercariae were found mainly in the parapods and also in the coelom, with a strong haemocyte host reaction.

## Discussion

*Proctotrema bartolii* uses the clam *D. solenoides* (Mactridae) as the first and second intermediate hosts in the waters along the Argentinean Patagonian coast. The cercariae possess a tiny knob tail and are only capable of crawling. The larvae are expelled by the excurrent siphon and enter the same individual or others of the same species, encysting as metacercariae mainly in the tip of the incurrent siphon. The adults develop in the silversides *O. smitti* and *O. nigricans* (Pisces: Atherinopsidae) and in the mullet *E. maclovinus* (Eleginopidae), which acquire the infection by ingesting the siphons of *D. solenoides* or the entire clam. Experimental infections with the metacercariae of *P. bartolii* in the siphons of *D. solenoides* and the development and maturation of the juveniles in *Odontesthes* spp. and *E. nigricans* were successful (Carballo *et al.*, 2011).

Cremonte *et al.* (2001) have summarized the life cycles of the monorchiid species known around the world. According to the four established morphological groups of monorchiid cercariae (Cremonte *et al.*, 2001), the cercariae of *P. bartolii* belong to group 4 because they possess a tiny knob tail and do not have ocelli. Other cercariae belonging to this group are *Cercaria caribbea*

XXXVI Cable, 1953, *Lasiotocus minutus* (Manter, 1931), *Lasiotocus elongatus* (Manter, 1931) and Monorchiid cercaria sp. (Cable, 1956; Holliman, 1961; Stunkard, 1981a, b; Cremonte *et al.*, 2001). The cercariae described herein can be distinguished from those of *L. minutus* because the latter has a sac-shaped excretory vesicle and its ventral sucker is smaller than the oral sucker (sucker ratio 1:0.75 versus 1:1) (Stunkard & Uzmann, 1959; Stunkard, 1981a). The cercariae described in the present report can be distinguished from those of *C. caribbea* XXXVI and *L. elongatus* because of the smaller body sizes of the latter (276  $\mu\text{m}$  *C. caribbea* XXXVI, 180–210  $\mu\text{m}$  *L. elongatus* and 478  $\mu\text{m}$  *P. bartolii*). In addition, *C. caribbea* XXXVI has a long and sinuous prepharynx with longitudinal muscles (Cable, 1956; Holliman, 1961; Stunkard, 1981b). Finally, the cercariae of *P. bartolii* differ from the unidentified monorchiid cercaria described by Cremonte *et al.* (2001) because the latter have a long excretory vesicle reaching the oral sucker level and the ventral sucker is located in the anterior part of the body. Additionally, the metacercariae described in the present report can be distinguished from those of *L. minutus* and Monorchiid cercaria sp. because the latter have a gelatinous, sticky sac that surrounds the cyst with two adhesive elongations. In contrast, *L. elongatus* individuals lack such elongations but have smaller cysts (70–75  $\mu\text{m}$  in diameter versus 152–188  $\mu\text{m}$ ).

The 18 known monorchiid species cercariae develop in bivalves belonging to the Order Veneroida (Cremonte *et al.*, 2001). The life cycle of *P. bartolii* is very similar to those of *Monorcheides cumingiae*, *Postmonorchis donacis* and *Paratimonia gobii* because the cercariae are expelled by the excurrent siphon, re-enter the same individual or others of the same species and encyst as metacercariae mainly in the incurrent siphon (Martin, 1938, 1940; Young, 1953; Stunkard, 1974; Maillard, 1975; Bartoli, 1984). Furthermore, in *L. longicystis*, for which cercariae have not been described, the metacercariae are mainly located in the incurrent siphon (Bartoli, 1965).

*Proctotrema bartolii* uses only the clam *D. solenoides* as the first and second intermediate hosts and the fish *O. smitti*, *O. nigricans* and *E. maclovinus* as its definitive hosts in its natural environment. Sporocysts with cercariae were found at a low prevalence (5%) on the first intermediate hosts. In the naturally infected clams, the intensity of metacercarial infection was 78% higher in the incurrent siphon than in the other organs affected. However, the intensity of metacercarial infection was more uniform in all of the affected organs in the experimentally infected clams. It is evident that metacercariae can encyst in any muscular tissue of their host.

Yet, the accumulation of metacercariae in the incurrent siphon has ecological implications: a siphon filled with metacercariae seems to be less contractile than a healthy siphon and, consequently, more exposed to the fish that are browsing the clam siphons.

With regard to the other macroinvertebrates that share the beach with *D. solenoides*, the clam *T. petitiiana* and the polychaete *G. americana* became experimentally infected by the metacercariae; however, these hosts are not naturally parasitized: *D. solenoides* is found over a large extent of the intertidal zone, whereas *T. petitiiana* is restricted to the lower intertidal zone (where *D. solenoides* is not present) (see Figure 1 in Cremonte, 2004). Taking into account that the cercariae have a tiny knob and are only capable of crawling, they seem to be incapable of infecting of *T. petitiiana*.

The metacercariae enter *G. americana* successfully, but the polychaete produces a highly haemocytic reaction in the invaded tissues that is capable of destroying the metacercariae. This polychaete is distributed in all of the intertidal *Darina* zones (Table 1 in Cremonte, 2004) and serves as food for coastal silversides (Carballo, 2008). Combes (2001) explained the mechanism responsible for the formation of host ranges, proposing the 'filters' concept. The probability that the encounter and compatibility filters will be opened exactly to the same degree in two or more host species is very low. In the case of *T. petitiiana*, the compatibility filter is opened, but the encounter filter is closed because the intertidal zones do not overlap. In the case of *G. americana*, the encounter filter is open, but the compatibility filter is closed, and the parasite–host association does not exist. Furthermore, the polychaete is capable of eliminating the parasite through its defence mechanisms.

*Darina solenoides* is located in isolated intertidal populations along its range on the Patagonian coast, wherever fine sand flats occur. The parasite (metacercariae on the incurrent siphon) was found at all of the sampled beaches from Fracasso Beach (42°25'S, 64°07'W) to Punta Loyola (51°38'S, 68°58'W), with prevalences ranging from 75 to 100%. *Proctotrema bartolii* seems to be an endemic parasite of the fauna of the Magellan Province and is distributed where its intermediate host (the clam) is present. Fracasso Beach is located in the northernmost part of the distribution area of *D. solenoides* (fig. 1), as evidenced by the smaller sizes of the clams and the lower intensities of parasite infections when compared with the situation in the southernmost sites (table 1).

This work is the first description of the life cycle of a monorchiid digenean and a *Proctotrema* sp. in the southern hemisphere. The metacercariae encyst in the substratum in three of the nine monorchiid species for which the life cycles are partially or completely known; in eight cases, the metacercariae encyst in the same venerid and in other clam species. It seems that the strategy of parasitizing the bivalve siphons was widely adopted in the monorchiiids that are successfully transmitted to fish acting as the definitive hosts (Martin, 1940; Young, 1953; Bartoli, 1965; Maillard, 1975). The life cycle of *P. bartolii* is characterized for a narrow spectrum of intermediate hosts, exhibiting both morphological and behavioural adaptations to enhance the transmission of the parasite.

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