

## Life cycle and geographic distribution of the gymnophallid *Bartolius pierrei* (Digenea) on the Patagonian coast, Argentina

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The life cycle of the gymnophallid *Bartolius pierrei* Cremonte, 2001 (Digenea) at Fracasso Beach (the type locality) (42°25'S, 64°07'W), Península Valdés, Argentina, was elucidated. This digenean uses the clam *Darina solenoides* (Mactridae) as both first and second intermediate host. The Kelp Gull, *Larus dominicanus*, and the Red Knot, *Calidris canutus rufa*, a Neotropical migratory bird, act as definitive hosts. A prevalence of infection of 92% was found in the Red Knot. The cercariae of *B. pierrei* did not parasitize other invertebrates, not even the tellinid clam *Tellina pettitiana*, which shares the intertidal habitat with *D. solenoides*. This fact could be explained by the behaviour of the cercaria and its strategy for penetration. The cercariae enter directly in the extrapallial space of the clam by piercing the exposed mantle border. *Tellina pettitiana* could not be invaded by *B. pierrei* cercariae because it does not expose the mantle border when feeding. Because the Red Knot does not feed on *T. pettitiana*, this behaviour may constitute an adaptive strategy of the parasite. Under laboratory conditions, cercariae lived up to 2 days, metacercariae became infective in 50 days, and the adult life is considered to be shorter than 3 days. The short adult life span in gymnophallids could be a result of adaptation to migratory birds. This fact would ensure parasite dispersion (i.e. by covering long distances in a short time) and inter-breeding with other *Darina*-infesting populations located far apart. *Bartolius pierrei* is an endemic parasite of the Magellan Region, distributed where its intermediate clam host is present, from San José Gulf in Península Valdés to the southern tip of South America.

KEYWORDS: Parasite, Gymnophallidae, *Darina solenoides*, south-west Atlantic coast.

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### Introduction

Gymnophallid trematodes are marine bird parasites that mainly utilize bivalves as first and second intermediate hosts. Some gymnophallids utilize polychaetes and gastropods as second intermediate hosts (Bartoli, 1974; Ching, 1995).

In South America, the only adult gymnophallid species reported is *Bartolius pierrei* Cremonte, 2001, occurring on the Patagonian coast of Argentina (Cremonte, 2001). This trematode was described from the metacercaria found in the clam

*Darina solenoides* (King and Broderip, 1832) (Mactridae) and from both experimentally and naturally obtained adults. The type host is the Kelp Gull *Larus dominicanus* Lichenstein, 1823; moreover, in faeces of *Calidris canutus rufa* (Wilson, 1813) dead expelled worms without eggs were found (Cremonte, 2001). Two developmental stages of metacercariae were found in the clam, one smaller and translucent (younger) and the other larger, dark coloured and enclosed in sacs (older) (Cremonte, 2001). Newly formed metacercariae ascend along the space between the outer mantle epithelium and the shell (the extrapallial space), and settle in the uppermost general extrapallial space, just ahead of the posterior adductor muscle.

Cremonte and Ituarte (2003) studied the reaction of the clam to the metacercariae. It was established that the reaction consists of the encapsulation of metacercariae in a sac, resulting from the proliferation and invagination of the outer mantle epithelium adjacent to the metacercariae. The sac progressively detaches from the mantle epithelium and becomes internal, in the postero-dorsal region of the visceral mass (Cremonte and Ituarte, 2003). Encapsulated metacercariae are much larger than young metacercariae with the genital system fully developed; these old metacercariae are infective for birds (Cremonte, 2001).

The aim of the present work is to elucidate the life cycle of *Bartolius pierrei* at the type locality (Fracasso Beach) on the Patagonian coast. The sporocyst and the cercaria of this species, as well as the development of the cercaria into an old metacercaria, are described for the first time. Other possible hosts (intermediate and definitive) that may be involved in the life cycle are identified, and the geographic distribution of *B. pierrei* along the Patagonian coast is provided.

## Materials and methods

### *Study site*

Fracasso Beach (42°25'S, 64°07'W) is located on the south-east coast of San José Gulf, Península Valdés, Chubut Province, Argentina. Península Valdés is a Protected Provincial Area and was declared Natural Human Patrimony (UNESCO). Fracasso is a gently sloping sandy beach, about 3.5 km long, limited at both sides by rocky shores. Bottom sediments are fine and medium-sized sands. The average tide amplitude is 6.14 m, reaching a maximum of 8.95 m; during low tides, the bottom is exposed for about 1 km seawards. Along the higher littoral levels there is a marsh, dominated by the grass *Salicornia*, which is flooded only on the occasion of extraordinary tides (figure 1). The dominant invertebrate of the intertidal zone is the bivalve *Darina solenoides* (Mactridae), reaching a density of 859 individuals per m<sup>2</sup> (Hernández, 2000). *Darina solenoides* is distributed in the south-western Atlantic Ocean from San Matías Gulf throughout the Magellan Region (Carcelles, 1950). Other relevant 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 *Laeonereis acuta* (Treadwell, 1923) (Nereidae). The distribution of these invertebrates along the sandy littoral and their relative abundances at each level are schematized in figure 1.

Fracasso Beach is a feeding area for about 23 species of migratory and non-migratory shorebirds (Pagnoni, 1997). *Darina solenoides* is the most important prey

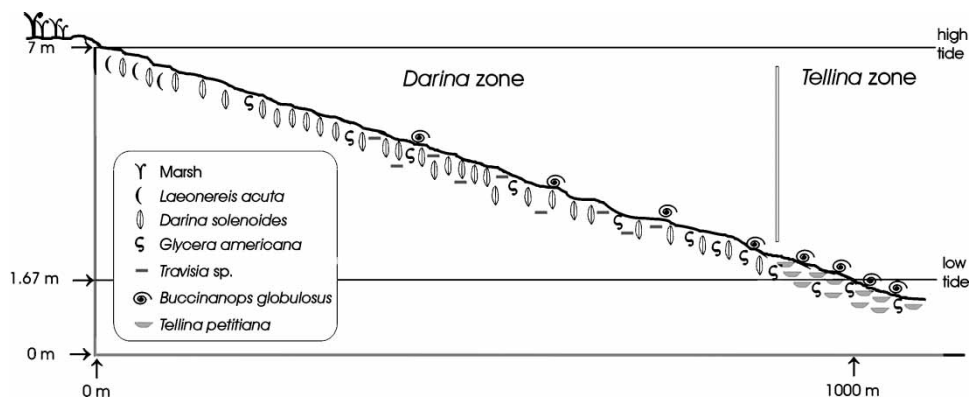


FIG. 1. Diagram showing the distribution of macroinvertebrates on the intertidal at Fracasso Beach. The abscissa indicates the tide level and the ordinate indicates the extension of exposed beach during low tide.

at Fracasso Beach, an important stopover for *Calidris canutus rufa*, *Calidris fuscicollis* Vieillot, 1819, *Calidris alba* (Pallas, 1764) (Scolopacidae) and *Charadrius falklandicus* Latham, 1790 (Charadriidae) migrating north in March and April (Hernández, 2000; D'Amico, 2001).

#### *Intramolluscan stages study*

Cercariae were studied from expelled specimens. Larvae (sporocysts, cercariae and metacercariae) were studied *in vivo* from both neutral red and Nile blue stained specimens, and non-stained ones. Larvae were fixed in hot 4% seawater formalin, measured, preserved in 70% ethanol, stained with Semichon's acetocarmine, cleared in creosote and mounted in Canada balsam. Drawings were made with the aid of a camera lucida. Mean lengths, with the range in parentheses, are given in micrometres ( $\mu\text{m}$ ).

The prevalence of *Bartolius pierrei* sporocysts was calculated for a sample of 60 specimens of *D. solenoides*, discriminated according to three size classes (8–12, 13–16 and 17–23 mm in maximum shell length). The clams were collected at Fracasso Beach, in March 2000, and immediately fixed in 10% seawater formalin. To establish the precise location of the sporocyst in the host, several specimens were dissected under a stereomicroscope. 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. Sections were stained with eosin and Harris' haematoxylin.

Sporocysts and cercariae of *B. pierrei*, as well as whole parasitized *Darina solenoides* from southernmost localities, were deposited in the Helminthological Collection, MLP (Nos 5020, 5064–5069), Museo de La Plata, La Plata, Argentina.

#### *Development of metacercariae*

Specimens of *Darina solenoides* were collected on several occasions from August 1999 to January 2002. On each occasion, about 200 clams were maintained in individual flasks to observe cercarial emission. Those clams emitting cercariae served as sources of infective material. Experimental infection was performed using

non-parasitized small clams (less than 12 mm in shell length). The uninfected condition of the clams was determined by direct inspection of their tissues under stereomicroscope through their translucent shells. The uninfected clams were kept in individual plastic containers and exposed to a great number of emitted cercariae. After 48–72 h, the experimentally infected clams were transferred to an aquarium provided with sediment and aerated seawater, in a refrigerated room (10–12°C). Water was changed weekly. Clams were dissected after 5, 10, 20, 30, 40 and 50 days post-infection, and the metacercariae were studied alive.

### *Host spectrum*

To establish the existence of other macroinvertebrates acting as second intermediate hosts for *Bartolius pierrei*, samples of *Tellina petitiana* ( $N=167$ ; August 1999, March 2000 and December 2001), *Buccinanops globulosus* ( $N=61$ ; April 2001), *Glycera americana* ( $N=56$ ; August 1999, April 2001 and December 2001), *Travisia* sp. ( $N=86$ ; August 1999, April 2001 and December 2001) and *Laeonereis acuta* ( $N=17$ ; April 2002) were collected. They were immediately fixed in 10% seawater formalin, stored in 70% alcohol, and examined for parasites. Moreover, experimental infection was performed by putting a great number of cercariae in a small flask with one specimen of each species (five replicates), and dissected 48 h after, looking for metacercariae.

To verify the role of migrating and non-migrating birds as definitive hosts for *B. pierrei*, faeces of *Larus dominicanus* ( $N=11$ ), *Larus maculipennis* Lichtenstein, 1823 ( $N=3$ ) (Laridae), *Charadrius falklandicus* ( $N=19$ ), *Calidris alba* ( $N=15$ ) and *Calidris canutus rufa* ( $N=13$ ) were collected in April 2002. All faeces were immediately fixed in 10% seawater formalin, stained with Bengal rose and examined under a stereomicroscope searching for expelled worms without eggs. Additionally, eight specimens of *Charadrius falklandicus* and one of *Calidris fuscicollis*, which were found dead in April 2000, were examined for adults of *Bartolius pierrei*. Prevalences of infected birds were calculated.

### *Geographic distribution*

To determine the range of *B. pierrei*, samples of *Darina solenoides* were taken at: Colombo Beach (42°36'S, 64°14'W), Nuevo Gulf, Rada Tilly (45°56'S, 67°34'W) in San Jorge Gulf, Punta Medanosa (48°06'S, 65°54'W) and Punta Loyola (51°38'S, 68°58'W) in Santa Cruz Province (figure 2). These sites encompass almost the entire distribution area of *D. solenoides*. Specimens of *D. solenoides* were immediately fixed in 10% seawater formalin, examined under a stereomicroscope, and the number of metacercariae recorded. Prevalence and intensity of infection were calculated for each locality.

In all cases, parasitological indices were used according to Bush *et al.* (1997).

## **Results**

### *Description of intramolluscan stages*

*Sporocysts* (10 specimens measured) (figure 3). Furcocercous cercariae develop in colourless, immobile and thin-walled sporocysts, 140 (103–179) long by 23 (18–26) maximum wide. Eighteen to 38 cercariae (average: 26) at different developmental stages occur in each sporocyst. Birth pore present.

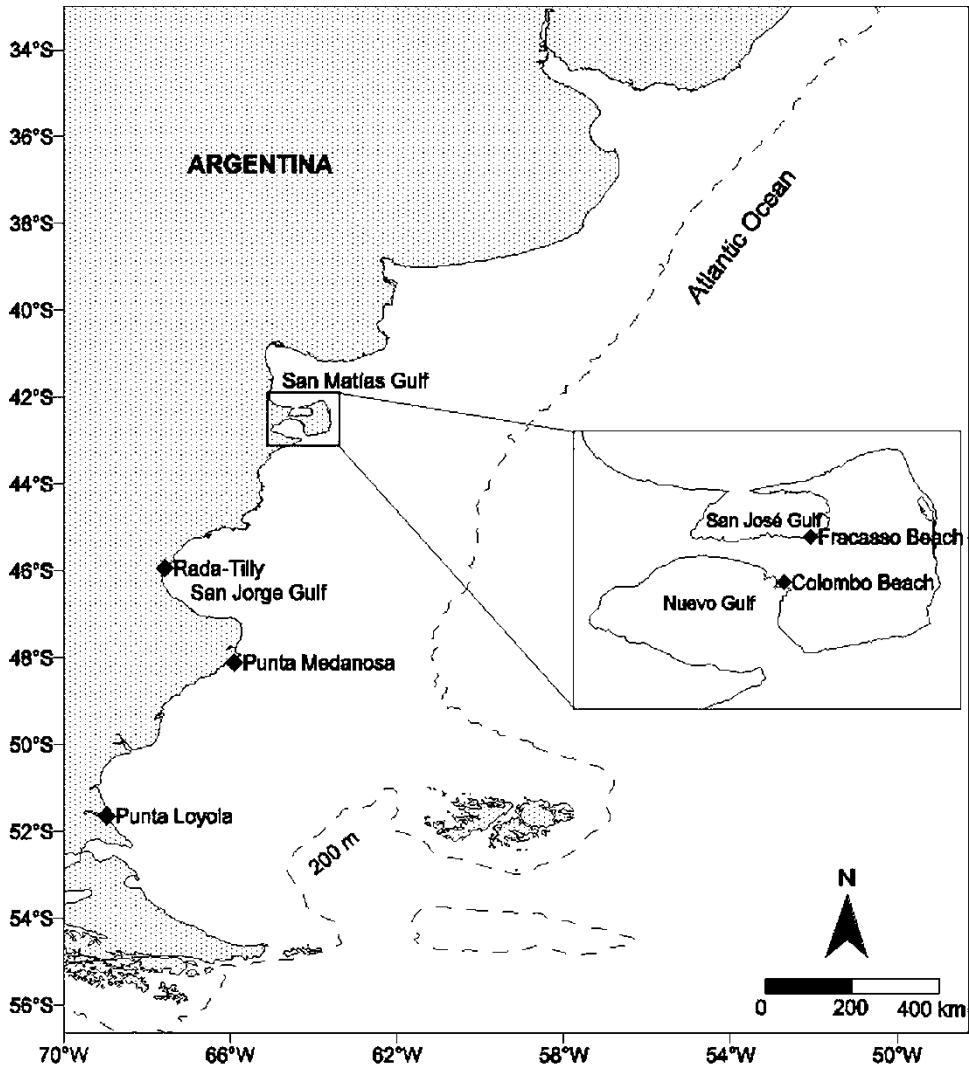


FIG. 2. Map showing the distribution of *Bartolius pierrei* along the Patagonian coast, Argentina; the area in the rectangle (Península Valdés) is enlarged on the right. (♦) Sites where parasitized *Darina solenoides* were found.

*Cercariae* (10 specimens measured) (figure 4). Body oval, 236 (205–282) long by 151 (126–180) wide at acetabular level. Spines arranged transversely, covering entire dorsal and ventral surfaces; also on tail. Oral sucker 52 (47–58) long by 53 (48–61) wide, without lateral projections. Pharynx ovoid, 32 (28–37) long by 20 (19–21) wide. Oesophagus 20 (12–34) in length. Four pairs of penetration glands opening dorsally to oral sucker. Caeca 69 (52–90) long by 48 (36–65) at maximum width, usually reaching middle of ventral sucker, never surpassing it. Distance from anterior body end to anterior edge of acetabulum 126 (100–152). Ventral sucker 54 (49–61) long by 61 (51–75) wide, with inner and outer circles of six papillae. Sucker ratio: 1:0.96 (0.85–1.14). Excretory formula:  $2 [(2+2)+(2+2)]=16$ . First pair of flame cells located at sides of oral sucker, second pair at level of the pharynx. Second group of

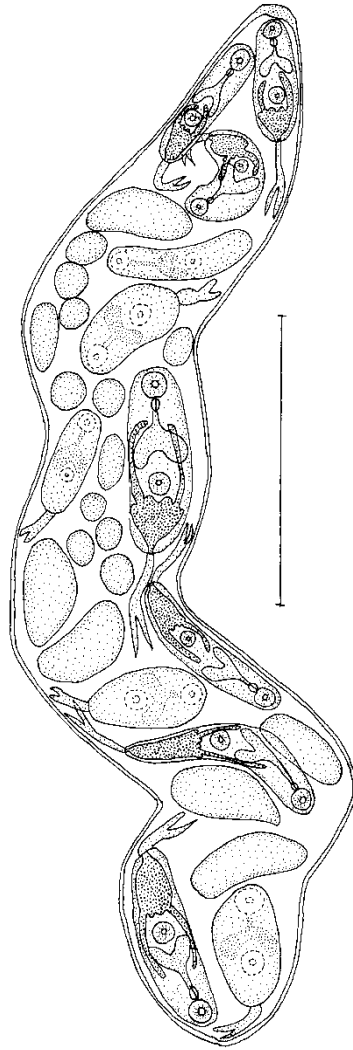


FIG. 3. Sporocyst of *Bartolius pierrei* parasitic in the clam *Darina solenoides*, from the Patagonian coast. Scale bar: 50  $\mu$ m.

flame cells with first and second pairs at acetabular and post-acetabular zone, respectively. Excretory vesicle with very short stem and two branches that extend to pharynx. Vesicle and ducts in tail filled with spherical and elongated excretion granules. Ducts opening on internal faces of furcae, just before tip. Tail stem 81 (58–120), furca 147 (110–200) long.

#### *Cercarial behaviour and penetration*

Sporocysts were found mainly in the gonad, and to a lesser extent in the kidney and the digestive gland of the clam. Cercariae were expelled by the current of the exhalant siphon. Cercariae swam in the water column when recently emitted, but shortly. The swimming movements were typical of gymnophallid cercariae (Bartoli,

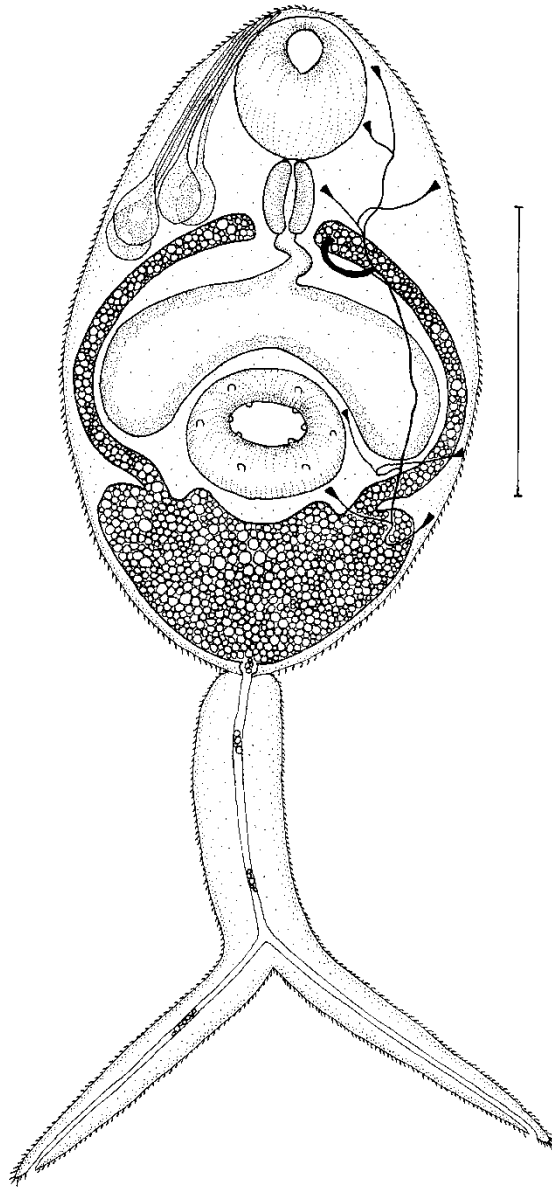


FIG. 4. Cercaria of *Bartolius pierrei* from the clam *Darina solenoides*, from the Patagonian coast, ventral view. Excretory cells on the right side and penetration glands on the left side omitted. Scale bar: 100  $\mu\text{m}$ .

1974), gradually decreasing, becoming less motile whereas creeping and quiescence increased. Cercariae spent most of the time on the bottom of the Petri dish, where they lay on their dorsal side, with the tail bent against the ventral surface of the body, and displaced by moving their tail. Cercariae enter into the extrapallial space of clams actively, by piercing the exposed mantle along the entire border (figure 5). Newly entered metacercariae ascend the extrapallial space (between outer mantle

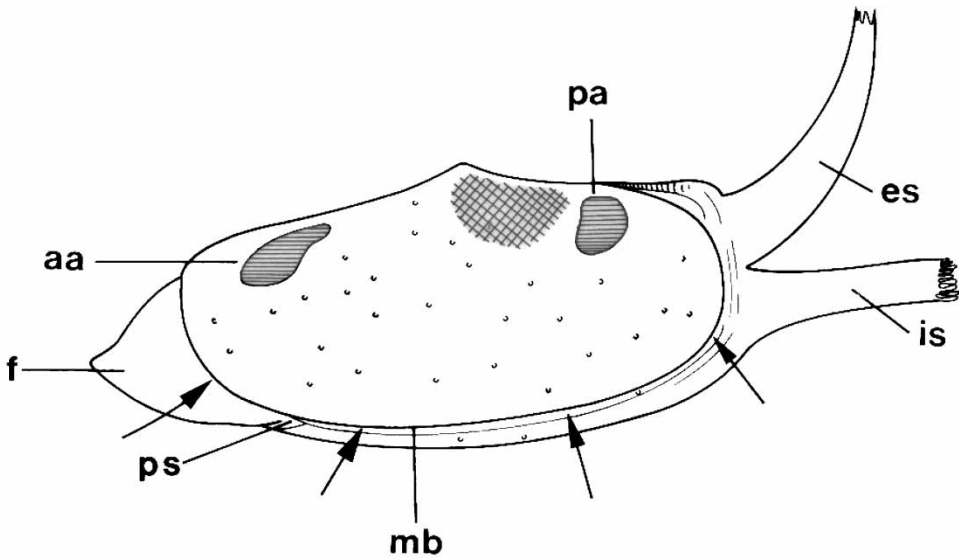


FIG. 5. Schematic view of *Darina solenoides* showing through the translucent valves the ways of cercaria penetration (arrows) and the metacercaria definitive habitat (shadow area). aa=anterior adductor muscle; es=exhalant siphon; f=foot; is=inhalant siphon; mb=mantle border; pa=posterior adductor muscle; ps=pedal slit;  $\cup$ =just entered metacercariae ascending.

epithelium and shell) until reaching their final habitat where they develop. The tail is lost before penetration or during the ascent. Cercariae lived up to 48 h at room temperature.

#### *Development of metacercariae*

By 5 days post-infection, metacercariae were located in the uppermost general extrapallial space and were the same size as the body cercariae. They had lost their penetration glands, but still did not have cephalic glands. The caeca were little dilated.

By 10 days post-infection, metacercariae were not easily detachable and showed a close contact with clam tissues. The only change at this stage was the presence of cephalic glands.

By 20 days post-infection, metacercariae were surrounded by clam tissue, but still had connection with the extrapallial space. They were only slightly larger than the cercarial body and the excretory vesicle was more developed but they still had no genital primordia.

By 30 days post-infection, the metacercariae showed no noticeable changes relative to those at 20 days post-infection.

By 40 days post-infection, metacercariae were somewhat smaller than those from natural infection. The caeca and the excretory vesicle were extended almost as much as in old metacercariae, and were filled with excretory granules. They already had genital primordia.

By 50 days post-infection, metacercariae were fully developed, enclosed in a sac and with a size similar to that found in natural infections.



Table 1. Prevalences of adult infection of *Bartolius pierrei* from birds in the type locality, Fracasso Beach, Patagonian coast, Argentina.

Bird species	N	Prevalence (%)
<i>Larus dominicanus</i>	11	36
<i>Larus maculipennis</i>	3	0
<i>Charadrius falklandicus</i>	27	0
<i>Calidris fuscicollis</i>	8	0
<i>Calidris alba</i>	15	0
<i>Calidris canutus rufa</i>	13	92

N, total number of birds examined via faeces or necropsies.

#### Life cycle and host spectrum

*Bartolius pierrei* utilizes only the clam *Darina solenoides* as both first and second intermediate host. 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 19–22 mm long). All examinations for natural infections, and attempts to obtain experimental infections in the clam *Tellina petitiiana*, the gastropod *Buccinanops globulosus*, and the polychaetes *Glycera americana*, *Travisia* sp. and *Laeonereis acuta* were negative. Definitive hosts of *B. pierrei* were the birds *Larus dominicanus* and *Calidris canutus rufa*. The highest prevalence was found in the latter (table 1).

#### Geographic distribution

*Bartolius pierrei* was present in all sites sampled, which extend through almost all the distributional range of *Darina solenoides* (figure 2). Table 2 shows prevalence and intensity at the different localities.

#### Discussion

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

Table 2. Prevalence and intensity of metacercarial infection of *Bartolius pierrei* in the clam *Darina solenoides* along the Patagonian coast, Argentina.

Site	Date	N	Clam size <sup>†</sup> (mm)	Intensity <sup>‡</sup>	Prevalence (%)
Fracasso Beach (42°25'S, 64°07'W)	March 2000	20	20 (19–22)	34 (3–40)	35
Colombo Beach (42°36'S, 64°14'W)	April 2002	15	21 (17–27)	7 (1–47)	50
Rada Tilly (45°56'S, 67°34'W)	December 1997	15	28 (24–33)	241 (153–460)	100
Punta Medanosa (48°06'S, 65°54'W)	March 1998	15	30 (18–37)	407 (223–680)	100
Punta Loyola (51°38'S, 68°58'W)	March 1998	15	32 (17–44)	261 (48–628)	100

N, number of clams examined.

<sup>†</sup>Mean of maximum shell length with the range in parentheses.

<sup>‡</sup>Mean with the range in parentheses (only encapsulated metacercariae were counted).

for many parasite species (Poulin, 1998). *Bartolius pierrei* uses only the clam *Darina solenoides* as first and second intermediate host, and the birds *Larus dominicanus* and *Calidris canutus rufa* act as its definitive hosts.

Bartoli (1973, 1974) found that the cercariae which invade a bivalve as second intermediate host lack penetration glands [e.g. *Meiogymnophallus fossarum* (Bartoli, 1965) and *Parvatrema duboisi* (Dollfus, 1923)] because they passively enter with the current through the inhalant siphon of the clams. Other species that only invade polychaetes as second intermediate host [e.g. *Meiogymnophallus nereicola* (Rebecq and Prévot, 1962) and *Gymnophallus choledochus* Odhner, 1900] have penetration glands (Loos-Frank, 1969; Bartoli, 1972). However, most gymnohallid cercariae have penetration glands. They passively enter by the inhalant current, reaching the pallial cavity, then penetrating the mantle epithelium and migrating to the extrapallial space (Loos-Frank, 1970, 1971; Bartoli, 1973, 1983; Campbell, 1985). Detailed descriptions of the penetration mode of cercariae were given by Bartoli (1973) and Pekkarinen (1987a).

Cercariae of *B. pierrei* have penetration glands and invade the bivalve, entering the extrapallial space by piercing the mantle border (figure 5). The active penetration mode seems to be more direct, because cercariae do not need to enter the mantle cavity to reach the extrapallial space. This is the first report of gymnohallid cercariae entering a bivalve in this way. This unique characteristic is considered a new behavioural apomorphic character that contributes effectively to the generic diagnosis of the monotypic genus *Bartolius*, together with morphologic apomorphic characters such as the post-testicular position of the ovary.

*Bartolius pierrei* showed a high degree of specificity for its second intermediate host, failing to invade other invertebrates, even the clam *Tellina petittiana* which shares the intertidal area with *D. solenoides* (figure 1). *Tellina petittiana* is a deposit-feeder which does not expose the mantle when feeding; thus, cercariae cannot enter. The observed host specificity could be explained by the behaviour of the cercariae, which, after being emitted, spent most time on the bottom without reaching the *Tellina* population, located in the lower intertidal. Moreover, the penetration mode requires that the clam exposes the mantle border to allow the larvae to penetrate. This is in agreement with observations made by Bartoli and Combes (1986), that the behaviour of cercariae is closely adapted to the ecology of their targets. The strategy of *B. pierrei* cercariae seems to be successful, and well adjusted when considering the role of the two clams as prey of birds. *Darina solenoides* is found over a large extent of the intertidal (figure 1), being the main prey for several marine bird species. In contrast, *T. petittiana* is restricted to the lower intertidal zone (where *D. solenoides* is not present) and it is only preyed upon by gulls (Pagnoni, 1997; Hernández, 2000; D'Amico, 2001).

In view of the fact that 70% of the diet of *Calidris canutus rufa* consists of *D. solenoides* (Pagnoni, 1997), it was not surprising to find a prevalence of 92% in this bird (table 1). In contrast, *Charadrius falklandicus*, *Calidris fuscicollis* and *C. alba* were not parasitized by *B. pierrei* despite also preying upon *D. solenoides* in a similar proportion (Hernández, 2000; D'Amico, 2001). However, these birds feed on very small clams, which are not parasitized (unpublished data). Prevalence of *B. pierrei* in *Larus dominicanus* may vary with their diet, because, in the study area, it alternatively feeds on *D. solenoides*, *T. petittiana* and small mussels.

The whole life cycle of *B. pierrei* requires about 2 months. In laboratory conditions, cercariae lived up to 2 days, metacercariae became infective at 50 days

and the adult life span is presumably shorter than 3 days because ovigerous specimens were experimentally obtained in 35–40 h (Cremonte, 2001). Other published data on the life span of gymnophallid cercariae report variable periods from 3 days to 4 weeks, being longer at lower temperatures. Bartoli (1974) found that *Meiogymnophallus fossarum* and *M. nereicola* cercariae lived up to 3 days at 20°C, and *M. fossarum* and *Gymnophallus rostratus* Bartoli, 1982 about 6 and 10 days, respectively, at 12–13°C. On the other hand, cercariae of *Lacunovermis macomae* (Lebour, 1908) and *Gymnophallus gibberosus* Loos-Frank, 1971 lived up to 4 weeks in winter (Pekkarinen, 1987b). The reported development time for gymnophallid metacercariae varies from 2 to more than 6 months. Bartoli (1972) obtained infective metacercariae of *M. nereicola* in 2 months, and those of *M. fossarum* in 4 months. On the other hand, Ching (1965) obtained infective metacercariae of *L. macomae* in 3 months, but Pekkarinen (1986), working with the same species, did not obtain fully developed metacercariae after 4 months. Moreover, metacercariae of *G. gibberosus* showed no clear size increase or changes until 6 months (Pekkarinen, 1987b). According to reported data, the adult life span varies from about 2 to 8 days. *Lacunovermis macomae* and *G. cholecochus* produced eggs after 2 and 8 days, respectively (Ching, 1965). Adults of *Meiogymnophallus rebecqui* (Bartoli, 1983) lived up to 5 or 6 days in the intestine of its hosts (Bartoli, 1983). In P. Bartoli's opinion (personal communication), the shortening of the life span of gymnophallids could be a result of an adaptive process to migratory birds. This hypothesis fully agrees with the results obtained in the present paper for *B. pierrei*, in which the life cycle is even shorter when compared with what is known for other gymnophallid species.

*Darina solenoides* has isolated intertidal populations along its range on the Patagonian coast, following the occurrence of fine sand flats. Migratory birds would ensure parasite dispersion by enhancing the possibilities of inter-breeding among well-separated parasite populations. As the adult life span is short, the parasite life strategy should be well adjusted to take advantage of migratory birds in order to ensure the parasite can reach, in a short time, other *Darina* populations located a thousand kilometres away.

*Bartolius pierrei* is an endemic parasite of the Magellanic faunal Province, distributed where its intermediate host (the clam) is present. Fracasso Beach is located in the northernmost part of the distribution area of *D. solenoides*. This is evidenced by the smaller size of the clams and by the lower prevalences and intensities of the parasite infections, when compared with the situation in southernmost sites (figure 2; table 2).

Loos-Frank (1969) categorized five types of gymnophallid life cycles: Ia. Swimming cercariae enter a second intermediate host different to the first one (e.g. *Parvatrema borinquenae* Cable, 1953); Ib. Swimming cercariae enter a second intermediate host that is the same species, even the same individual (e.g. *L. macomae*); IIIa. Cercariae lose their tail, remaining within the sporocyst, and becoming infective metacercariae [e.g. *Meiogymnophallus affinis* (Jameson and Nicoll, 1913)]; IIIb. Cercariae lose their tail within the germinal sac, becoming infective metacercariae (e.g. *Parvatrema homoeotecnum* James, 1964); II. A combination of types Ia and IIIa, with two intermediate hosts alternating seasonally (e.g. *G. choledochus*). According to the classification of Loos-Frank (1969), the life cycle of *B. pierrei* belongs to type Ib. About half of the known

gymnophallid life cycles belong to this type. However, in most of them, there are other species acting as second intermediate hosts, mostly bivalves.

Gymnophallid digeneans are usually considered to be marine bird parasites that mainly utilize bivalves as first and second intermediate hosts (Lauckner, 1983; Ching, 1995). Perhaps it would be more accurate to consider gymnophallids as bivalve parasites that use birds as definitive hosts. The gymnophallid metacercaria is not a 'waiting' stage, as it is in some other digeneans. On the contrary, development and growth of gymnophallids occur at this life stage, and they spend most of their life cycle within the bivalve, from which they feed (Ching, 1965; Bartoli, 1974; Cremonte and Ituarte, 2003). Gymnophallid metacercariae occupy, and have the capacity to exploit, a habitat, the extrapallial space, that almost no other digeneans use; this may be due to the fact that the presence of non-gymnophallid larvae generally provokes a nacrezation response which leads to the parasite's death (Bartoli, 1974; Lauckner, 1983; Ituarte *et al.*, 2001; Cremonte and Ituarte, 2003). However, gymnophallids avoid this nacrezation response and may take advantage of the capsule produced by their molluscan host, because the metacercariae are never encysted: the capsule acts as a cyst (Bowers and James, 1967; Ituarte *et al.*, 2001; Cremonte and Ituarte, 2003). Gymnophallids are able to reach adulthood in only a few days (Ching, 1965; Bartoli, 1983; Cremonte, 2001) because the metacercariae have the genital system completely (or almost) developed. Birds are necessary as dispersal agents. It was postulated that, in parasites with complex life cycles, evolution favoured the use of intermediate hosts as a resource basis and definitive hosts as vehicles for dispersal in space and time (Poulin, 1998).

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