

A survey of larval digeneans in the commonest intertidal snails from Northern Patagonian coast, Argentina

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Abstract

Over a two-years period, a survey was carried out in order to increase the knowledge of digeneans parasitising the commonest intertidal gastropods on the Patagonian coast, Southwestern Atlantic Ocean. A total of 4,725 gastropods were examined. Six species of digenean parasitising four snail species were found; four of them were registered for first time: *Maritrema* sp. 1 (Microphallidae) in *Crepidula dilatata* (Calyptaeidae), *Parorchis* sp. (Philophthalmidae) and sporocyst of Rencolidae in *Trophon geversianus* (Muricidae), and *Diptherostomum* sp. (Zoogonidae) in *Buccinanops globulosus* (Nassariidae). Two other species were found in *Siphonaria lessoni* (Siphonariidae): *Maritrema* sp. 2 and Hemiuridae. One snail species, *Tegula patagonica* (Trochidae) was not parasitised. These gastropods act as first intermediate host, and *C. dilatata*, *S. lessoni* and *B. globulosus* also frequently host metacercariae within the sporocyst. Overall prevalences varied from 0.16% of *Diptherostomum* in the intertidal population of *B. globulosus* to 33.45% of *Maritrema* sp. 1 in *C. dilatata*.

Keywords

Trematoda, Digenea, intertidal gastropods, larval stages, Patagonian coast

Introduction

Digeneans mainly use snails as first intermediate hosts, in which their parthenogenetic stages (sporocysts or rediae) develop (Yamaguti 1975, Galaktionov and Dobrovolskij 2003). These parasites may affect survival, behavior, reproductive function, phenotype and abundance or population structure of their hosts (e.g., Granovitch *et al.* 2000, Combes 2001, Galaktionov and Dobrovolskij 2003, Fredensborg *et al.* 2005). Furthermore, parasites are good markers of environmental stress, structure of food webs and biodiversity (Lafferty 1997, Marcogliese and Cone 1997, Lafferty *et al.* 2006).

The intertidal of Patagonian shores is a zone of high diversity that supports a rich fauna of invertebrates and birds (Balech and Ehrlich 2008), promoting the complex digenean life cycles. Despite the importance of digeneans in coastal marine areas, only six have been reported as infecting gastropods from the Southwestern Atlantic coast: a notocotylid cercaria from *Laevilitorina caliginosa* (Gould) (Littorinidae) (Graefe 1968), a lepecreidiid cercaria from *Buccinanops monilifer* (Kiener) and *Buccinanops cochlidium* (Dillwyn) (Nassariidae) (Martorelli 1991, Aberbuj and Cremonte 2010), a gymnophal-

lid metacercaria from the patellids *Nacella (Patinigera) magellanica* (Gmelin) and *Nacella (Patinigera) deaurata* (Gmelin) (Martorelli and Morriconi 1998), a microphallid cercaria and metacercaria from the siphonariids *Siphonaria lessoni* (Blainville) and *Kerguelenella lateralis* (Gould), and schistosomatid and hemiurid cercariae from *S. lessoni* (Alda and Martorelli 2009).

Coasts of Nuevo Gulf in northern Patagonia have extensive sandy and rocky intertidal zones with a gradual slope. The commonest species of gastropods are *Crepidula dilatata* Lamarck (Calyptaeidae), living on vertical walls at lower intertidal levels, *S. lessoni* (Siphonariidae) inhabiting the entire intertidal zone, with the largest specimens located in the upper zone, *Trophon geversianus* Pallas (Muricidae) living on mussel beds, *Buccinanops globulosus* (Kiener) (Nassariidae) inhabiting lower sandy intertidal flats and *Tegula patagonica* (d'Orbigny) (Trochidae) occurring at lower intertidal rocky zones.

Bearing in mind the importance of digeneans in the life histories of marine snail hosts, the goal of the present study was to describe the larval digenean fauna affecting the commonest intertidal snails at Puerto Madryn, on the northern Patagonian coast, Argentina.

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Materials and methods

Snails were collected by hand during low tides from the intertidal of Punta Cuevas (42°46'S, 64°54'W), Puerto Madryn, Chubut Province, Argentina. From November 2007 to October 2009, approximately 75 specimens of *C. dilatata* and 100 specimens of *S. lessoni* were monthly collected. Samples were randomly taken using a 12 cm² square in order to include all size classes. From August 2008 to July 2010, monthly samples of about 30 specimens of each of *T. geversianus*, *B. globulosus* and *T. patagonica* were randomly collected along a transect perpendicular to the coast line. An additional sample of *B. globulosus* (n = 59) was collected from the sandy shallow subtidal at the same beach.

Gastropods were transported to the laboratory and placed in small flasks filled with seawater at room temperature (20–23°C). Twice daily they were inspected under stereomicroscope looking for emerging of cercariae. Emerged cercariae were studied alive, stained with neutral red or Nile blue under a light microscope before being fixed. After 48 hs, all snails were necropsied in order to detect prepatent infections and to study sporocysts or rediae and metacercariae. The morphology was studied alive and photographed. The behaviour of emerged cercariae was studied in the flask under a stereomicroscope. The life span of cercariae was determined at room temperature (20–22°C) and at 10°C.

Several naturally emerged cercariae, sporocysts, rediae and metacercariae were killed with heated seawater, immediately fixed with 10% formalin (Cribb and Bray 2010), stained with acetic carmine, dehydrated through ascendant ethanol series, cleared with methylsalicylate and mounted on glass-slides with Canada balsam. Drawings and measurements (recorded for n = 15–20, unless otherwise indicated) were obtained from unflattened, fixed, stained and mounted specimens with the aid of a light microscope with drawing device. Measurements are presented in micrometers as mean values followed by the range in parentheses. Prevalences were calculated following Bush *et al.* (1997).

For scanning electron microscopy (SEM), larval digeneans were fixed in a 2.5% glutaraldehyde solution buffered with 0.1 M sodium cacodylate, dehydrated through an ascendant ethanol series and dried by rinsing for few minutes in hexamethyldisilazane. Photomicrographs were obtained with a Jeol JSM-6460LV SEM operating at 15 KV.

Mounted specimens were deposited in the Helminthological Collection, Museo de La Plata (MLP), La Plata, and in the Parasitological Collection, Centro Nacional Patagónico (CNP-Par), Puerto Madryn, Argentina.

Results

A total of 4,725 gastropods were examined during a two-year period. Six species of digeneans, belonging to five families, were found parasitising *C. dilatata*, *S. lessoni*, *T. geversianus*,

and *B. globulosus*. No parasites were found in *T. patagonica* (n = 689). The sites of infection of all intramolluscan larval stages found were the gonad and digestive gland.

Crepidula dilatata acted as first and second intermediate host for a species of *Maritrema* (Microphallidae). *Siphonaria lessoni* served as first and second intermediate host for *Maritrema* sp. 2 and also harboured sporocysts containing a cystophorous hemiurid cercaria. *Trophon geversianus* was parasitised by two digenean species, rediae with cercariae of *Parorchis* sp. (Philophthalmidae) and sporocysts with renicolid cercariae. Finally, *Buccinanops globulosus* was parasitised by sporocysts, cercariae and metacercariae of *Diptherostomum* sp. (Zoogonidae).

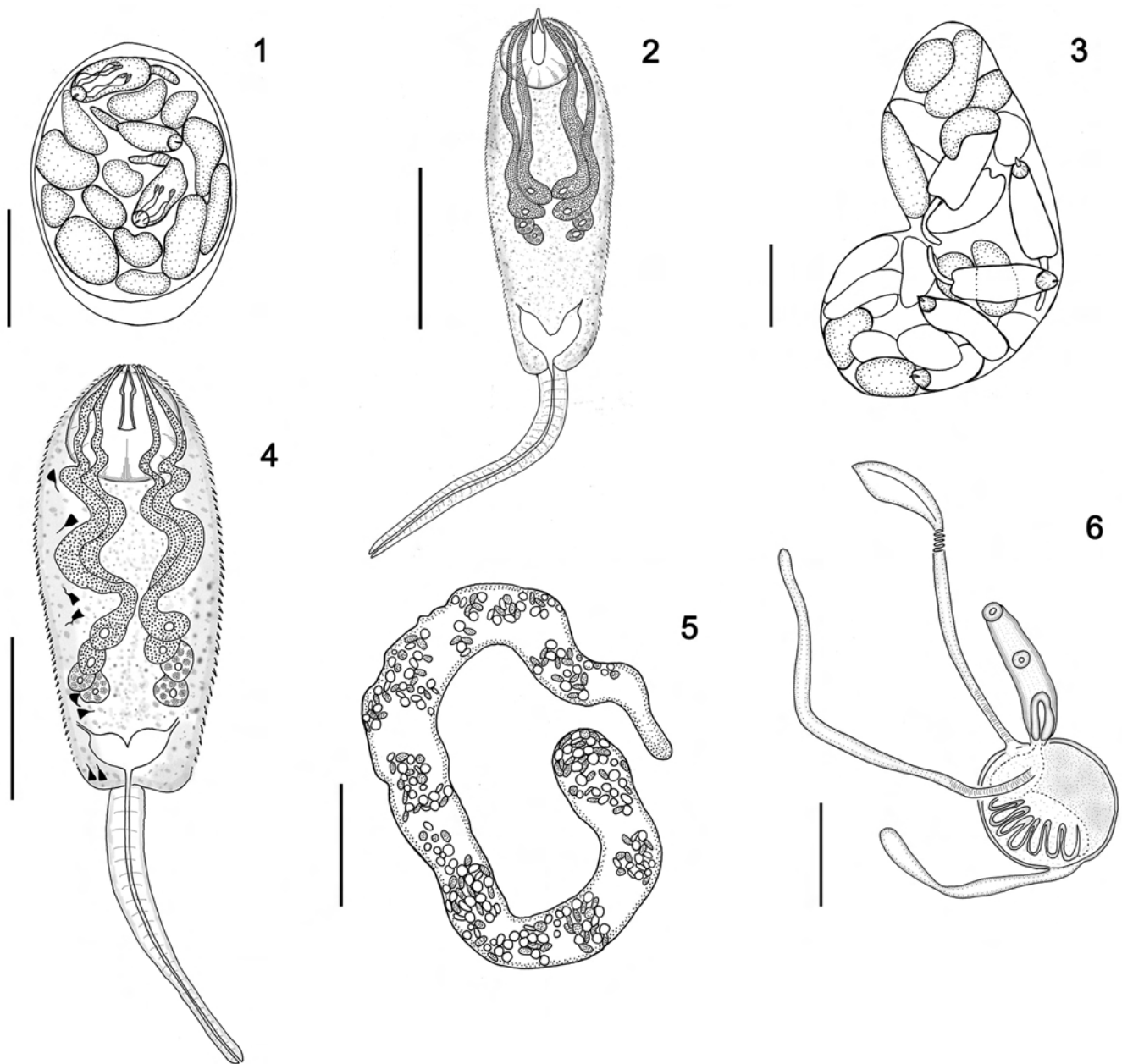
Family Microphallidae Travassos, 1920

Maritrema sp. 1 (Figs 1, 2, 7–11)

Sporocyst (Figs 1, 7, 8): Motionless, colourless, ovoid with anterior extremity often slightly pointed. Body 279 (160–379) long by 198 (130–279) maximum wide. Body wall 7 (5–10) thick. Movement of active cercariae may distort body shape; fully developed cercariae leave sporocyst via terminal birth pore. Two kinds of sporocysts observed, mother sporocysts filled only with germinal balls and daughter sporocysts containing germinal balls 6 (3–9), cercariae 9 (4–22) and/or metacercariae 7 (3–11). Sporocysts containing many germinal balls have few developed cercariae and *vice versa*. Total number of developing stages (germinal balls, cercariae, and metacercariae) per sporocyst 16 (6–31).

Cercaria (Fig. 2, 9–11): Monostome xiphidocercaria. Body small, elongate, 116 (93–143) long by 36 (26–49) maximum wide, uniformly covered with tiny spines from oral sucker to posterior end. Oral sucker subterminal, 22 (17–28) in diameter. Stylet well developed, lanceolate and refractive thick-walled in upper half, 18 (15–21) long by 6 (5–8) wide at base (Fig. 10). Ventral sucker absent. Four pairs of penetration glands irregular in shape, with nuclei near mid-body (Figs 2, 9); 2 anterior pairs sac-like with swollen ducts extending dorsally, opening on either side of ventral lip of oral sucker; 2 posterior pairs sac-like with thinner ducts extending ventrally and masked by dorsal ducts, opening on either side of stylet. Secretory material in anterior gland-ducts in form of rough grains, which stain with neutral red. In posterior gland-ducts, secretory material in form of small grains, not staining with neutral red (Fig. 9). Distance from anterior body end to posterior extremity of penetration glands 70 (66–77). Mouth and other parts of digestive system not developed. Excretory vesicle thin-walled, V-shaped, stem and arms short (Fig. 11). Excretory pore at tip of tail. Tail cylindrical and contractile, slightly shorter than the body, 87 (70–100) long by 11 (9–15) wide at base, diameter decreasing towards distal end. Tegument with numerous annular folded membranes.

Behaviour: Under laboratory conditions, naturally emerged cercariae swim actively for a few minutes and then fall to the bottom. While swimming, the posterior extremity of the



Figs 1–6. Line drawings: 1. Sporocyst of *Maritrema* sp. 1. 2. Cercaria of *Maritrema* sp. 1, dorsal view. 3. Sporocyst of *Maritrema* sp. 2. 4. Cercaria of *Maritrema* sp. 2, dorsal view, flame cells of right side omitted. 5. Sporocyst of Hemiuridae. 6. Cercaria of Hemiuridae. Scale bars = 50 μ m (2, 4), 100 μ m (1, 3, 6), 500 μ m (5)

cercarial body is bent ventrally and tail lies parallel to the ventral body surface. Cercariae move alternating body contractions with vigorous tail lashes. Their life-span is less than 24 hs at room temperature, but at 10°C survive up to 48 hs.

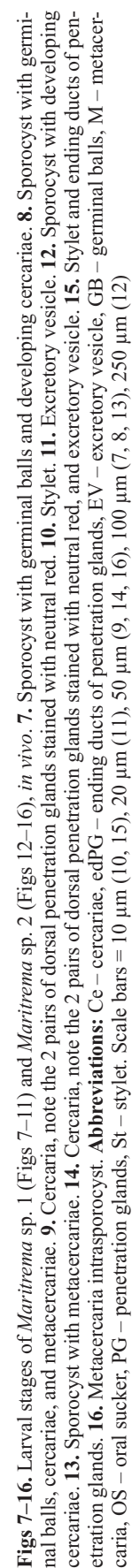
Metacercaria: Spherical cyst, 74 (67–80) long by 69 (63–75) wide, wall 5 (3–6) thick. Under laboratory conditions cercariae encyst inside sporocyst; cercaria gradually cease movement, becoming rounded and secreting a thin wall.

Host: *Crepidula dilatata* Lamarck (Caenogastropoda, Calyptraeidae).

Prevalence: 33.45% (n = 1,665).

Specimens deposited: 6285 (MLP) and 28 (CNP-Par).

Comments: The following characteristics define the described cercaria as belonging to the Microphallidae: oral sucker with a poorly defined musculature and prominent stylet (xiphidocercaria); ventral sucker absent (monostoma cercaria); tegument spined; digestive system undeveloped (anenteric cercaria); excretory vesicle V-shaped and tail small and simple (leptocercous cercaria). The cercaria belongs to the genus *Maritrema* Nicoll, 1907 because of the shape and location of penetration glands (Bridgman 1971). This is a third report of a *Maritrema* parthenitae in snails from Argentina.



Previously, Etchegoin and Martorelli (1997) described *Maritrema bonaerensis* Etchegoin et Martorelli, 1997 from the brackish water snail *Heleobia australis* d'Orbigny (Cochliopidae) from Mar Chiquita coastal lagoon (37°46'S, 57°27'W). In addition, Alda and Martorelli (2009) described *Maritrema* sp. 2 parasitising *Siphonaria lessoni* and *Kerguelenella lateralis* Gould (Siphonariidae) from the rocky intertidal at Comodoro Rivadavia (45°51'S, 67°29'W), Chubut Province.

***Maritrema* sp. 2** (Figs 3, 4, 12–16)

Sporocyst (Figs 3, 12, 13): Body elongate, light yellowish, motionless, 709 (520–1000) long by 245 (170–290) maximum wide. Movement of active cercariae may distort body shape; fully developed cercariae leave sporocyst via terminal birth pore. Two kinds of sporocysts observed; sporocysts with germinal balls and cercariae at different developmental stages or sporocysts filled with metacercariae (Fig. 13). Total number of larval stages per sporocyst (germinal balls, cercariae, and metacercaria) 37 (15–50).

Cercaria (Figs 4, 14, 15): Monostome xiphidocercaria. Body small, elongate, 132 (105–200) long by 52 (48–70) maximum wide, uniformly covered with tiny spines from oral sucker to posterior end. Oral sucker subterminal, 34 (29–38) in diameter. Stylet well developed 18 (14–22) long by 4 (2.5–4.5) wide at base, arrow-shaped with refractive layer, wide at base with anterior end pointed (Fig. 15). Ventral sucker absent. Four pairs of penetration glands irregular in shape, with nuclei in posterior part of body; 2 anterior pairs sac-like with swollen ducts extending dorsally, forming three loops and opening on either side of ventral lip of oral sucker; 2 posterior pairs sac-like with thinner ducts extending ventrally and masked by dorsal ducts, opening on either side of stylet (Figs 4, 14). Secretory material in anterior gland-ducts in form of rough grains, which stain with neutral red and in posterior gland-ducts in form of small grains, not staining with neutral red. Distance from anterior body end to posterior extremity of penetration glands 93 (82–109). Mouth and other parts of digestive system not developed. Excretory vesicle thin walled, V-shaped, stem and arms short (Fig. 14). Excretory formula: $2[(2+2) + (2+2)] = 16$. Excretory pore at tip of tail. Tail cylindrical and contractile, shorter than body, 103 (95–125) long by 10 (8–11) wide at base, diameter decreasing towards distal end. Tegument provided with numerous annular folds.

Behaviour: Movement and life-span similar to *Maritrema* sp. 1.

Metacercaria (Fig. 16): Oval cyst, 74 (65–85) long by 125 (110–135) wide, with hyaline layer 10 (7–13) thick. Pharynx, caeca and spinous cirrus, present.

Host: *Siphonaria lessoni* Blainville (Pulmonata, Bassomatophora, Siphonariidae).

Prevalence: 8.95% (n = 1,050).

Specimens deposited: 6284 (MLP) and 27 (CNP-Par).

Comments: *Maritrema* sp. 2 was described by Alda and Martorelli (2009) from *S. lessoni* and *K. lateralis* at the inter-

tidal of Comodoro Rivadavia, about 500 km south of the present locale. *Maritrema* sp. 2 differs from *Maritrema* sp. 1 in the body size, the shape of the stylet and the location of the penetration glands and ducts. Sporocysts of *Maritrema* sp. 2 are larger than those of *Maritrema* sp. 1 and bear a larger number of larval stages (37 vs 16). The stylet is of similar size (18), although in *Maritrema* sp. 1 is it lanceolate and covered with a refractive layer at the pointed anterior end, and in *Maritrema* sp. 2 it is arrow shaped and is entirely covered by a refractive layer. The nuclei of the penetration glands are situated near the middle of the body in *Maritrema* sp. 1 and near the posterior end in *Maritrema* sp. 2. Metacercariae of *Maritrema* sp. 1 were found developing inside the sporocyst mainly under laboratory conditions; whereas sporocysts of *Maritrema* sp. 2 were usually found filled with encysted metacercariae. *Maritrema* sp. 2 is most similar to *M. bonaerensis* in the size of the larval stages and the shape of the stylet. However, the species differ in the position of the nuclei of the penetration glands and in the loops of the ducts. In the latter characters, *M. bonaerensis* is similar to *Maritrema* sp. 1 (Etchegoin and Martorelli 1997).

Family Hemiuridae Looss, 1899 (Figs 5, 6, 17–20)

Sporocyst (Figs 5, 17, 18): Body long, elongate, irregularly shaped, with anterior end narrow and posterior end slightly expanded, 4,461 (2,700–7,410) long by 269 (180–310) wide. Terminal birth pore long and narrow (Fig. 17). Number of cercariae per sporocyst 250 (130–400).

Cercaria (Figs 6, 19, 20): Cystophorous, elongate body 138 (117–162) long by 27 (22–30) wide, developing in spherical caudal cyst, 105 (92–128) in diameter. Delivery tube attached at posterior end of cyst cavity, slightly flattened, 329 (267–369) long (n = 3), with median longitudinal furrow and apical finfold on each side. Excretory vesicle epithelial, 43 (32–50) long by 21 (14–26) wide. Oral sucker 20 (15–25) in diameter and ventral sucker 16 (14–20) in diameter. Pharynx, oesophagus and intestinal caeca not observed. Two caudal filaments at both side of cyst, thin and delicate, one longer, 416 (320–490) long another shorter 226 (196–290) long.

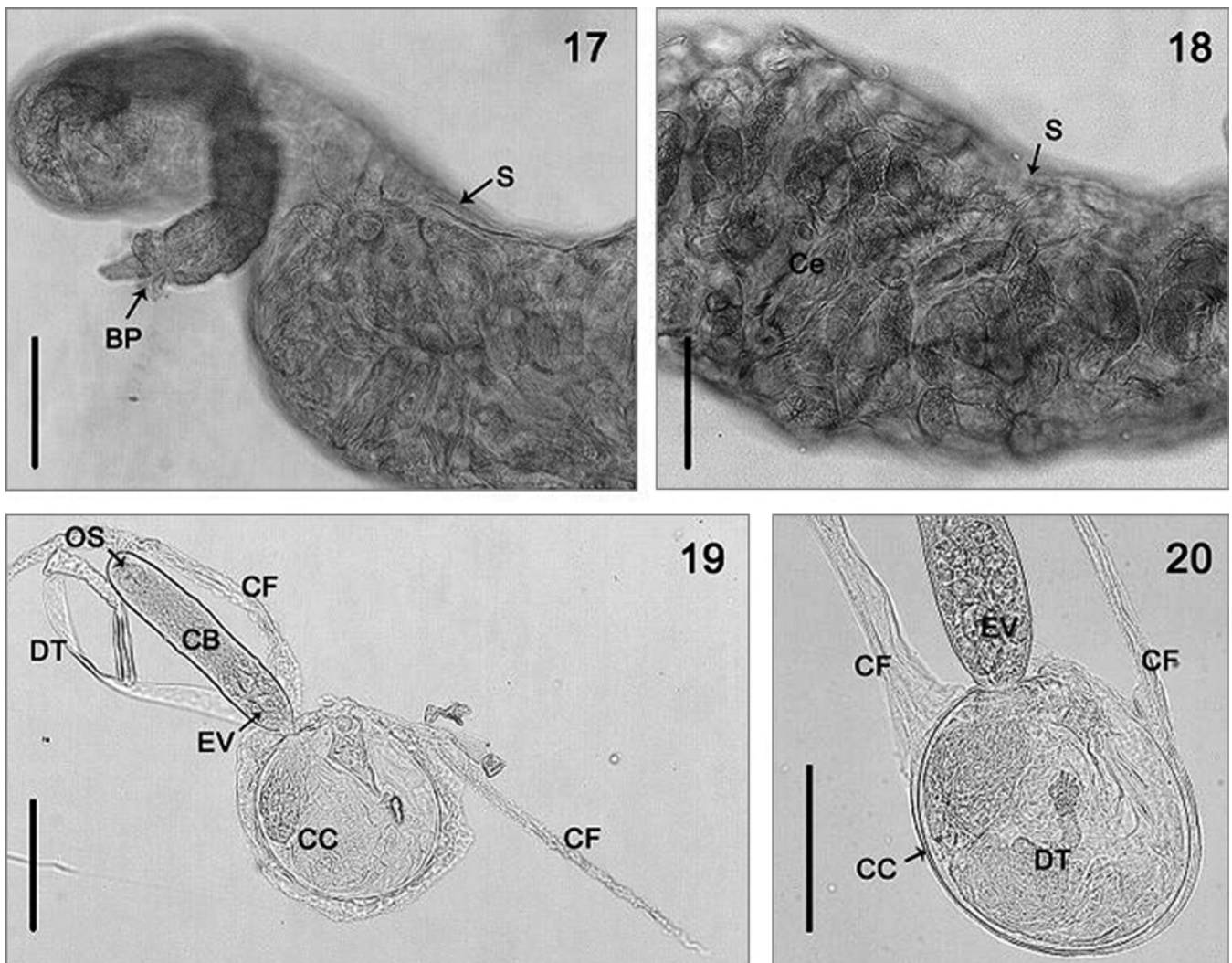
Behaviour: Natural cercarial emergence was not observed. Larvae were obtained from sporocysts; after leaving the sporocyst, cercariae moved slowly for a few minutes.

Host: *Siphonaria lessoni* Blainville (Pulmonata, Bassomatophora, Siphonariidae).

Prevalence: 0.48% (n = 1,050).

Specimens deposited: 6283 (MLP) and 26 (CNP-Par).

Comments: This cystophorous cercaria belongs to the family Hemiuridae. Three cercariae of this family have been reported from Argentinean waters: *Genarchella genarchella* in *Heleobia parchappei* d'Orbigny (Cochliopidae), and cystophorous cercariae in *Potamolithus agapetus* Pilsbry (Cochliopidae) from lagoons at Buenos Aires Province (Martorelli 1989, 1994) and in *Siphonaria lessoni* from Patagonian coast at Comodoro Rivadavia, Chubut Province (Alda and Mar-



Figs 17–20. Larval stages of Hemiuridae (Figs 17–18 *in toto*, Figs 19–20 *in vivo*). **17.** Anterior end of sporocyst with developing cercariae. **18.** Sporocyst. **19.** Cercaria. **20.** Detail of caudal cyst with delivery tube inside. **Abbreviations:** BP – birth pore, Ce – cercaria, CB – cercarial body, CC – caudal cyst, CF – caudal filament, DT – delivery tube, EV – excretory vesicle, OS – oral sucker, S – sporocyst. Scale bars = 50 μ m (20), 100 μ m (17–19)

torelli 2009). The larvae here described are probably the same as reported from Comodoro Rivadavia; however, in the specimens here examined, 2 caudal filaments were observed.

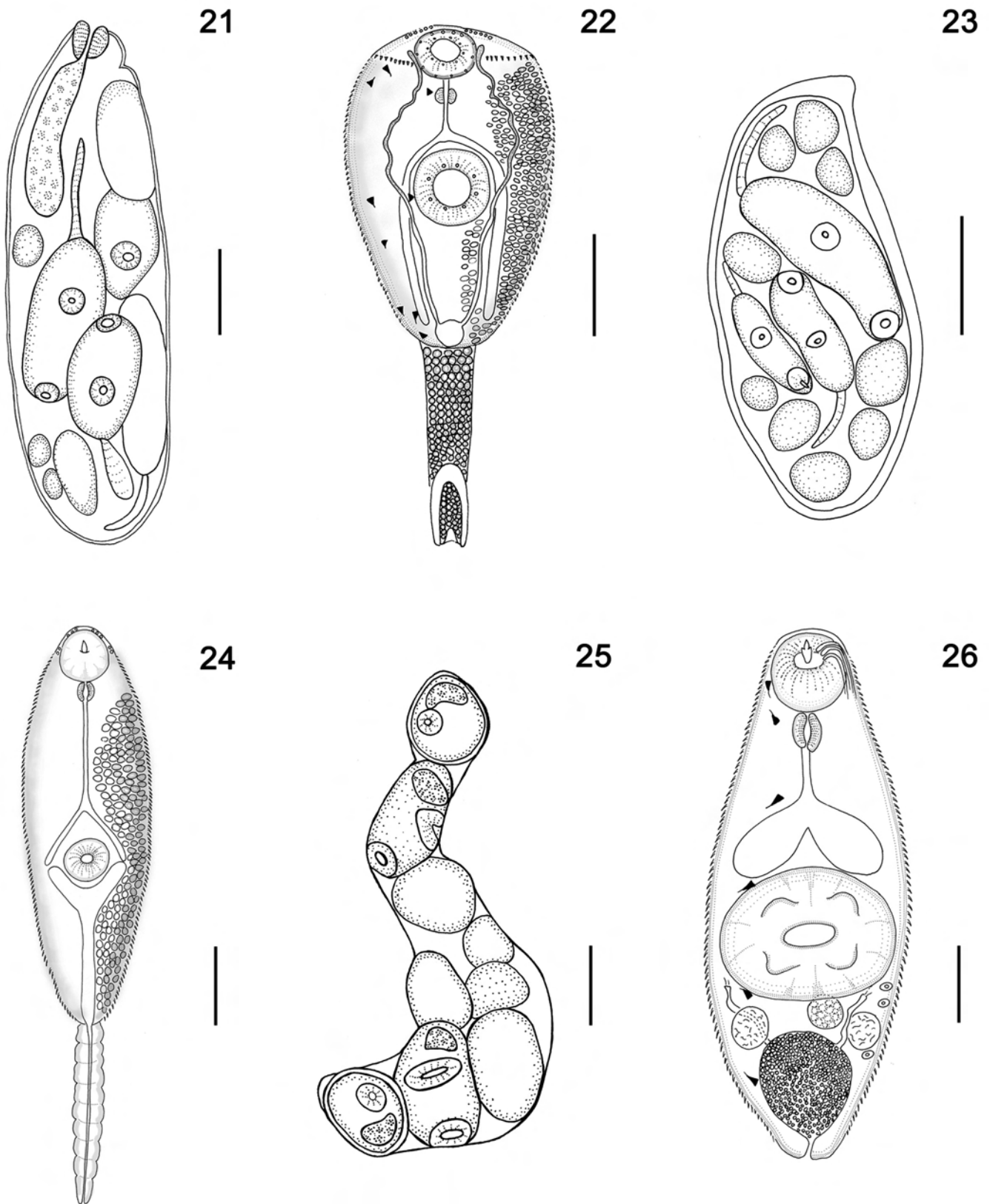
Family Philopthalmidae Travassos, 1918

Parorchis sp. (Figs 21, 22, 27–41)

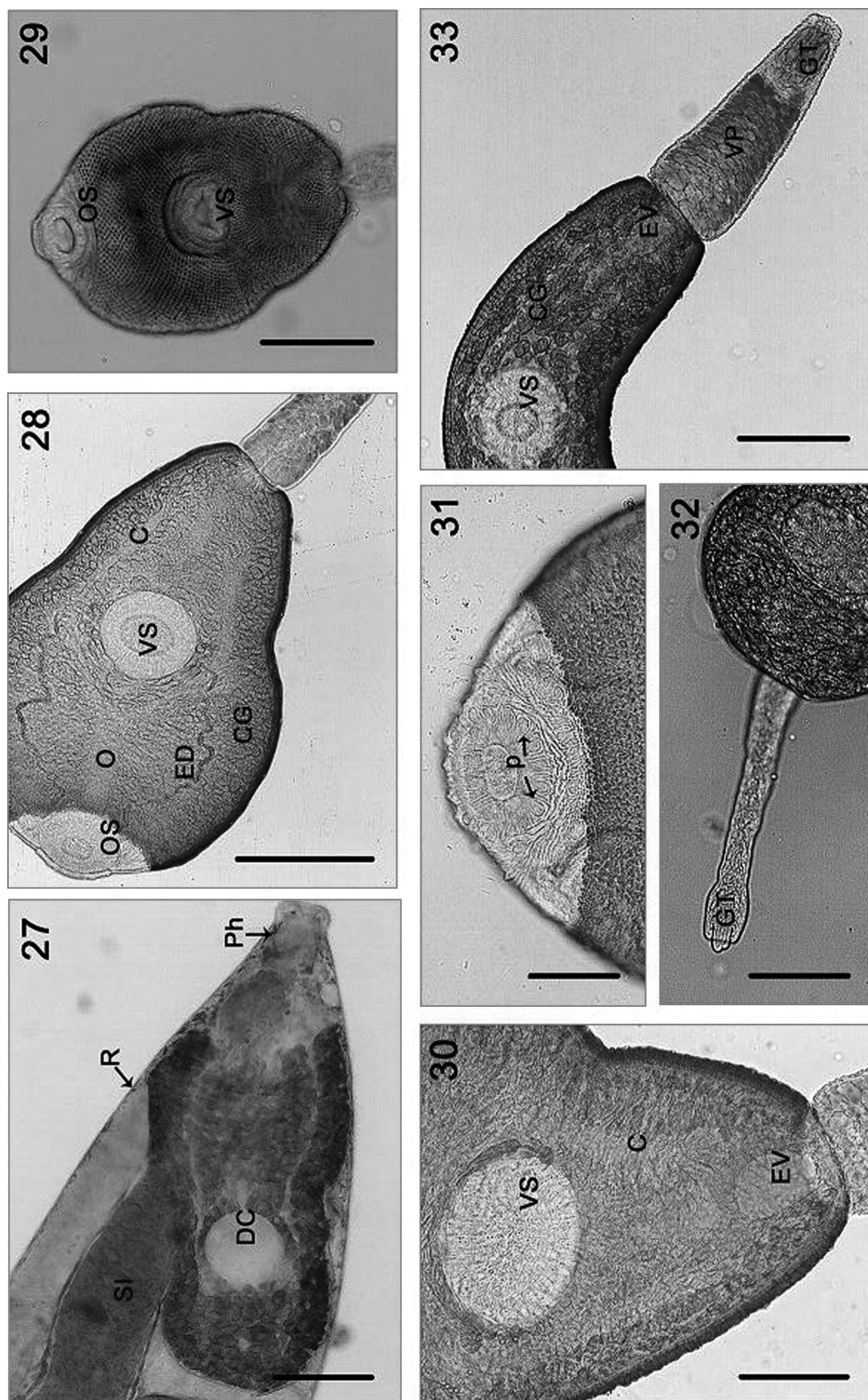
Redia (Figs 21, 27, 34–36): Body large, elongate, 1,128 (620–1,350) long by 271 (140–380) maximum wide. Birth pore anterior and lateral (Fig. 35); wall 9 (6–16) thick. Pharynx small, 83 (38–135) long by 67.5 (32–110) wide. Intestine saccular, 273 (180–340) long by 43 (35–60) wide ($n = 5$), orange in colour. Usually, 10 (8–14) embryos per redia at different developmental stages, 5 (4–9) cercariae and 4 (2–8) germinal balls.

Cercaria (Figs 22, 28–33, 37–40): Echinostome-like cercaria. Body spinous, 328 (260–408) long by 172 (130–230)

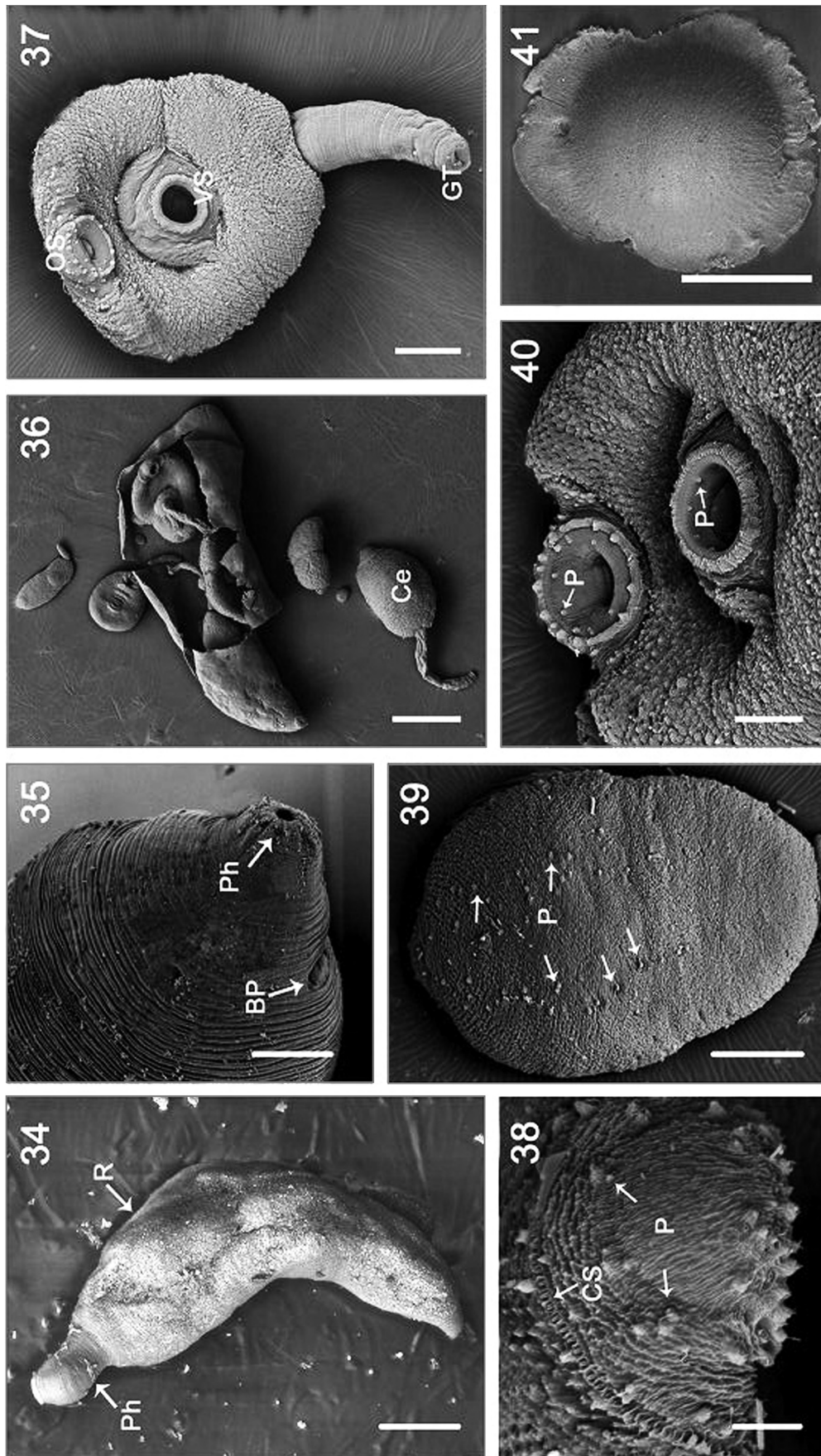
wide at level of ventral sucker. Numerous cystogenous glands from pharynx to body end, staining well with neutral red, filled with granular inclusions (Figs 28, 33). Oral sucker 49 (36–60) long by 58 (50–70) wide. Mouth subterminal, surrounded by an outer circle of 12 papillae and an inner circle of 8 papillae (only seen at SEM, Fig. 40). Collar with about 77 spines per row, interrupted ventrally (Figs 22, 38). Prepharynx short, pharynx muscular 52 (28–80) long by 39 (24–55) wide ($n = 10$), oesophagus long, bifurcating anterior to ventral sucker. Intestinal caeca narrow, 119 (62–156) long ($n = 3$) extending to level of excretory vesicle. Ventral sucker at mid-ventral level of body, 73 (46–93) long by 78 (60–105) wide, with an inner circle of 8 papillae (only seen at SEM, Fig. 40). Body covered with spines, except around ventral sucker (Figs 29, 37). Two longitudinal rows of papillae distributed dorsally and ventrally from anterior end to ventral sucker level and group of papillae scattered from collar spine to anterior end (only



Figs 21–26. Line drawings: **21.** Redia of *Parorchis* sp. **22.** Cercaria of *Parorchis* sp., ventral view, flame cells of left side and cystogenous cells of right side omitted. **23.** Sporocyst of Renicolidae. **24.** Cercaria of Renicolidae. **25.** Sporocyst of *Diptherostomum* sp. **26.** Cercaria of *Diptherostomum* sp., ventral view, flame cells of left side omitted. Scale bars = 50 μ m (24, 26), 100 μ m (22, 23, 25), 200 μ m (21)



Figs 27–33. Larval stages of *Parorchis* sp., *in vivo*. **27.** Anterior end of redia. **28.** Cercaria, ventral view. **29.** Ventral body spination. **30.** Posterior end of body. **31.** Detail of papillae of oral sucker. **32.** Tail with glandular tip contracted. **33.** Tail with glandular tip evaginated. **Abbreviations:** C – caeca, CG – cystogenous glands, DC – developing cercaria, ED – excretory ducts, EV – excretory vesicle, GT – glandular tip, O – oesophagus, OS – oral sucker, P – papillae, Ph – pharynx, R – redia, SI – saccular intestine, VP – vacuolar parenchyma, VS – ventral sucker. Scale bars = 50 µm (30, 31, 33); 100 µm (27–29, 32)



Figs 34–41. Larval stages of *Parorchis* sp., at SEM. **34.** Redia. **35.** Anterior end of redia with lateral birth pore and pharynx. **36.** Broken redia with developing cercariae. **37.** Cercaria, ventral view. **38.** Cercaria, papillae and papillae, dorsal view. **39.** Cercaria, papillae on dorsal body surface. **40.** Detail of oral and ventral sucker papillae. **41.** Metacercaria. **Abbreviations:** BP – birth pore, Ce – cercaria, CS – collar spines, GT – glandular tip, OS – oral sucker, VS – ventral sucker. Scale bars = 10 μ m (38), 20 μ m (40), 100 μ m (37, 39, 41), 200 μ m (34, 35), 300 μ m (36)

seen at SEM, Fig. 39). Six pairs of penetration glands opening dorsally at anterior end of body; none of these glands stained with neutral red; cytons and ducts masked by cystogenous glands. Excretory vesicle small, spherical; limbs extending straight to middle of ventral sucker and then as irregular sinuous ascending tubules reaching posterolateral margins of oral sucker (Figs 28, 30). Limbs with minutes spherical concretions from ventral sucker level to pharynx level. Excretory formula: $2 [(3+3+3)] = 18$. Tail 303 (200–455) long by 52 (40–60) wide at base, with vacuolar parenchyma, ending in a glandular tip (Figs 32, 33, 37).

Behaviour: Under laboratory conditions, naturally emerged cercariae swim for a few minutes, undulate the body with quick contracting and stretching movements. Then, cercariae fall to the bottom, creeping and attaching with the aid of ventral sucker, being very difficult to dislodge with pipette suction; then rapidly encyst. After attachment to the substrate, secretions from cystogenous cells form the cyst. Life-span is about 24 hs at room temperature, but at 10°C survival is up to 72 hs.

Metacercaria (Fig. 41): Cyst slightly oval, flattened on side of attachment, 234 (212–260) long by 204 (198–213) wide, with two-layer wall. Outer wall 9 (8–10) thick, inner wall 13 (8–19) thick.

Host: *Trophon geversianus* Pallas (Caenogastropoda, Muricidae).

Prevalence: 0.87% (n = 689).

Specimens deposited: 6282 (MLP) and 24–25 (CNP-Par).

Comments: The following characteristics define the described cercaria as belonging to the Philophthalmidae and are diagnostic for the genus *Parorchis* Nicoll, 1906: echinostome-like cercaria, with collar spines, glandular invaginated tip of tail and metacercarial cyst slightly oval found on the substrate. There are at least seven previous descriptions of cercariae belonging to *Parorchis*, all from the Northern Hemisphere (e.g., Nicoll 1907, Cable 1963, Lauckner 1980). These descriptions were extensively discussed because cercariae present only slight morphological differences (e.g., Stunkard and Cable 1932, Holliman 1961). This is the first record of philophthalmid parthenitae from South America.

Family Renicolidae Dollfus, 1939 (Figs 23, 24, 42–50)

Sporocyst (Figs 23, 42): Oval, 381 (227–502) long by 207 (132–330) wide, wall 10 (6–15) thick, anterior end narrow, containing 10 (2–24) germinal balls and 5 (1–8) cercariae; total number of larval per sporocyst 13 (3–27). Birth pore terminal.

Cercaria (Figs 24, 43–50): Distome xiphidocercaria. Body elongate, 258 (197–286) long by 58 (46–77) wide, covered with tiny spines from oral sucker to posterior end, more scattered behind ventral sucker. Four rows of longitudinal papillae on ventral surface, from oral sucker to ventral sucker (only seen at SEM, Fig. 47). Cystogenous glands filling the parenchyma from level of pharynx to posterior end of body,

most dense at sides (Fig. 45). Oral sucker subterminal, 37 (22–44) long by 25 (18–42) wide, with 4 groups of 3 papillae around mouth (seen at SEM, Fig. 48). Stylet small, lanceolate, 7 (6–8) long by 2 (1–3) wider at base (Fig. 43). Ventral sucker 27 (20–32) long by 21 (16–30) wide, with 2 concentric rows of spines (only seen at SEM, Fig. 50). Five pairs of penetration glands; ducts of 3 pairs opening dorsally close to stylet, 2 pairs opening ventro-laterally to oral sucker. Nuclei of penetration gland-cells not observed, masked by cystogenous glands. Prepharynx absent, pharynx 11 (9–13) long by 9 (7–12) wide, oesophagus 50 (35–55) long; caeca, 45 (35–52) long reaching mid-level of ventral sucker. Excretory vesicle Y-shaped with long stem and short arms reaching mid-level of ventral sucker (Figs 24, 44), opening at tip of tail. Excretory cells not observed, masked by cystogenous glands. Tail 135 (104–164) long by 19 (16–24) wider at base.

Behaviour: Under laboratory conditions, naturally shed cercariae swim for several hours with vigorous movements of the body, bending the posterior body end downward and tail vibrating laterally. On the bottom, cercariae maintain the ventral face upward and tail bent over the body. Their life-span is about 48 hs at room temperature.

Host: *Trophon geversianus* Pallas (Caenogastropoda, Muricidae).

Prevalence: 0.29% (n = 689).

Specimens deposited: 6278–6279 (MLP) and 20 (CNP-Par).

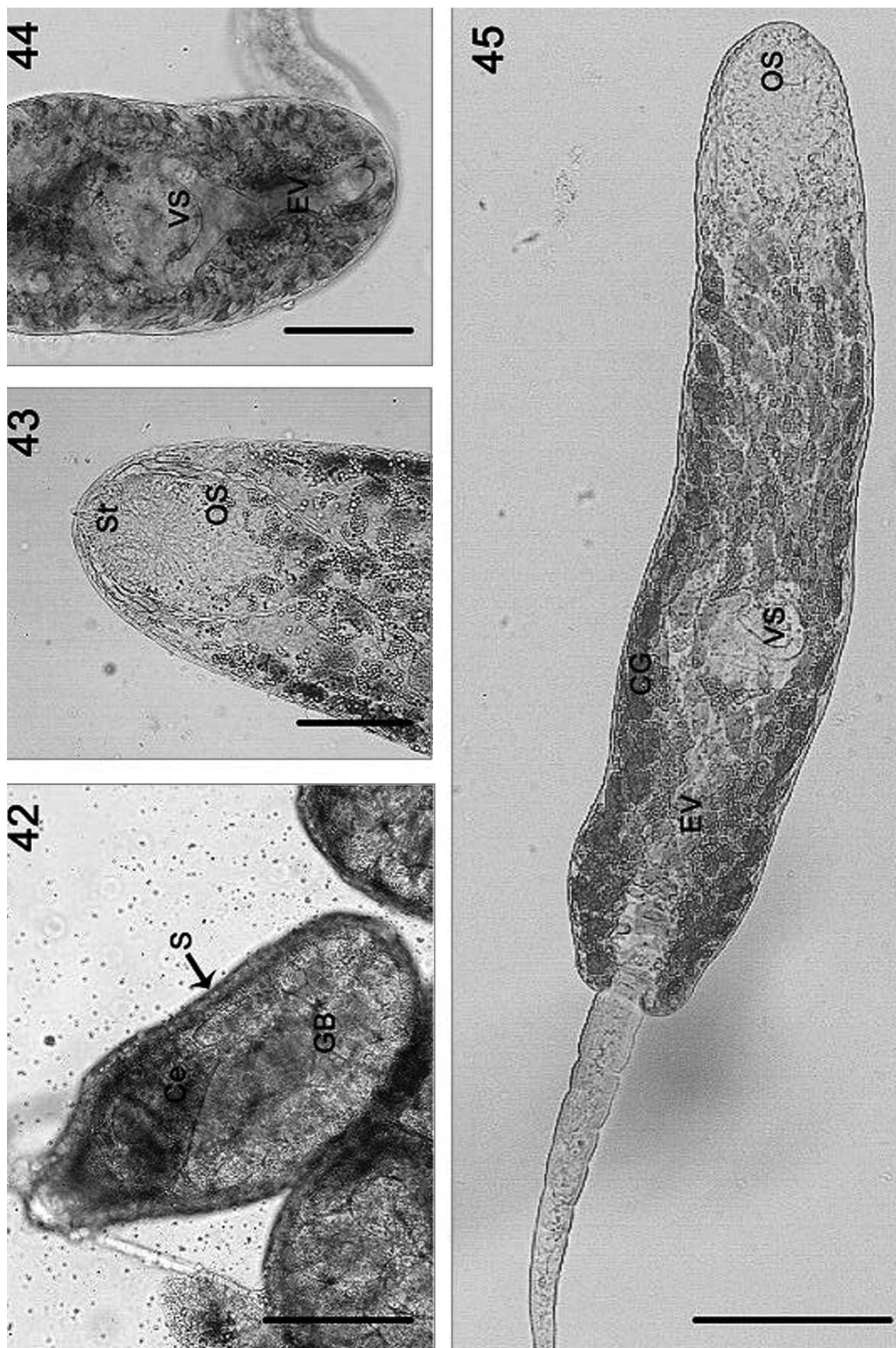
Comments: The following morphological features define the parthenitae here described as belonging to Renicolidae: xiphidocercaria (with stylet present), Y-shaped excretory vesicle, prepharynx absent and cystogenous glands numerous. This is the first report of renicolid parthenitae from South America. At least 23 renicolid cercariae have been described worldwide (Cable 1956, Sannia and James 1977, Galaktionov and Skirnisson 2000, Martorelli *et al.* 2008).

Family Zoogonidae Odhner, 1902

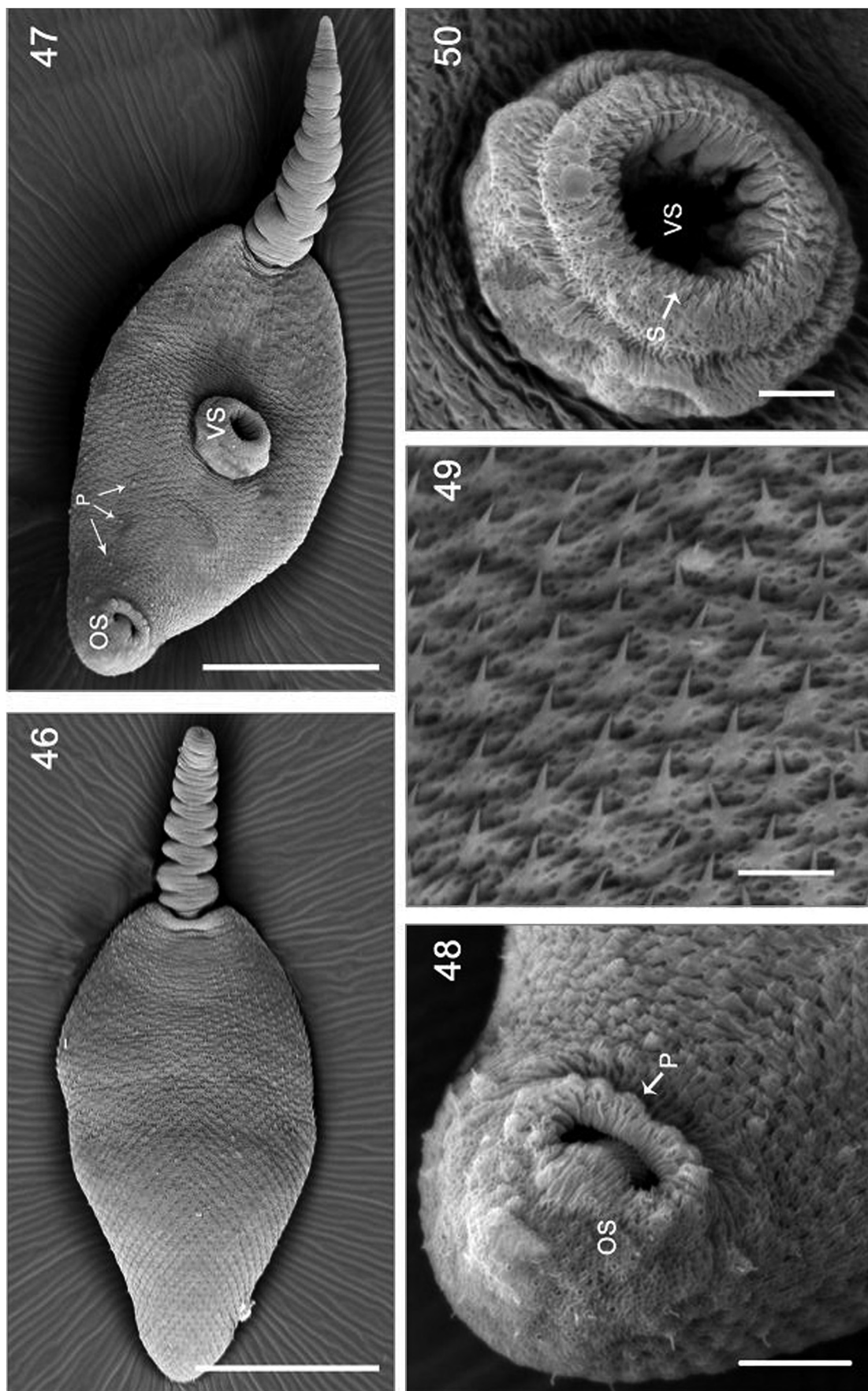
***Diptherostomum* sp.** (Figs 25, 26, 51–60)

Sporocyst (Figs 25, 56, 57): Body elongate, motionless, yellowish, 962 (530–1,420) long by 222 (170–260) wide, body wall 6 (2–14) thick; movement of cercariae may distort body shape. Three types of sporocysts observed: (a) filled with germinal cells and developing cercariae, (b) filled with metacercariae or (c) filled with both stages. Total number of larval stages (cercariae and metacercariae) 7 (6–11).

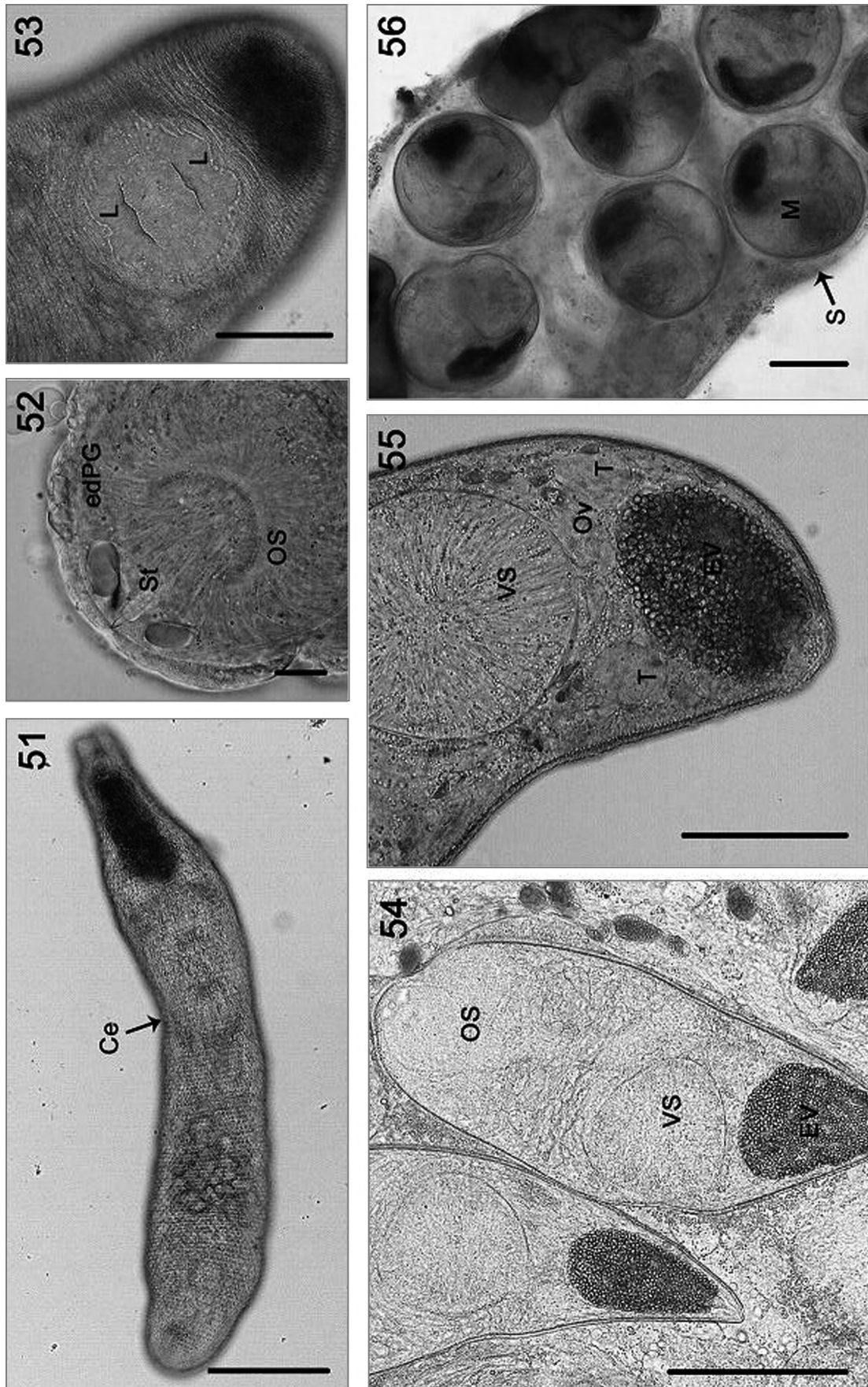
Cercaria (Figs 26, 51–55, 58–60): Tailless xiphidocercaria, small and elongate body, 323 (263–392) long by 120 (90–160) wide, entire body covered with minute spines (Figs 51, 60). Cystogenous cells scattered in parenchyma. Oral sucker subterminal, 63 (50–76) long by 50 (36–69) wide. Stylet small, lanceolate, 14 (12–20) long by 4 (3–6) wide at base (Fig. 52). Prepharynx absent, pharynx globose, 26 (22–30) long by 21 (20–23) wide (n = 3), oesophagus short, bifurcating into two short saccular intestinal caeca, 55 (46–66) long (n = 5), not



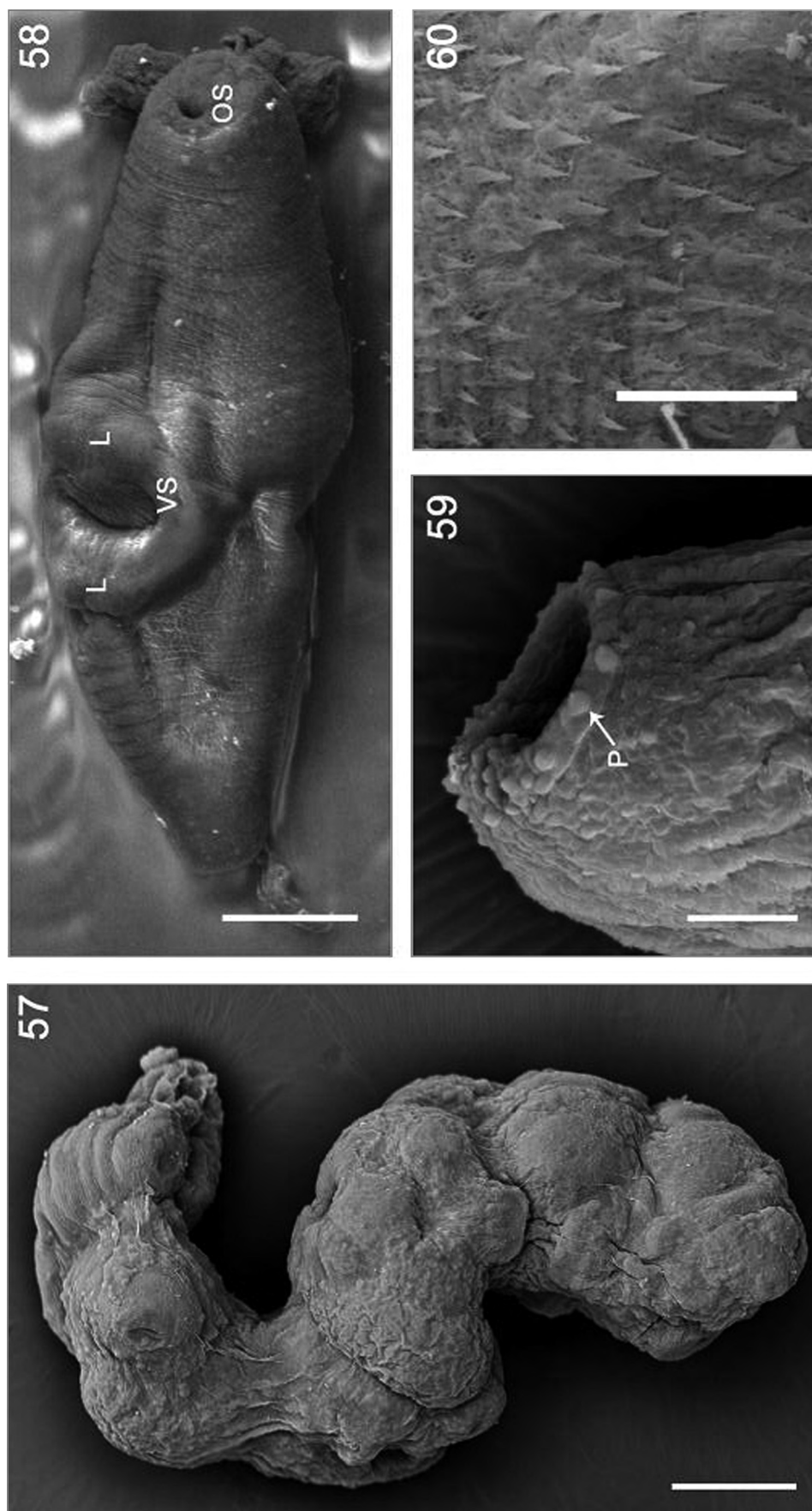
Figs 42–45. Larval stages of Renicolidae, *in vivo*. **42.** Sporocyst with germinal balls and developing cercariae. **43.** Cercaria, detail of anterior body end. **44.** Cercaria, detail of posterior body end. **45.** Cercaria, ventral view. **Abbreviations:** CG – cystogenous glands, Ce – cercaria, EV – excretory vesicle, GB – germinal balls, OS – oral sucker, S – sporocyst, St – stylet, VS – ventral sucker. Scale bars = 50 μ m (43–45), 100 μ m (42)



Figs 46–50. Larval stages of Rencolidae, at SEM. **46.** Cercaria, dorsal view. **47.** Cercaria, ventral view. **48.** Detail of anterior end of body. **49.** Detail of ventral body spines. **50.** Detail of ventral sucker. **Abbreviations:** OS – oral sucker, P – papilla, S – spines, VS – ventral sucker. Scale bars = 5 μ m (49, 50), 10 μ m (48), 40 μ m (47), 50 μ m (46)



Figs 51–56. Larval stages of *Diplotherostomum* sp., *in vivo*. **51.** Cercaria. **52.** Detail of styllet and ending ducts of penetration glands. **53.** Detail of lips of ventral sucker. **54.** Developing cercariae inside sporocyst. **55.** Detail of gonads. **56.** Metacercariae inside sporocyst. **Abbreviations:** Ce – cercaria, edPG – ending ducts of penetration glands, EV – excretory vesicle, L – lips, M – metacercaria, OS – oral sucker, Ov – ovary, S – sporocyst, St – styllet, T – testis, VS – ventral sucker. Scale bars = 10 μ m (52), 50 μ m (53, 55), 100 μ m (51, 54, 56)



Figs 57–60. Larval stages of *Diphterostomum* sp., at SEM. **57.** Sporocyst. **58.** Cercaria. **59.** Oral sucker with papillae. **60.** Spines of ventral body surface. **Abbreviations:** L – lips, OS – oral sucker, P – papilla, VS – ventral sucker. Scale bars = 2 μ m (60), 10 μ m (59), 50 μ m (58), 100 μ m (57)

surpassing anterior edge of ventral sucker. Ventral sucker large and strong muscular, 96 (54–143) long by 106 (69–126) wide, with 4 muscular lips (2 anterior and 2 posterior), sometimes invaginated (Fig. 53). Three pairs of penetration glands (determined by openings of ducts), opening ventrally at stylet base. Immature ovary 23 (18–30) long by 24 (20–30) wide ($n = 5$) at left side of body, between ventral sucker and excretory vesicle; two developing testes, at each side, left testis, 27 (23–32) long by 21 (17–24) wide, right testis, 29 (25–31) long by 19 (17–22) wide ($n = 3$) (Fig. 55). Cirrus pouch incompletely developed, located between intestinal caeca. Genital pore opening at left side of body at pharyngeal level. Excretory vesicle oval, 70 (46–86) long by 59 (44–80) wide, filled with refringent granules (Fig. 55). Excretory formula: $2 [(2+2+2)] = 12$.

Behaviour: Cercariae move actively inside sporocyst and emerge by rupture of the wall. Some cercariae encysted inside the sporocyst were observed. Naturally shed cercariae move rapidly on the bottom of Petri dishes by contracting and stretching the body. Their life-span is less than 24 hs at room temperature, but at lower temperature (10°C) survival is up to 96 hs.

Metacercaria (Fig. 56): Cyst spherical, 269 (245–285) in diameter, wall 9 (6–13) thick.

Host: *Buccinanops globulosus* Kiener (Caenogastropoda, Nassariidae).

Prevalence: 0.16% ($n = 632$, intertidal samples) and 5.1% ($n = 59$, subtidal sample).

Specimens deposited: 6280–6281 (MLP) and 21–23 (CNP-Par).

Comments: The following morphological features define parthenitae here described as belonging to Zoogonidae: tailless xiphidocercariae with large ventral sucker at posterior half of body, cystogenous glands scattered in parenchyma, excretory vesicle saccate filled with excretory granules, 2 opposite testes and a median ovary, cirrus pouch with lateral genital pore. Larvae were identified as belonging to the genus *Diptherostomum* Stossich, 1903 by the presence of 2 short and divergent caeca (Bray 2008). It is similar to *Diptherostomum brusinae*, having four lips on the ventral sucker, which are also present in the adult form described by Pina *et al.* (2009) from northern Portugal.

Discussion

This study reports 4 new records of larval stages of digeneans parasitising the most common intertidal gastropods from the northern Patagonian coast: *Maritrema* sp. 1, *Parorchis* sp., *Renicolidae* gen. et sp. and *Diptherostomum* sp. We also found the larval stages of *Maritrema* sp. 2 and *Hemiuridae* gen. et sp., previously described by Alda and Martorelli (2009) from intertidal gastropods occurring 500 km south of the locale of the present study. In the study area, during the year, both species of *Maritrema* showed the highest overall prevalences

(33.45% and 8.95%), while the remaining species presented lower values: *Hemiuridae* larvae (0.48%), *Parorchis* sp. (0.87%), *Renicolidae* larvae (0.29%) and *Diptherostomum* sp. (0.16%). *Crepidula dilatata* harbours the most prevalent species, *Maritrema* sp. 1. This is the first record of a parasite in gastropods of the genus *Crepidula* (Pechenik *et al.* 2001).

Descriptions of larval digeneans represent a valuable contribution toward the understanding of their life cycles on Patagonian coasts. Both species of *Maritrema* most probably use the kelp gull, *Larus dominicanus*, as definitive host (Diaz 2006). The cercaria of *Maritrema* sp. 2 seems to correspond to the metacercariae found in the crab *Cyrtograpsus altimanus* Rathburn (Grapsidae) (Diaz and Cremonte 2010). From these metacercariae, Diaz and Cremonte (2010) experimentally obtained the adult stage, identified as *Maritrema madrynsensis* Diaz et Cremonte, 2010. The adult corresponding to the larval stages of *Hemiuridae* here studied could belong to one of the nine species reported parasitising fishes from Patagonian coasts (Kohn *et al.* 2007). With regard to the cercaria of *Parorchis* sp., previous worldwide records also reported muricid gastropods as first intermediate hosts (Stunkard and Cable 1932, Cooley 1962). In the study area, adults of *Parorchis acanthus* were found in *L. dominicanus* (Diaz 2006), which may correspond to the larval stages found in *T. geversianus*. At present, there are no records of adult *Renicolidae* from the Southwestern Atlantic coasts. Only metacercarial stages were found in mytilids (*Bivalvia*) on the Patagonian coast (Cremonte 1999), these could correspond to the larval stages here reported in *T. geversianus*. *Diptherostomum* sp. described in the present study from *B. globulosus* seem to belong to *D. brusinae*. In a previous parasitological survey of the Brazilian sandperch *Pinguipes brasilianus* Cuvier (*Pinguipedidae*) from the present study area, Timi *et al.* (2008) cited the presence of adult digeneans identified as *Opecoeliidae* gen. et sp. indet. The examination of additional material revealed that, in fact, these adult digeneans actually belong to *D. brusinae*. In agreement with our findings, the life cycles of *D. brusinae* studied by Palombi (1930, 1934), Prévot (1966) and Pina *et al.* (2009), included nassariid gastropods as first intermediate hosts.

Larval trematodes in intertidal snails were reported by many authors (e.g., Curtis 1990, Galaktionov and Skirnisson 2000, Fredensborg *et al.* 2005). The commonest families found were *Microphallidae*, *Renicolidae*, *Echinostomatidae*, *Philophthalmidae*, *Lepocreadiidae*, and *Zoogonidae* (e.g., Lauckner 1980, Galaktionov and Skirnisson 2000, Fredensborg *et al.* 2005, Russell-Pinto *et al.* 2006, Curtis 2007). We found parthenitae corresponding to six species and to five families, *Microphallidae* (2), *Hemiuridae*, *Philophthalmidae*, *Renicolidae* and *Zoogonidae*. These species use seabirds (*Maritrema* sp. 1 and *Maritrema* sp. 2, *Parorchis* sp. and *Renicolidae*) and fishes (*Hemiuridae* and *Diptherostomum* sp.) as definitive hosts.

The study of digenean life cycles associated with marine coastal areas is particularly important in regions where infec-

tion of intertidal molluscs with digeneans is increasing (e.g., Galaktionov and Skirnisson 2000, Hechinger *et al.* 2008). This phenomenon is related with an increase in the seabird population (mainly gulls) favoured by some human activities (fishing ports, coastal settlements, fish farms, etc.), leading to an increase in parasite pressure on populations of intertidal animals in adjacent coastal regions (Hechinger and Lafferty 2005, Bustnes 2000). However, the high diversity of parasites is an indicator of healthy ecosystems because the conditions to promote the life cycles of digeneans are preserved (Lafferty 1997). In the present case, most of the more prevalent parasites reported upon here, especially the *Maritrema* species, seem to use the kelp gull as definitive host, reflecting the increase in abundances of gull populations at Puerto Madryn city, due to human activities (Yorio *et al.* 2005).

Acknowledgements. The authors thank to Jaime Groizard (SEM unit, ALUAR SA) for the SEM photographs and Estefanía Bagnato for her help in the field. This study was funded by ANPCyT (PICT 1374), PIP (CONICET, to F. Cremona) and N628 (UNLP). Authors are members of CONICET.

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(Accepted January 26, 2011)