

DEVELOPMENT FROM METACERCARIA TO ADULT OF A NEW SPECIES OF *MARITREMA* (DIGENEA: MICROPHALLIDAE) PARASITIC IN THE KELP GULL, *LARUS DOMINICANUS*, FROM THE PATAGONIAN COAST, ARGENTINA

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ABSTRACT: *Maritrema madrynensis* n. sp. is described from the Patagonian coast, Argentina, based on naturally obtained adults from the kelp gull *Larus dominicanus* and cultured metacercariae from the crab *Cyrtograpsus altimanus*. The new species fits into the “eroliae complex” and can be distinguished from other related species mainly in shape, size, and distribution of the spines on the cirrus and vitellarium in a complete ring in all specimens. Based on morphological and morphometric features of the naturally and experimentally obtained specimens and the trophic relationship between invertebrate and vertebrate hosts, we suggest that the crab *C. altimanus* acts as a second intermediate host of *M. madrynensis* n. sp. This is the first report of a *Maritrema* species in Patagonia and the southernmost record for the genus.

Maritrema Nicoll, 1907 (Microphallidae), is a large genus of digenean parasites mainly of marine birds (Yamaguti, 1971; Deblock, 2008). Their life cycles involve gastropods as first intermediate hosts and mainly crustaceans as second intermediate hosts (Deblock, 1971; Yamaguti, 1975). Only 2 species in this genus have been previously reported from Argentinean waters, i.e., *Maritrema bonaerensis* Etchegoin and Martorelli, 1997, from *Larus atlanticus* Olrog, *Larus maculipennis* Lichtenstein, and *Larus dominicanus* Lichtenstein (Etchegoin and Martorelli, 1997; La Sala et al., 2009), and *Maritrema orensensis* Cremonte and Martorelli, 1998, from *L. dominicanus* (Cremonte and Martorelli, 1998) and *L. atlanticus* (La Sala et al., 2009). During a survey of helminth communities in birds from the Argentinean Patagonian coast, a new species of *Maritrema* was found parasitizing the intestine of the kelp gull, *L. dominicanus*. The aims of the present paper are to describe the naturally obtained adult of the new species in the kelp gull from the Patagonian coast, to report the infective metacercaria found in the second intermediate host (the crab *Cyrtograpsus altimanus* [Rathbun] [Crustacea: Grapsidae]) from the same area, and to illustrate its development to the sexual adult stage under controlled conditions.

MATERIALS AND METHODS

From March 2000 to April 2004, 29 kelp gulls, *Larus dominicanus* (Aves: Laridae), were collected from Puerto Madryn coast and adjacent areas (42°04'–42°53'S, 63°21'–65°04'W), Chubut Province, Argentina. The birds were captured using a net, killed with ether, and transferred as soon as possible to the laboratory for dissection. Samples of an adult digenean belonging to *Maritrema* were recovered from the intestine. The specimens were fixed in 5% hot formalin, stored in 70% ethanol, stained with Semichon's acetocarmine, cleared in methyl salicylate, and mounted in Canada balsam.

In addition, during May 2007, May 2008, and April 2009, specimens of *C. altimanus* (n = 60) were collected at Punta Cuevas, Puerto Madryn (42°46'S, 65°02'W), Chubut Province, Argentina. The crabs were collected from tidal pools during low tides and transferred to an aquarium at 10°C with aerated seawater. Some of the encysted metacercariae collected from the body cavities were studied alive using light microscopy, whereas the rest were placed in small Petri dishes containing physiological solution and incubated in vitro at 39°C. They were observed at different time intervals to study their development to the sexually mature adult stage. The specimens were

observed alive, stained with neutral red and Nile blue. Metacercariae and incubated specimens at different developmental stages were fixed and stained as above. Illustrations were made with the aid of a drawing tube. Dimensions, measured on mounted specimens, are given in micrometers, with the mean value followed by the range in parentheses. The forebody was measured from the anterior extremity to the anterior margin of the ventral sucker. Some fixed adult specimens collected from the gulls were dehydrated, critical point dried, gold coated, and observed and photographed using a JEOL JSM 6360 LV (Jeol, Tokyo, Japan) scanning electron microscope (SEM).

DESCRIPTION

Maritrema madrynensis n. sp.

(Figs. 1–8)

Diagnosis (naturally obtained adults; measurement based on 10 specimens): Body linguiform, 535 (400–690) long by 309 (235–390) wide at ventral sucker level (Fig. 1). Spines transversally arranged covering entire body surface (Figs. 3–6). Anterior spines multidentated (Figs. 3–4), wider and shorter than posterior ones (Figs. 5–6). Oral sucker subterminal, 53 (40–62) long by 48 (35–61) wide. Two rows of 4 papillae surrounding anterior border of oral sucker and 4 single papillae forming a quadrant between the others (Fig. 7). Ventral sucker 59 (50–75) long by 51 (43–60) wide, located in second third of body. Forebody 257 (215–320). Sucker ratio (ventral sucker/oral sucker width) 1.14 (0.95–1.71). Prepharynx 36 (18–50) long. Pharynx 37 (30–42) long by 25 (21–30) wide. Esophagus 48 (25–75) long. Intestinal ceca reaching middle of ventral sucker. Testes postovarian, lateral, symmetrical, oval with lobed edges. Right testis 63 (55–75) long by 84 (60–120) wide; left testis 62 (50–75) long by 87 (60–120) wide. Vasa deferentia joining anterior region of seminal vesicle. Cirrus sac shaped as inverted “J,” 235 (147–300) long by 66 (55–75) maximum wide (Figs. 2, 3); located between intestinal ceca and ventral sucker; muscular wall strongly developed. Cirrus sac enclosing oval seminal vesicle, 85 (62–110) long by 52 (48–55) wide; numerous prostatic cells surrounding curled ejaculatory duct, conspicuous pars prostatica, and spinous cirrus (Figs. 2, 3). Invaginated cirrus 126 (100–155) long, 25 (20–25) wide. Spines at base of cirrus (proximal ones) triangular and larger than conical spines at tip (distal ones) (7–8 vs. 5–6, respectively). Ovary submedian, postacetabular, trilobed, partially displaced at right and covered by posterior border of ventral sucker; 55 (40–65) long by 93 (75–110) wide. Oviduct short. Seminal receptacle present. Ootype and Mehlis' gland situated immediately posterior to ventral sucker (Fig. 2). Laurer's canal present. Metraterm thick walled and diverticulated. Genital pore conspicuous, simple, slightly differentiated, and unarmed, located at left side of ventral sucker. Uterus occupying most of hindbody, from posterior border of cirrus sac and anterior border of testes to the end of body. Eggs numerous, operculated, 20 (18–23) long by 11 (10–12) wide (Fig. 3). Vitellarium with numerous small follicles forming 2 symmetrical ribbons, confluent posteriorly, enclosing uterus and testes. Excretory vesicle “Y” shaped. Excretory formula: $2[(2+2)+(2+2)] = 16$.

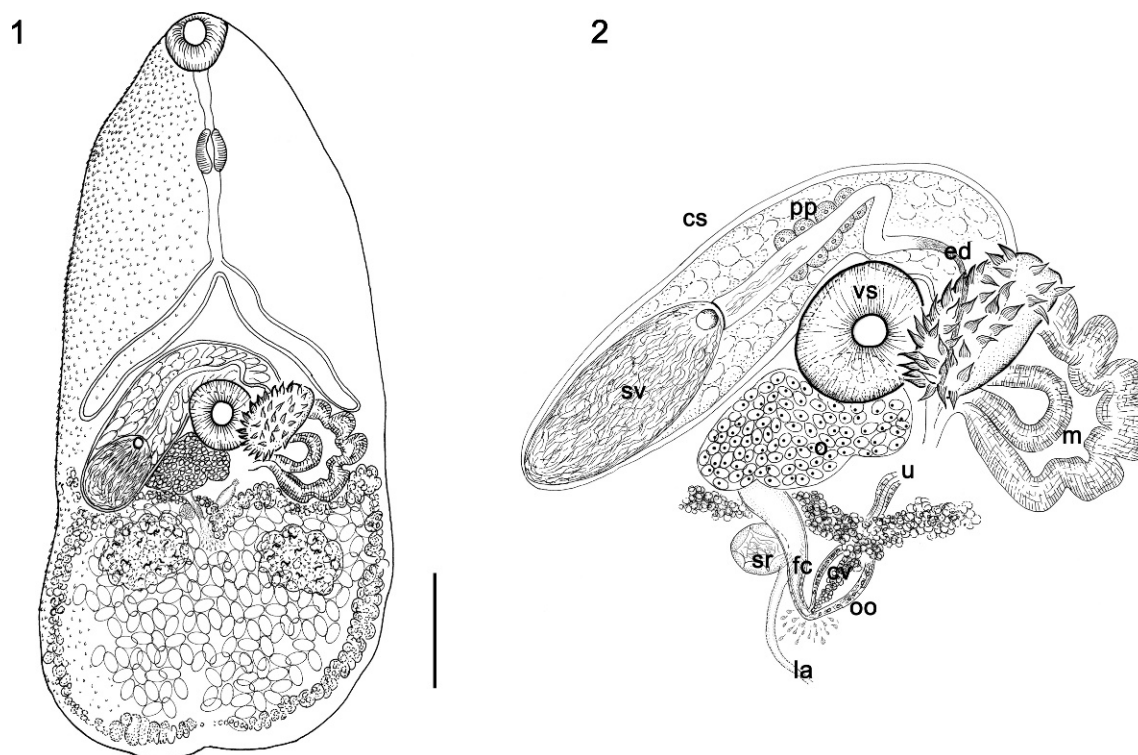
Taxonomic summary

Type host: *Larus dominicanus* Lichtenstein (Aves: Laridae).

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FIGURES 1–2. *Maritrema madrynensis* n. sp. (1) Naturally obtained adult, ventral view. (2) Genital system, ventral view. Abbreviations: cs, cirrus sac; cv, common vitelline duct; ed, ejaculatory duct; fc, fertilization chamber; la, Laurer's canal; m, metraterm; o, ovary; oo, ootype; pp, pars prostatica; sr, seminal receptacle; sv, seminal vesicle; u, uterus; vs, ventral sucker. Scale bars: (1) = 100 μ m; (2) = 50 μ m.

Type locality: Puerto Madryn coast (42°46'S, 65°02'W), Chubut Province, Argentina.

Site of infection: Small intestine.

Prevalence: 44.8%.

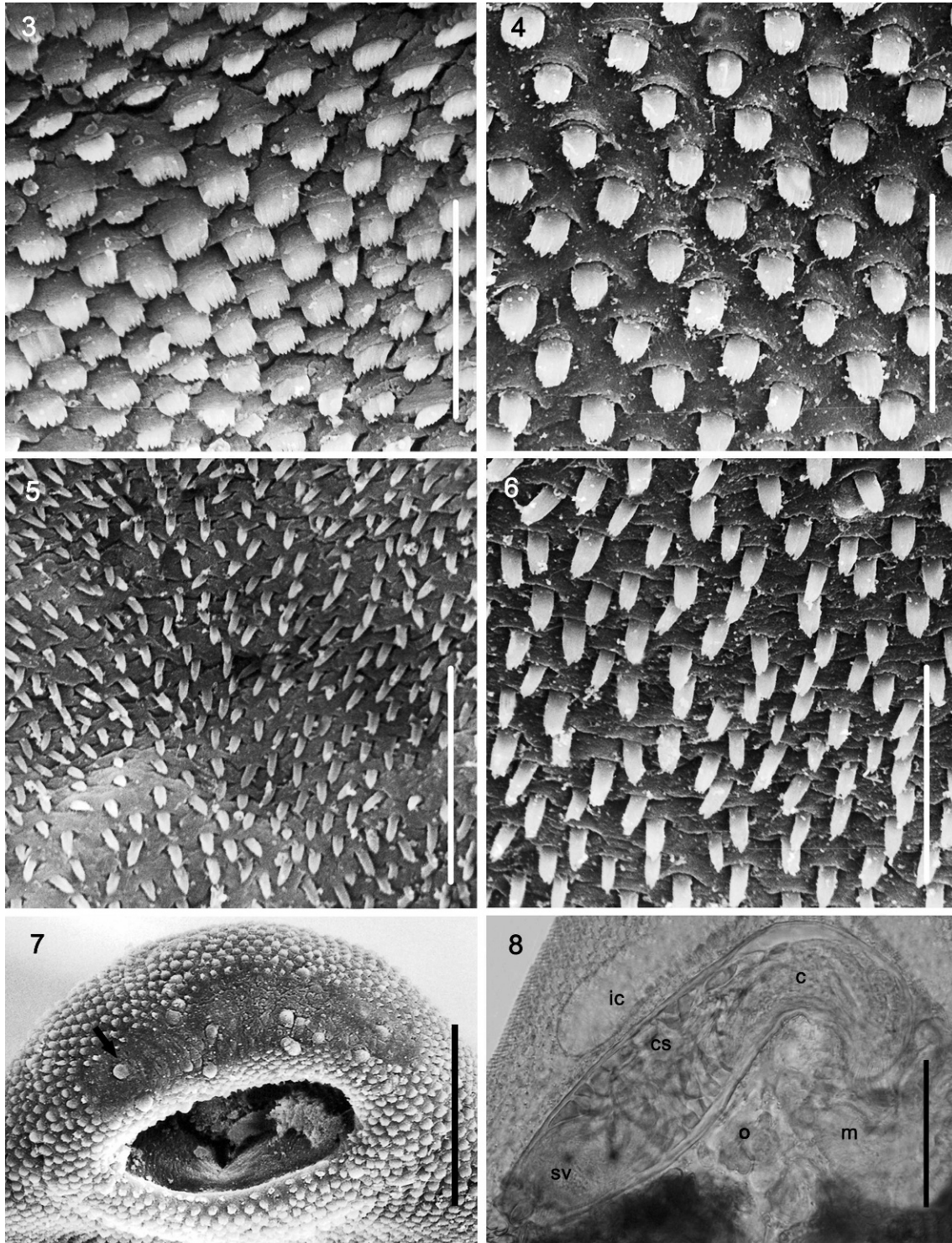
Specimens deposited: Holotype no. 6087 and paratypes no. 6088 (stained whole-mounts of naturally obtained adults), voucher specimens nos. 6089, 6090 (stained whole-mounts of infective metacercariae and experimentally obtained adults) in the Helminthological Collection of Museo de La Plata (CHMLP), Museo de La Plata, La Plata, Argentina.

Etymology: The specific name is derived from the name of the city (Puerto Madryn) where some hosts were collected and the work was carried out.

Remarks

According to the classification of microphallid digeneans proposed by Deblock (1971, 2008), the new species can be included in the genus *Maritrema* Nicoll, 1907, and in the subgenus *M.* (*Maritrema*) by the presence of uterine coils postcaecal; vitellarium in symmetrical ribbons surrounding uterine coils and testes; a cirrus sac enclosing an evaginable cirrus like a glove finger turned inside out; a simple, superficial, and unarmed genital pore; and the absence of a spined acetabulo-atrial plate. The classification and identification of the species in the genus are confusing because of the great number of described species and the homogeneity of their features (Deblock, 1971). The presence of a spinous cirrus enclosed in a cirrus sac places the new species with the "eroliae complex" proposed by Deblock and Canaris (1992), parasitizing mainly Charadriiformes. Within this complex are grouped several species, difficult to distinguish from each other, and very similar to *Maritrema eroliae* Yamaguti, 1939. At present, the "eroliae complex" includes *Maritrema misenensis* (Palombi, 1940); *M. eroliae* (syn.: *M. urayensis* Ogata, 1951, *M. magnicirrus* Belopolskaya, 1952, and *M. kitanensis* Shibue, 1953); *Maritrema echinocirrata* Leonov, 1958; *Maritrema interrupta* Oshmarin, 1970; *Maritrema borneoense* Fischthal and Kuns, 1973; *Maritrema minuta* Ke, 1976; *Maritrema papillorobusta* Ke, 1976; *Maritrema jilinensis* Zhong, 1988; *Maritrema spinosulum* Deblock and Canaris, 1996; *Maritrema*

rubeum Deblock and Canaris, 1996; and *Maritrema novaezealandensis* Martorelli, Fredensborg, Mouritsen, and Poulin, 2004 (Coil, 1955; Deblock, 1971, 1975a, 1975b; Werdnig, 1973; Prévot et al., 1976; Deblock and Canaris, 1992, 1996; Gracenea et al., 1993; Martorelli et al., 2004). Moreover, the "eroliae complex" is divided into 3 subgroups according to the body size, morphology of the vitellarium, and geographical distribution, i.e., group A = species smaller than 400 μ m and horseshoe-shaped vitellarium; group B = species larger than 600 μ m and the vitellarium in a complete or subcomplete ring; and group C = some species with intermediate features between A and B (Deblock and Canaris, 1992). In considering this classification, *Maritrema madrynensis* n. sp. is closely related to group "C," which includes *M. misenensis*, *M. eroliae* (sensu Deblock and Pearson, 1968), *M. interrupta*, and *M. papillorobusta*. The new species differs from all mentioned species by the arrangement of the cirrus spines, by having a vitellarium in a complete ring (Prévot, et al., 1976; Deblock and Canaris, 1992, 1996), and by its Neotropical distribution. *Maritrema madrynensis* n. sp., differs also from *M. eroliae* studied by Deblock and Canaris (1968) by the development of the vitellarium (complete vs. horseshoe-shaped, subcomplete, or complete) and because the new species has smaller proximal cirrus spines (7–8 vs. 8–9) and greater distal ones (5–6 vs. 1.5–2). Some of studied specimens in this work exceed 600 in length; although all of them show a vitellarium in a complete ring, they could be related with group "B." *Maritrema madrynensis* n. sp. differs from *M. echinocirrata* Leonov, 1958, by the smaller size (400–690 vs. 670–850) and by the distribution and morphology of cirrus spines (Deblock, 1975a; Deblock and Canaris, 1992). Also, in this group were included several specimens determined, as *M. eroliae* on the base of metacercariae recovered from crustaceans in Asia and the Pacific Ocean (Deblock and Canaris, 1992). The size, vitellarium, arrangement of cirrus spines (when known), and/or the geographical distribution of these metacercariae place them away from present specimens. Deblock and Canaris (1992) have expressed doubts regarding the correct identification of the specimens reported as *M. eroliae* sensu stricto. The variability found among the specimens reported as *M. eroliae* and the fact that many reports and descriptions belong to metacercariae or immature specimens make the correct comparison of the new species with

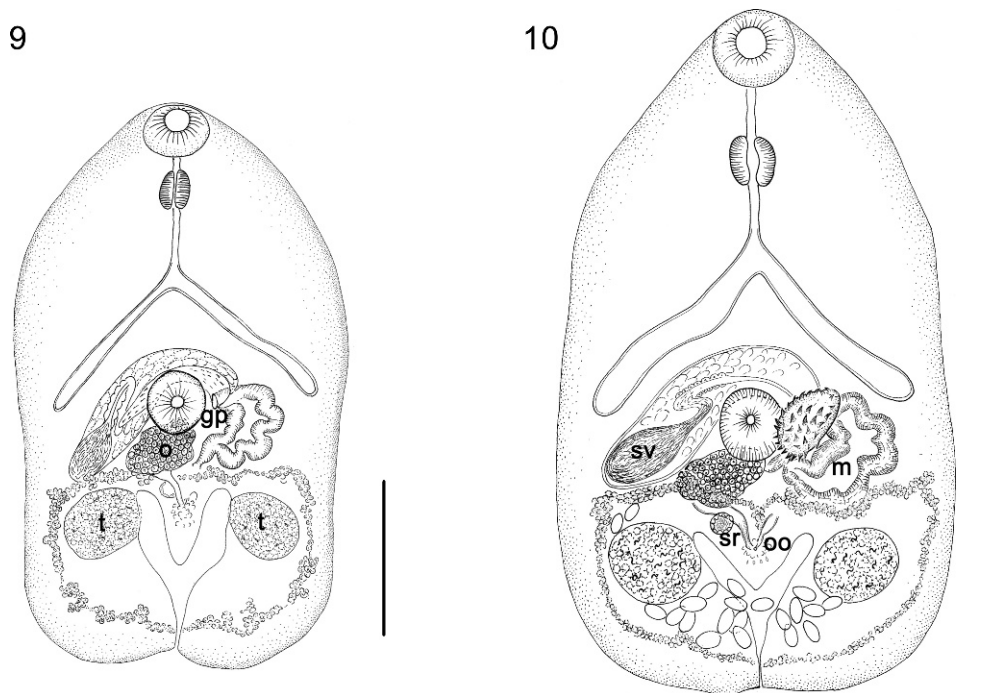


FIGURES 3–8. *Maritrema madrynsensis* n. sp. (3–6) Body spines (SEM). (3) Preacetabular ventral spines. (4) Preacetabular dorsal spines. (5) Postacetabular ventral spines. (6) Postacetabular dorsal spines. (7) Oral sucker (OS) at SEM, showing anterior papillae (arrow). (8) Cirrus sac, ventral view. Abbreviations: c, cirrus; cs, cirrus sac; ic, intestinal ceca; m, metraterm; o, ovary; sv, seminal vesicle. Scale bars: (3–6) = 20 μ m; (7) = 50 μ m; (8) = 50 μ m.

this species difficult. *Maritrema madrynsensis* n. sp. can be distinguished from other species using a combination of features like size, shape of vitellarium, shape and distribution of cirrus spines, and geographical distribution.

Development from metacercaria to adult (Figs. 9–14)

Infective metacercaria (20 specimens measured, from the body cavity of *C. altimanus*) appear within an oval cyst, 310 (260–350) long by 260 (210–



FIGURES 9–10. *Maritrema madrynensis* n. sp. (9) Juvenile, obtained after 24 hr of metacercaria cultured in vitro. (10) Adult specimen, obtained after 44 hr of metacercaria cultured in vitro. Abbreviations: c, cirrus; gp, genital pore; m, metraterm; o, ovary; oo, oötype; sr, seminal receptacle; sv, seminal vesicle; t, testis. Scale bars: 100 µm.

320) wide. The wall of the cyst consists of 2 layers; the internal is 6 (4–8) thick, and the outer is 7 (4–12) thick. Cirrus and metraterm are completely developed in the metacercaria within the cyst. Also the intestinal ceca are well developed (Fig. 11).

After less than 30 min in vitro at 39 C, metacercariae had escaped from their envelope using the anterior end. After 60 min, in vitro excysted metacercariae increased their movements greatly and the oral sucker attached to the bottom of the Petri dish, while the body continued contracting. Most structures of the adults were visible at this time, including testes, ovary, cirrus sac, seminal vesicle, and a rudimentary vitellarium. By 24 hr, metacercariae enlarged in size and closely resembled the adult, having the vitellarium in a complete ring and all sexual features well developed. The testes possessed active spermatozoa, although the seminal vesicle and common vitelline duct were not completely full. The specimens measured 413 (380–440) long by 246 (230–260) wide (Fig. 9).

At 36 hr, a fully visible excretory vesicle containing excretion granules was observed, and the seminal vesicle and common vitelline duct were completely full. Several specimens possessed 1–22 eggs in the uterine coils (Fig. 10) by 40–44 hr. The specimens measured 432 (340–540) long by 239 (200–280) wide. In spite of a slight difference in size, there were no significant morphological differences between adults obtained from naturally infected hosts and those obtained under experimental conditions (Figs. 12–14).

DISCUSSION

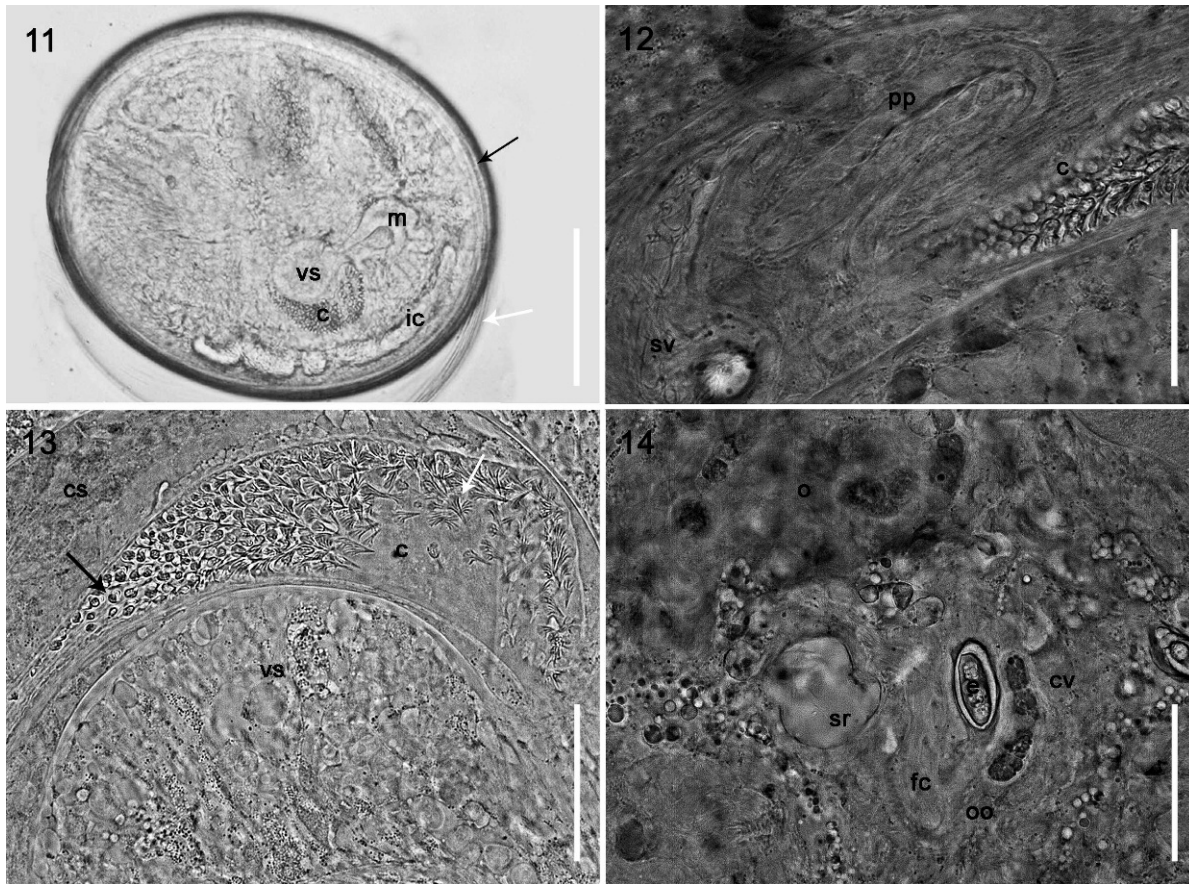
A large number of species of *Maritrema* have been reported parasitizing different hosts around the world (e.g., Yamaguti, 1971; Deblock, 1971; Deblock and Canaris, 1992; Gracenea et al., 1993; Tkach, 1998; Martorelli et al., 2004). In contrast, only a few species have been reported in South American birds, i.e., *Maritrema nicolli* Travassos, 1920; *Maritrema bravoae* Caballero and Ibáñez, 1970; *Maritrema magdalenae* Werdnig, 1973; *M. bonaerensis*; and *M. orensensis* (see Caballero and Ibáñez, 1970; Deblock, 1971; Etchegoin and Martorelli, 1997; Cremonte and Martorelli, 1998). However, none of them belong to the “eroliae

complex,” and only *M. bonaerensis* and *M. orensensis* have been reported parasitizing birds of the genus *Larus* in Argentina (Lunaschi et al., 2007). These 2 species were found in *L. dominicanus* from the Buenos Aires Province coast, located 1,500 km north of Península Valdés (Cremonte and Martorelli, 1998; Cremonte et al., 1999), and belong to the “acadiae complex,” characterized by the absence of spines in the cirrus. This is the first report of *Maritrema* in Patagonia and the southernmost report for the genus.

Based on the experimental development study described above, on the diet preferences of *L. dominicanus* in nearby areas (Yorio and Bertellotti, 2002), and on the prey items observed in the stomach of analyzed gulls, it is suggested that infected crabs *C. altimanus* are ingested by the kelp gull, which acts as definitive host; inside it the metacercariae of *Maritrema madrynensis* n. sp. excyst quickly and rapidly develop into egg-producing adults. Several other species of *Maritrema* have been reported to use decapods as intermediate hosts (Yamaguti, 1975; Schell, 1985). The transmission of *M. madrynensis* n. sp. is consistent with previous reports of other species of *Maritrema* (Etchegoin and Martorelli, 1997; Martorelli et al., 2004).

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FIGURES 11–14. *Maritrema madrynensis* n. sp. (11) Infective metacercaria from crab *Cyrtograpsus altimanus*, showing internal and external layers (black and white arrows, respectively). (12–14) Cultured adult, ventral view. (12) Detail of cirrus sac, showing invaginated cirrus, pars prostatic, and fully seminal vesicle. (13) Detail of invaginated cirrus, showing proximal and distal spines (white and black arrows, respectively). (14) Female genital system. Abbreviations: c, cirrus; cs, cirrus sac; cv, common vitelline duct; e, egg; fc, fertilization chamber; ic, intestinal ceca; m, metraterm; o, ovary; oo, oötype; pp, pars prostatica; sr, seminal receptacle; sv, seminal vesicle; vs, ventral sucker. Scale bars: (11) = 100 μ m; (12–13) = 25 μ m; (14) = 40 μ m.

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