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Redescription and life cycle of the monorchiid *Postmonorcheides maclovini* Szidat, 1950 (Digenea) from the Southwestern Atlantic Ocean: Morphological and molecular data

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ABSTRACT

The adult monorchiid, *Postmonorcheides maclovini* Szidat, 1950, digenean parasite of the Patagonian blennie *Eleginops maclovinus* (Cuvier) (Eleginopidae) from Puerto Deseado (47° 45′ S, 65° 55′ W), Argentina, was characterized and its life cycle elucidated. *P. maclovinus* is the only species of the genus *Postmonorcheides*, proposed by Szidat (1950) from Tierra del Fuego province (~54° S), Argentina. This digenean uses the Patagonian blennie as definitive host, and the intertidal bivalve *Lasaea adansoni* (Gmelin) (Lasaeidae) as both first and second intermediate hosts (metacercariae encyst inside sporocysts), being the first record of this clam as intermediate host of trematode parasites. The cercaria may, in addition to encysting in the sporocyst, emerge and presumably infect other intermediate hosts. This is the second report of a monorchiid species with metacercariae encysting inside the sporocyst. Adults were found parasitizing the fish stomach, pyloric caeca and intestine with a prevalence of 100%; sporocysts with cercariae and/or metacercariae were found parasitizing the gonad of the bivalve with a prevalence of 2.78%. The cercariae possess a well-developed tail and eye-spots are absent. The ITS1 sequence from the adult digeneans found in the Patagonian blennie, identified as *P. maclovini*, was found to be identical to the ITS1 sequences obtained both from sporocysts containing cercariae and encysted metacercariae found in *L. adansoni*.

1. Introduction

Szidat [2] proposed the genus *Postmonorcheides* for a new species found in the blennie *Eleginops maclovinus* (Cuvier) from Tierra del Fuego (~54° S) and described a new species of *Monorcheides* found in the same host and locality. Later, MacKenzie [1] reported *P. maclovini* parasitizing the same host in sub-Antartic waters. To date, there are three adults of monorchiid species described from the Argentine Patagonian coast, *Monorcheides popovicii* Szidat, 1950; *Postmonorcheides maclovini* Szidat, 1950 and *Proctotrema bartolii* Carballo, Laurenti & Cremonte, 2011 [2,3]. Nineteen monorchiid cercariae have bivalves as first intermediate host, all belonging to the Order Veneroida and seven of these to the family Veneridae; only 3 of them from the Southern Hemisphere [4–6]. The life cycles of 8 species are known, 7 from the Northern Hemisphere [7–13] and 1 from Southern Hemisphere [6]. The metacercariae can be found either free in the environment, in the same species of bivalve, or in other bivalves; the adults are found in the intestine of teleost fish [4].

The life cycles of most digenean species are complex; frequently only a portion of the cycle is known with certainty, particularly because distinguishing morphological characters are often lacking. However, recent molecular tools make it possible to determine whether a stage from one host species is genetically the same as a stage from another host species [13]. For example, the use of a highly variable molecular marker, such as the ITS1 (internal transcribed spacer), allows one to determine if samples of adults, metacercariae, and sporocysts belong to the same or different species [14].

The aim of this work was to redescribe *P. maclovini* from the Patagonian blennie *E. maclovinus*, and elucidate its life cycle on the Patagonian coast, Southwestern Atlantic Ocean, using morphological and molecular information.

2. Materials and methods

The sampling site was the intertidal rocky littoral zone at Puerto Deseado (47° 45′ S, 65° 55′ W), Santa Cruz province, Argentina. Samples were collected at low tide, during 2012 and 2013. A total of 14 specimens of *E. maclovinus* (mean total length 6.13 ± 1.12 cm) were caught

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using a net and 648 bivalves *Lasaea adansoni* were separated from the crowds of mytilids collected by hand, among which they live fixed to the byssus threads. Dissections were performed either from freshly collected fish or from hosts fixed in 10% formalin, immediately after capture. Digeneans were isolated from stomach, pyloric caeca and intestine. The bivalves were removed by dissecting forceps from mytilids byssal threads, and dissected under stereomicroscope. An additional sample of 800 bivalves was isolated in small flasks with seawater at room temperature (20–22 °C) to observe cercarial emission; the percentage of emitting clams was calculated. The life span of emitted cercariae was observed at room temperature (20–22 °C) and at 10 °C, counting about 40 cercariae which were checked every 2 h. Prevalence (P) and intensity of infection were calculated according to [15].

For light microscopy, adults, sporocysts, emitted cercariae and metacercariae intra-sporocysts were killed with hot seawater and immediately fixed with 10% formalin, preserved in 70% ethanol, and stained with Semichons' acetocarmine and Gomori's trichrome, dehydrated through ethanol series, cleared with metylsalicilate, mounted in Canada balsam and observed. Drawings were made with the aid of a camera lucida and the measurements are given in μm and expressed as the mean followed by the range in parentheses. A sample of adult digeneans (n = 33) from *E. maclovinus* and sporocysts (n = 15) from *L. adansoni* was preserved in ethanol 96% and frozen for molecular analyses.

2.1. Loaned material

Type material (holotype and paratypes) of monorchiids from Patagonian blennie, studied by Szidat (1950) and deposited in the Parasitological Collection, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-Pa), Buenos Aires, Argentina (*M. popovicii* MACN-Pa 27.776 and *P. maclovini* MACN-Pa 27.777), were studied to compare with newly collected specimens (Table 1).

2.2. Scanning electron microscopy

For scanning electron microscopy (SEM), some adults were fixed with 10% formalin-physiological solution, dehydrated in increasing concentrations of alcohol and dried by rinsing for a few minutes in hexamethyldisilazane, and gold coated for observation and photomicrograph using a Jeol JSM-6460LV SEM operating at 15 KV.

2.3. DNA extraction, amplification, and sequencing

DNA from 33 adults from *E. maclovinus* and 15 sporocysts from *L. adansoni* were extracted using a Sigma (St. Louis, Missouri, USA) kit (GenElute Mammalian Genomic DNA Miniprep Kit). Adult digeneans were divided into 4 pools (1 pool of 15 specimens from stomach and 3 pools of 18 specimens from intestine). Due to the fact that adults isolated from intestine presented different sizes and were morphologically indistinguishable under stereomicroscope (or unstained under light microscope), they were separated in 3 pools: immature specimens (n = 10), small gravid specimens (n = 3) and large gravid specimens (n = 5). Polymerase chain reaction (PCR) amplifications were performed in a total volume of 50 μl with an amplification profile consisting of 40 cycles of 30 s at 94 °C, 30 s at 54 °C, 120 s at 72 °C, followed by 10 min at 72 °C for final extension. The ITS1 region of the rDNA was amplified using a forward primer 18S-ITS1 (5′-CCGTCGCTACTACCGATTGAA -3′), located at 142 base pairs (bp) from the 3′ end of the 18S rDNA, and a reverse primer 5.8S-ITS1 (5′-CGCAATGTGCGTTCAAGATGTC -3′) located at 96 bp from the 5′ end of the 5.8S rDNA. Amplified PCR products were purified using a Qiagen (Valencia, California, USA) kit (QIAquick Gel Extraction Kit) and sequenced. ITS1 sequences were submitted to GenBank and were compared using Multalin software [16].

3. Results

Redescription of adult of *Postmonorcheides maclovini* Szidat, 1950 and description of larval stages from its life cycle (Figs. 1–3).

3.1. Redescription of adult (Figs. 1c, 2c–f, 3a-h)

[Measurements based on 19 stained and mounted gravid specimens]: Body small, oval, elongated, 417 (285–650) long and 213 (135–320) in maximum wide, anterior and posterior extremity rounded. Forebody 155 (120–190) long. Tegument spinous; spines decreasing in size to posterior extremity (Fig. 3a, b, c). Spines with digitiform projections at tip (Fig. 3b, c). Spines absent around genital pore (Fig. 3a, g). Eye-spots absent. Oral sucker sub-terminal, 101 (80–140) long and 91 (70–120) wide, larger than ventral sucker, externally surrounded by 12 papillae (5 pairs located in outer circle distributed in latero-posterior part of sucker and 2 papillae located in inner circle at each side of mouth) (only seen at SEM, Fig. 3d); internal papillae present. Ventral sucker rounded and pre-equatorial, 73 (50–95) long and 69 (42–90) wide, externally surrounded by 5 papillae (3 located in upper part of sucker and 2 located anterolateral, one at each side) (only seen at SEM, Fig. 3e) and internally surrounded by nearly 9–10 papillae uniformly distributed (only see at SEM, Fig. 3f). Sucker ratio 1.38:1 (1.11:1–2.02:1). Four pairs of cephalic glands, positive to staining with neutral red, reaching to posterior forebody (Fig. 2c). Pre-pharynx absent. Pharynx well developed, ovoid, transversely elongate, 36 (25–50) long and 27 (20–32) wide. Esophagus very short; intestinal bifurcation anterior to ventral sucker. Caeca elongated, 300 (210–450) long and 12 (8–20) wide, reaching posterior margin of testes. Testes two, large, varying from opposite to partially overlapped, entirely in hindbody (Figs. 1c, 2c); left testis 87 (60–110) long and 63 (40–90) wide; right testis 106 (75–170) long and 64 (52–98) wide. Cirrus sac

Table 1
Comparison of relevant taxonomic features among adult specimens of the present study and type material of *Monorcheides popovicii* Szidat, 1950 and *Postmonorcheides maclovini* Szidat, 1950.

Features	Studied adults (19 gravid specimens)	*Postmonorcheides maclovini* (holotype and 2 paratypes)	*Monorcheides popovicii* (holotype and 7 paratypes)
Body size			
Length	417 (285–650)	1003 (956–1085)	661 (437–853)
Width	213 (135–320)	476 (433–524)	361 (308–392)
Testes	From symmetrically opposite to partially overlapped	Very large, partially overlapped	Symmetrically opposite
Cirrus sac	Well developed, slightly exceeds posterior edge of ventral sucker	Relatively short and bulky, reaching just until the middle of ventral sucker	Well developed, exceeds posterior edge of ventral sucker
Ovary	Located from right side to middle of body	Located in middle of body	Located on right side of body
Vitellaria	1 mass	1 mass	2 opposite masses
Eggs			
Length	19 (12–28)	21 (20–23)	26 (20–35)
Width	10 (8–15)	15 (14–15)	14 (11–19)

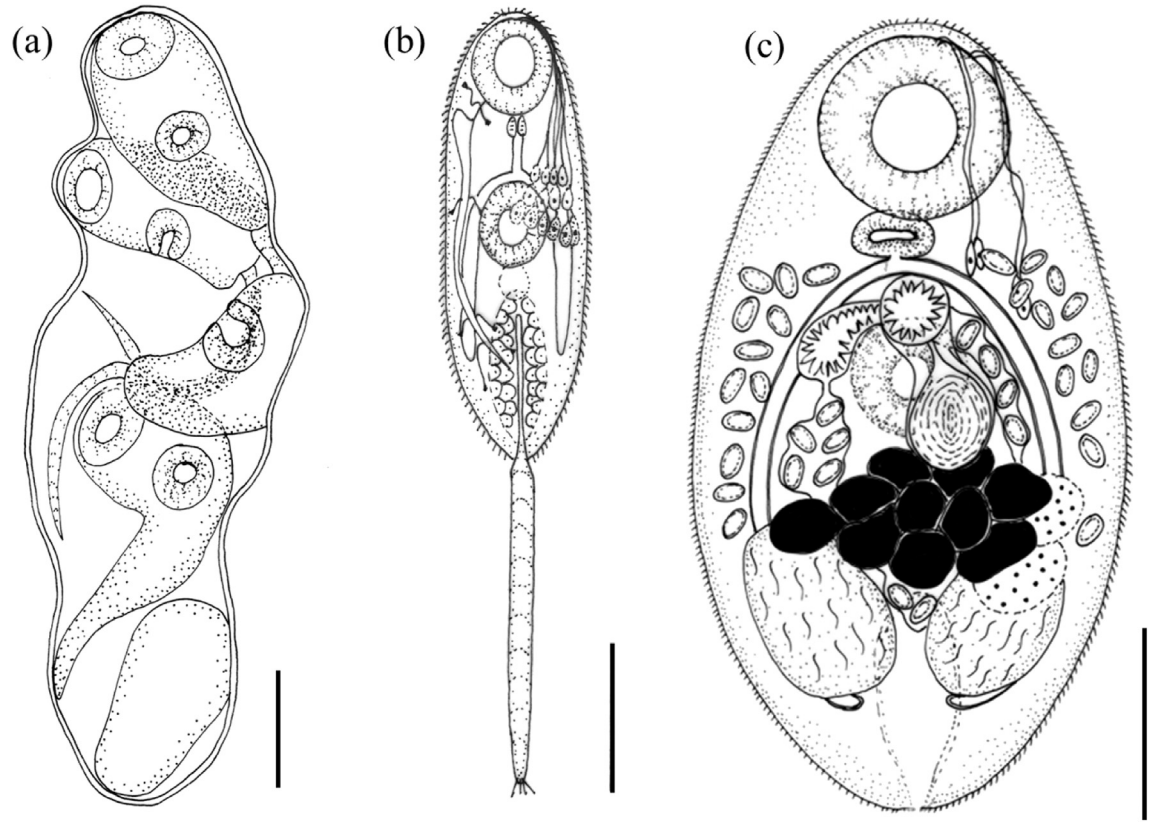

Fig. 1. *Postmonorcheides maclovini.* Line drawings of the larval and adult stages: (a) sporocyst; (b) cercaria, ventral view, the flame cells and the penetration glands of the left and right side, respectively, are omitted; the posterior cell glands have dots, indicating that they are colored with Neutral Red; (c) adult, dorsal view. Scale bars: 50 μm (a, b), 100 μm (c).

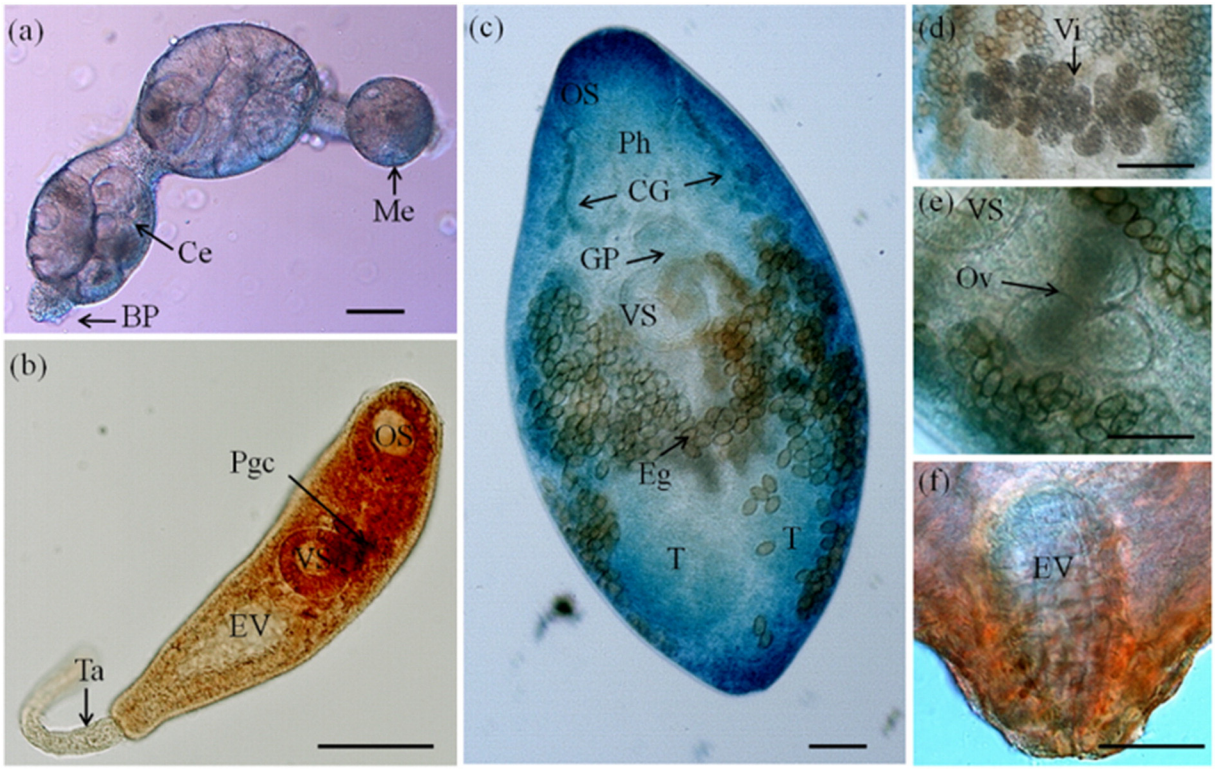

Fig. 2. *Postmonorcheides maclovini.* Light microscopic photographs of live specimens (a) sporocyst filled with cercariae and metacercariae; (b) cercaria, ventral view; (c) adult, ventral view; (d) detail of vitellaria; (e) detail of trilobed ovary; (f) detail of excretory vesicle. Abbreviations: BP, birth pore; Ce, cercaria; CG, cephalic glands; Eg, eggs; EV, excretory vesicle; GP, genital pore; Me, metacercaria; OS, oral sucker; Ov, ovary; Pgc, penetration glands citons; Ph, pharynx; T, testis; Ta, tail; Vi, vitellaria; VS, ventral sucker. Scale bars: 100 μm (a), 50 μm (b, c, d, f), 25 μm (e).

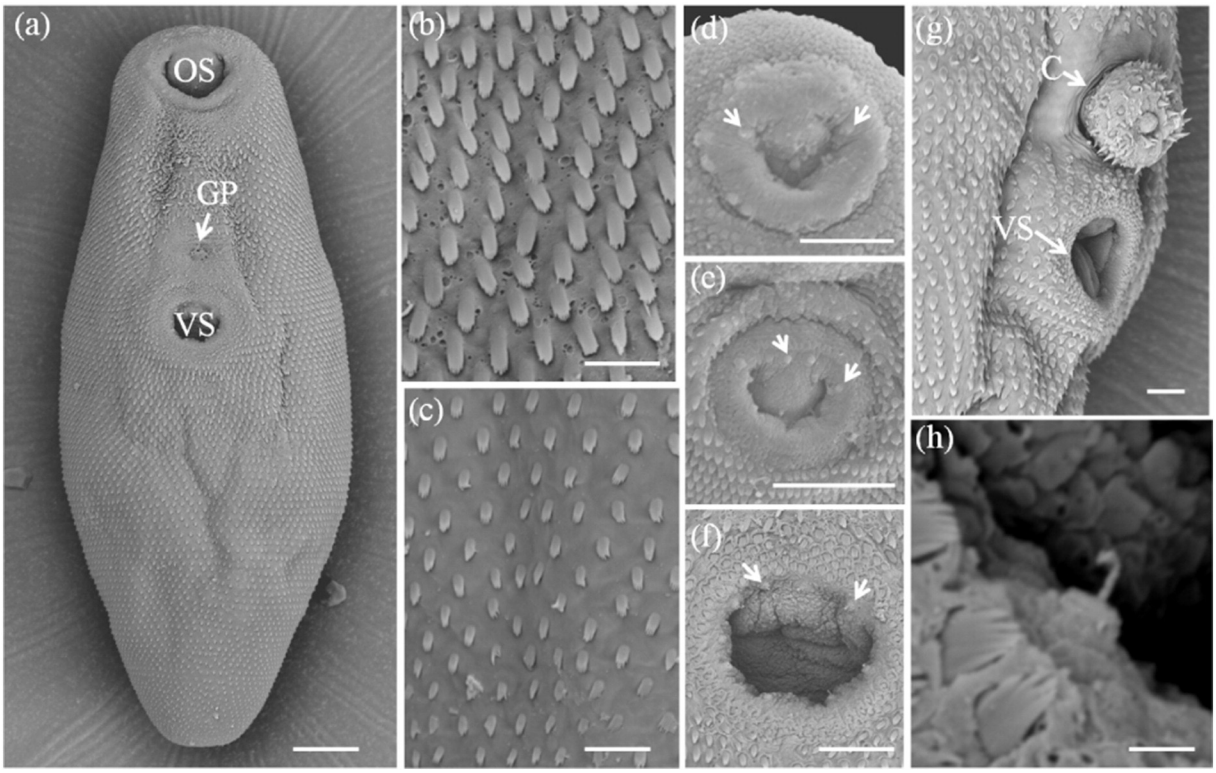

Fig. 3. *Postmonorcheides maclovini*. SEM photographs of adult (a) entire specimen, ventral view; (b) detail of anterior body spines; (c) detail of posterior body spines; (d) external papillae of oral sucker (arrows); (e) external papillae of ventral sucker (arrows); (f) internal papillae of ventral sucker (arrows); (g) everted spinous cirrus showing the surrounding area devoid of spines; (h) detail of an internal papilla of ventral sucker. Abbreviations: C, cirrus, GP, genital pore; OS, oral sucker; VS, ventral sucker; white arrows indicate papillae. Scale bars: 50 μm (a, d, e), 5 μm (b, c); 25 μm (f), 15 μm (g), 1 μm (h).

well developed, 88 (60–130) long and 44 (35–60) wide, slightly exceeding posterior edge of ventral sucker. Seminal vesicle internal, sacciform, 54 (40–90) long and 38 (30–55) wide. Cirrus 33 (23–48) long and 31 (19–45) wide (not everted), strongly covered with sharp triangular spines, 11 (8–15) long and 6 (3–10) wide (Fig. 3g). Genital pore mid-ventral, midway between intestinal bifurcation and ventral sucker (Fig. 3a). Terminal organ elongated, sinistral and curved, unipartite, thick-walled, 50 (45–70) long and 28 (18–35) wide, armed with triangular spines similar to cirrus, 10 (8–15) long and 5 (3–8) wide. Ovary trilobed, with regular lobes, 73 (40–90) long and 46 (28–70) wide, usually on the right side of the body (Fig. 2e), sometimes in the middle, often obscured by vitellaria (dark brown), pre-testicular or slightly overlapping anterior margin of testes. Vitellaria 135 (60–200) long and 67 (50–90) wide, formed by 1 central mass of approximately 10 follicles; mainly pre-testicular, from ventral sucker to half testes level (Fig. 2d). Uterus enters the distal part of the terminal organ, filling space between pharynx and posterior edge of testes. First eggs in forebody, brownish, oval, filament absent, 19 (12–28) long and 10 (8–15) wide (n = 25). Excretory vesicle saccular, 104 (97–109) (n = 3) long (Fig. 2f). Excretory pore terminal.

3.2. Sporocysts (Figs. 1a, 2a)

[Measurements based on 12 stained and mounted sporocyst]: Colorless, thin-walled, ovoid to elongate, 348 (237–525) long and 104 (73–126) wide. Three types of sporocysts were observed: 1—daughter sporocysts filled with 2 to 8 (mean = 5) cercariae in different developmental stages per sporocyst; fully formed cercariae leave sporocyst via a terminal birth pore; 2—daughter sporocysts containing cercariae and encysted metacercariae; 3—daughter sporocysts filled with encysted aligned metacercariae, resulting in a chaplet shaped figure (Fig. 2a).

3.3. Naturally emerged cercariae (Figs. 1b, 2b)

[Measurements based on 16 naturally emitted cercariae]: Body small, elongate, 157 (109–234) in length and 47 (32–58) in maximum width. Forebody 58 (41–92) in length. Tegument uniformly covered with long, sharp spines. Eye-spots absent. Oral sucker sub-terminal, rounded 39 (31–43) long and 33 (28–39) wide. Ventral sucker pre-equatorial, 32 (27–38) long and 29 (23–34) wide. Sucker ratio 1:1.1 (1:1.06–1:1.16). Numerous pairs of penetration glands; at least 8 anterior pairs negative and 6 posterior pairs positive to staining with Neutral Red. Cell bodies of penetration gland-cells form a mass near mid-body, overlapping anterior edge of ventral sucker. Pre-pharynx very short. Pharynx well developed, 9 (8–10) long and 9 (6–11) wide. Esophagus 22 (11–54) long. Caeca reach about half of hindbody; 63 (41–87) long. Genital primordium located between ventral sucker and anterior end of excretory vesicle. Excretory vesicle thick-walled, sac-shaped, elongated with narrow lumen, 63 (31–95) long and 18 (7–30) wide. Flame cell formula: 2 [(2 + 2) + (2 + 2)] = 16. Tail cylindrical and contractile, slightly shorter than body, 119 (51–197) long and 6 (5–8) wide at base. Tuft of spines at tip of tail.

3.3.1. Behavior

0.25% of bivalves (2 from 800) emitted cercariae. A mean of 20 (6–40) cercariae were emitted for each bivalve per day. The cercariae move slowly on the bottom of flask, with the posterior extremity of the cercarial body folded ventrally. The tail, which lies parallel to ventral body surface, lashes vigorously during swimming. Cercariae were never observed reaching the surface of water. Their life span was about 6 h at room temperature (20–22 °C) and about 24 h at 10 °C. After this time, they sink to the bottom and die without encysting.

3.4. Metacercariae (Fig. 2a)

[Measurements based on 4 specimens inside sporocysts]: Cyst slightly oval, 133 (110–152) in length and 105 (93–115) in width. Wall thin 6 (4–7) in thickness. Oral sucker 36 (22–45) in length and 29 (19–40) in width. A 15% of parasitized bivalves (2 from 13) presented 1 to 2 (mean = 2) encysted metacercariae inside sporocysts.

3.5. Molecular information

The sequence of the amplified ITS1 fragment provided a single product 811 bp long. After sequence analysis, putative 18S and 5.8S regions were identified through comparison with identical regions of other digeneans, and found to be 134 and 116 nucleotides long, respectively. The sequence encoding for the ITS1 region had 561 nucleotides in length. No identical sequence was found in GenBank. The ITS1 sequences of immature specimens, small gravid adults and large gravid adults morphologically identified as *P. maclovini* and isolated from the intestine of *E. maclovinus*, were identical to the sequence of specimens isolated from the stomach of the same host, and to the sequence obtained from sporocysts collected in naturally infected *L. adansoni*.

3.6. Taxonomic summary

Hosts L. adansoni (Gmelin) (Bivalvia: Lasaeidae) as first and second intermediate hosts; *E. maclovinus* (Cuvier) (Fish: Eleginopsidae) as definitive host.

Site of infection Sporocysts with cercariae and encysted metacercariae in gonad of *L. adansoni*; adults in stomach, pyloric caeca and intestine of *E. maclovinus*.

Type-locality Bahía Aguirre (54° 56′ S, 65° 51′ W), Tierra del Fuego Province, Argentina, Southwestern Atlantic Ocean.

Other locality Puerto Deseado (47° 45′ S, 65° 55′ W), Santa Cruz Province, Argentina, Southwestern Atlantic Ocean.

Prevalence 2.01% for sporocysts (13 of 648 *L. adansoni*) and 100% for adults [14 of 14 *E. maclovinus*, in stomach (64.3%), pyloric caeca (28.6%) and intestine (100%)].

Mean and range intensity of infection of adults 39 (7–70).

Voucher specimens Sporocysts with cercariae and encysted metacercariae from *L. adansoni* (CNP-Par 61) and adults from *E. maclovinus* (CNP-Par 62) were deposited in the Parasitological Collection of the Centro Nacional Patagónico, Puerto Madryn, Argentina.

GenBank accession numbers KC920684 (adult specimens from intestine of *E. maclovinus*), KC920685 (sporocysts with cercariae and metacercariae from *L. adansoni*).

3.7. Taxonomic remarks

Szidat [2] found two monorchiid species in blennies from Tierra del Fuego (54.9° S), one belonging to *Monorcheides* Odhner, 1905 and other belonging to *Postmonorcheides*, a genus proposed by the author, and named *P. maclovini*. Both species were observed together in the pyloric caeca of the *E. maclovinus* and, even colored, were difficult to distinguish under the microscope. In our study, we carefully compared our species with the two species described by Szidat. The monotypic genus *Postmonorcheides* is mainly distinguished by the vitellaria, which are formed by a central mass of follicles in the hindbody. In contrast, *Monorcheides* present vitellaria composed by two opposite masses of follicles at each side of the body. According to Szidat [2], the two species may be easily confused when not stained, because the body shape and size are very similar in young and gravid adults. In Table 1, the main measurements and characteristics of the holotype and paratypes of both monorchiid adults from *E. maclovinus*, *P. maclovini* and *M. popovicii* described by Szidat [2], are compared with those of the present specimens. Adults studied in the present paper are similar to *M. popovicii* in body size, in the well developed cirrus sac, slightly exceeding the posterior edge of ventral sucker and in the uterus coils, exceeding the posterior edge of testes. On the other hand, adults here described are similar to *P. maclovini* in vitellaria, which are formed by a central mass, and in the size of eggs. The testes vary from opposite (as in *M. popovicii*) to partially overlapping (as in *P. maclovini*) and the position of the ovary varies from located at the right side of the body (as in *M. popovicii*) to a central position (as in *P. maclovini*). Specimens here studied belong to *P. maclovini* mainly due to the characteristics of the vitellarium, which is composed by one central mass, being this, the most relevant character for distinguishing *Postmonorcheides* from the close similar genus, *Monorcheides*.

Cremonte [4] established 4 groups of monorchiid cercariae according to their morphology: (1) cercariae possessing a well-developed tail and usually ocellate; (2) cercariae with shorter or collar-like tails; (3) cercariae with short furcae without tail stem; and (4) non-ocellate cercariae with a tiny knob tail. From the Patagonian coast, 3 cercariae have been described: monorchiid cercaria sp. 2 Gilardoni, Posadas, Kroeck & Cremonte, 2011 and *P. maclovini*, Szidat, 1950 (present study) belonging to Cremonte's [4] group 1; and *P. bartolii* Carballo, Laurenti & Cremonte, 2011 belonging to group 4; making a total of 19 monorchiid cercariae recorded to date [4–6]. The cercaria here described, together with *Cercaria caribbea* LXIV Cable, 1963 are the only known non-ocellate cercariae among the 10 cercariae possessing a well developed tail, belonging to the group 1 according to Cremonte [4]. The cercaria here described can be distinguished from *C. caribbea* LXIV by the presence of a higher number of penetration glands (several vs. two pairs). All the monorchiid larvae belonging to group 1, use bivalves of the Order Veneroida as first intermediate host, including *P. maclovini* which is found parasitizing *L. adansoni*.

4. Discussion

In the studied specimens of *E. maclovinus* from Puerto Deseado, we did not find *M. popovicii*, one of the two species reported by Szidat [2] in blennies from Tierra del Fuego (54.9° S). As these geographical regions are 1200 km apart, it is possible that these species are present in further southwestern and sub-Antarctic waters. However, MacKenzie [1] reported parasites of blennies from Malvinas Islands (51° 49'S), and he found *P. maclovini*, but not *M. popovicii*.

P. maclovini uses the bivalve *L. adansoni* as first and second intermediate hosts, and the blennie *E. maclovinus* as definitive host. Since sporocysts contained cercariae and encysted metacercariae, this species present an abbreviated life cycle (metacercariae were never found in other tissues like siphons, mantle or foot of *L. adansoni*). According to several authors [4,6–8,10,17], most monorchiid cercariae use the same bivalve species as the first and second intermediate hosts, in which metacercariae usually encyst in the siphons, mantle or foot. The monorchiid cercaria here described, along with *Monorchis parvus* Looss, 1902, are the only species that present abbreviated cycle [13]. The cercariae of *P. maclovini* have some characteristics associated to free-living habits (long tail), and to active penetration into a second intermediate host (penetration glands). The metacercariae were not found in examined bivalves or other invertebrates from the same study area (unpubl. data). Indeed, the emission of cercariae observed in laboratory conditions was very poor, and the cercariae remained alive for about 24 h at 10 °C. In this case, it seems that the shortened life cycle is an adaptation of the cercaria to stressed environment (intertidal, low temperatures), to ensure the parasite transmission and thus promote the abbreviated life cycle [18,19].

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References

[1] K. MacKenzie, The Parasite Fauna of Mullet, *Eleginops maclovinus* (Cuv. & Val., 1830) In the Falkland Islands: a pilot study. In: the Falkland *Eleginops maclovinus*: biology and fishery in Falkland Islands' waters, Falklands Islands Govermewnt Printing Office Stanley, Falklands Islands, 2003 41–53.
[2] L. Szidat, Los parásitos del róbalo ("*Eleginops maclovinus*" Cuv. & Val.), Prim. Congr. Nac. Pesq. Marít. Ind. Deriv. 2 (1950) 235–270.
[3] M.C. Carballo, S. Laurenti, F. Cremonte, A new species of monorchiid digenean from marine fishes in the Southwestern Atlantic Ocean off Patagonia, Syst. Parasitol. 78 (2011) 233–240, http://dx.doi.org/10.1007/s11230-010-9285-4.
[4] F. Cremonte, M.A. Kroeck, S.R. Martorelli, A new monorchiid cercaria (Digenea) parasitising the purple clam *Amiantis purpurata* (Bivalvia: Veneridae) in the Southwest Atlantic Ocean, with notes on its gonadal effect, Folia Parasitol. 48 (2001) 217–223, http://dx.doi.org/10.14411/fp.2001.035.
[5] C. Gilardoni, G. Posadas, M. Kroeck, F. Cremonte, Monorchiid and aporocotylid cercariae (Digenea) parasitising the purple clam *Amiantis purpurata* (Bivalvia, Veneridae) from the Southwestern Atlantic coast, Acta Parasitol. 56 (2011) 385–391, http://dx.doi.org/10.2478/s11686-011-0074-2.
[6] C. Gilardoni, M.C. Carballo, F. Cremonte, The life cycle and geographical distribution of the monorchiid *Proctotrema bartolii* (Digenea) in the clam *Darina solenoides* from the Patagonian coast, Argentina, J. Helminthol. 87 (2013) 392–399, http://dx.doi.org/10.1017/S0022149X12000569.
[7] W.E. Martin, Studies on trematodes of woods hole. III. The life cycle of *Monorcheides cumingiae* (Martin) with special reference to its effect on the invertebrate host, Biol. Bull. 79 (1940) 131–144.
[8] R.T. Young, *Postmonorchis donacis*, a new species of monorchid trematode from the Pacific coast, and its life history, J. Wash. Acad. Sci. 43 (1953) 88–93.
[9] J.D. DeMartini, I. Pratt, The life cycle of *Telolecithus pugetensis* Lloyd and Guberlet, 1932 (Trematoda: Monorchiidae), J. Parasitol. 50 (1964) 101–105.
[10] C. Maillard, Cycle évolutif de *Paratimonia gobii* Prévot et Bartoli 1967 (Trematoda-Monorchiidae), Acta Trop. 32 (1975) 327–333.
[11] H.W. Stunkard, The morphology, life history, and systematic relations of *Lasiotocus elongatus* (Manter, 1931) Thomas, 1959 (Trematoda: Digenea), Biol. Bull. 160 (1981) 146–154.
[12] H.W. Stunkard, The life history, developmental stages, and taxonomic relations of the digenetic trematode *Lasiotocus minutus* (Manter, 1931) Thomas, 1959, Biol. Bull. 160 (1981) 146–154.
[13] P. Bartoli, O. Jousson, F. Russell-Pinto, The life cycle of *Monorchis parvus* (Digenea: Monorchiidae) demostrated by developmental and molecular data, J. Parasitol. 86 (2000) 479–489.
[14] O. Jousson, P. Bartoli, J. Pawlowski, Use of the ITS rDNA for elucidation of some life-cycles of Mesometridae (Trematoda, Digenea), Int. J. Parasitol. 28 (1998) 1403–1411.
[15] A.O. Bush, K.D. Lafferty, J.M. Lotz, A.W. Shostak, Parasitology meets ecology on its own terms: Margolis et al. revisited, J. Parasitol. 83 (1997) 575–583.
[16] F. Corpet, Multiple sequence alignment with hierarchical clustering, Nucleic Acids Res. (1988) 10881–10890.
[17] R.M. Cable, Marine cercariae from Curaçao and Jamaica, Z. Parasitenkd. 23 (1963) 429–469.
[18] R. Poulin, T.H. Cribb, Trematode life cycles: short is sweet? Trends Parasitol. 18 (2002) 176–182.
[19] K. Skirnisson, K.V. Galaktionov, Life cycles and transmission patterns of seabird digeneans in SW Iceland, Sarsia 87 (2002) 144–151.