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Systematic Parasitology
An International Journal

ISSN 0165-5752
Volume 78
Number 3

Syst Parasitol (2011)
78:233-240
DOI 10.1007/
s11230-010-9285-4



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A new species of monorchiid digenean from marine fishes in the Southwestern Atlantic Ocean off Patagonia

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Received: 3 September 2010 / Accepted: 2 December 2010
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Abstract *Proctotrema bartolii* n. sp. (Digenea: Monorchiidae) is described based on naturally and experimentally obtained adults from the marine fishes *Odontesthes smitti* (Lahille), *O. nigricans* (Richardson) (Atherinopsidae) and *Eleginops maclovinus* (Cuvier) (Eleginopidae) off Patagonia, Argentina, in the Southwestern Atlantic Ocean. Its generic identification is based on the presence of a unipartite terminal organ with the metraterm uniting with its distal region, an unarmed genital atrium, a single testis, a vitellarium follicular lateral to the ovary and ventral sucker, and uterine coils occupying most of the hindbody. The new species differs from *P. bacilliovatum* Odhner, 1911, *P. amphitruncatum* Fischthal & Thomas, 1969 and *P. guptai* Ahmad & Dhar, 1987 in having a smaller body (305–650 vs 1,600–3,080, 1,500–1,800 and 2,150–2,670 μm , respectively), a round vs funnel-shaped oral sucker, a smooth vs lobed ovary, a saccular rather than tubular excretory vesicle, the number of vitelline follicles (12–16 vs 8–9, 9 and 6–8, respectively), and wider eggs

(25–31 \times 15–20 vs 28–37 \times 9–12, 24–28 \times 7–10, and 24–30 \times 8–10 μm , respectively). Moreover, the new species differs from *P. bacilliovatum* and *P. amphitruncatum* in having a saccular rather than a coiled seminal vesicle, and from *P. bacilliovatum* and *P. guptai* in having its tegument completely vs partly spined. *Proctotrema* Odhner, 1911 is considered to be restricted to these four species. This is the first report of a species of this genus from South American waters.

Introduction

In the Southwestern Atlantic Ocean, Antarctic and sub-Antarctic waters, 18 species of monorchiid digeneans have been recorded parasitising marine fishes (Szidat, 1950; Gosztanyi, 1979; Amato, 1982; Knoff & Amato, 1991; MacKenzie, 1997; Zdzitowiecki, 1997; Kohn et al., 2007). Only two of these have been recorded from off the Argentinean coast, i.e. *Monorcheides popovicii* Szidat, 1950 and *Postmonorcheides maclovini* Szidat, 1950, both parasites of *Eleginops maclovinus* (Cuvier) (Eleginopidae) (Szidat, 1950; Gosztanyi, 1979; MacKenzie, 1997).

Five species of *Proctotrema* Odhner, 1911 have been previously described from marine fishes: *P. bacilliovatum* Odhner, 1911 (type-species) in *Mullus barbatus* L. and *M. surmuletus* L. in the Mediterranean Sea (Gulf of Marseille and off Trieste); *P. amphitruncatum* Fischthal & Thomas, 1969 in *Pomadasys jubelini* (Cuvier & Valenciennes) from

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off the Cape Coast, Ghana; *P. odhneri* Ramadan, 1985 in *Rhabdosargus haffara* (Forsskål) (syn. *Sparus haffara*) from the Red Sea, *P. guptai* Ahmad & Dhar, 1987 in *Pomadasys maculatus* (Bloch.) from the Puri Coast, Bay of Bengal, and *P. pritchardae* Nahhas & Cable, 1964 in *Haemulon album* (Cuvier) and *Plectorhynchus cinctus* (Temminck & Schlegel) (Haemulidae) from off Curaçao and Jamaica and off the coast of the Emirates (Nahhas & Cable, 1964; Bartoli & Prévot, 1966; Fischthal & Thomas, 1969; Ramadan, 1985; Ahmad & Dhar, 1987; Kardousha, 2003). The validity of two of these species is discussed below.

The aim of this paper is to describe the adult of a new species of *Proctotrema* (Monorchiidae) from the silversides *Odontesthes smitti* Lahille and *O. nigricans* Richardson (Atherinopsidae) and the Patagonian mullet *Eleginops maclovinus* Cuvier (Eleginopidae) from off the Patagonian coast, Southwestern Atlantic Ocean, obtained under both experimental and natural conditions.

Materials and methods

A total of 356 adult specimens of *Odontesthes smitti* were caught between April, 2003 and February, 2005 off the Puerto Madryn coast, Nuevo Gulf (NG) (42°47'S, 65°02'W) (n = 183; mean total length 24.3 ± 16.26 cm) and off Larralde beach, San José Gulf (SJG) (42°25'S, 64°07'W) (n = 173; mean total length 23 ± 3.6 cm), Southwestern Atlantic Ocean. Additionally, during 2003, 105 specimens of *O. nigricans* (mean total length 16.6 ± 1.48 cm) were collected from NG and 20 specimens (mean total length 16.3 ± 2.8 cm) from SJG, plus 5 specimens of *Eleginops maclovinus* from SJG and 19 specimens from NG. Fishes were slit open prior to being fixed with 5% formalin and then examined in the laboratory. Fixed immature and gravid digeneans were collected from the stomach, intestine and pyloric caeca, stored in 70% ethanol, stained with Semichon's acetocarmine, dehydrated through an alcohol series, mounted in Canada balsam and studied using a light microscope. Some fishes were examined while fresh, and the digeneans obtained were studied *in vivo* with neutral Red, killed with hot physiological solution, fixed in 5% formalin, stored in 70% alcohol and stained as indicated above.

Additionally, 5 specimens of the silversides *O. smitti*, 12 of *O. nigricans* (11–13.5 cm in total length) and 9 of the mullet *E. maclovinus* (1 specimen 20 cm long and 8 specimens 11.5–15 cm in total length) were caught using coastal nets at Puerto Madryn (NG), the locality where the natural infection with the new taxon had the lowest prevalence (pers. obs.). Fishes were kept in aquaria at 10°C for a month prior to experimental infection. This period would allow the discrimination of any specimens resulting from natural infections (expected to be larger and have many eggs) from experimental ones (immature or young adults with few eggs). Fishes were fed daily with VitaFish®. For experimental infections, siphons of the clam *Darina solenoides* (King) (Mactridae) from SJG (100% infected with monorchiid metacercariae, unpublished data) were cut into pieces and placed in the fish pharynx with the aid of a syringe. The smallest fishes (for which this procedure was not possible) were maintained without food for two days and then offered only live, infected clams as food. These fishes were observed to feed on the clams. Each week, one silverside and one mullet were killed and examined for helminths. The digeneans obtained were studied *in vivo* with neutral Red, then killed, fixed, stored and stained as indicated above. These experimental specimens were used for the description.

Drawings were made with the aid of a drawing tube. Figures were based on fixed specimens, except where otherwise indicated. Prevalence (P) and mean intensity (MI) were calculated according to Bush et al. (1997). Only ovigerous specimens were used for the measurements, which are given in micrometres, with the mean followed by the range in parentheses. The terminology for the distal and proximal parts of the terminal organ is according to Madhavi (2008).

Proctotrema bartolii n. sp.

Type-host: *Odontesthes smitti* (Lahille) (Atherinopsidae).

Other hosts: *Odontesthes nigricans* (Richardson) (Atherinopsidae) and *Eleginops maclovinus* (Cuvier) (Eleginopidae).

Site of infection: Stomach, intestine (*Odontesthes* spp.) and pyloric caeca (*Eleginops maclovinus*).

Type-locality: San José Gulf (42°25'S, 64°07'W), Southwestern Atlantic Ocean, off Chubut Province, Argentina.

Other locality: Nuevo Gulf (42°47'S, 65°02'W), Southwestern Atlantic Ocean, off Chubut Province, Argentina.

Prevalence and mean intensity: See Table 1.

Type-material: Holotype No. 5881, paratypes No. 5882 and vouchers from other hosts Nos. 5988, 5989, 5990 and 5991, Helminthological Collection of the Museo de La Plata (MLP), La Plata, Argentina. Voucher specimens, CNP-Par 10, Parasitological Collection of the Centro Nacional Patagónico.

Etymology: The specific epithet is for Professor Pierre Bartoli in recognition of his contributions to the taxonomy and systematics of digeneans, and especially of monorchids.

Description (Figs. 1–2; Table 2)

[Measurements based on 7 whole-mounted experimental gravid specimens from *Odontesthes smitti*; Table 2 presents measurements from specimens obtained naturally from *O. smitti* and from the other hosts.] Body small, oval, 497 (370–591) in length. Maximum width at ventral sucker level, 192 (165–240), tapering towards posterior end (Fig. 1A). Body length/width ratio 1:2.6 (1:2.1–1:3). Tegument covered by spines. Eyespots absent. Oral sucker subterminal, rounded, 74 (60–80) in length, 70 (65–75) in width. Ventral sucker equatorial or pre-equatorial in fully gravid specimens, rounded, 66 (60–70) in length, 61 (50–70) in width. Sucker ratio (oral sucker length/ventral sucker length) 1:1 (1:0.9–1:1.3). Forebody (distance from anterior end to anterior margin of ventral sucker) 166 (120–215). Forebody as % of body length 34.1 (32.4–36.4)%.

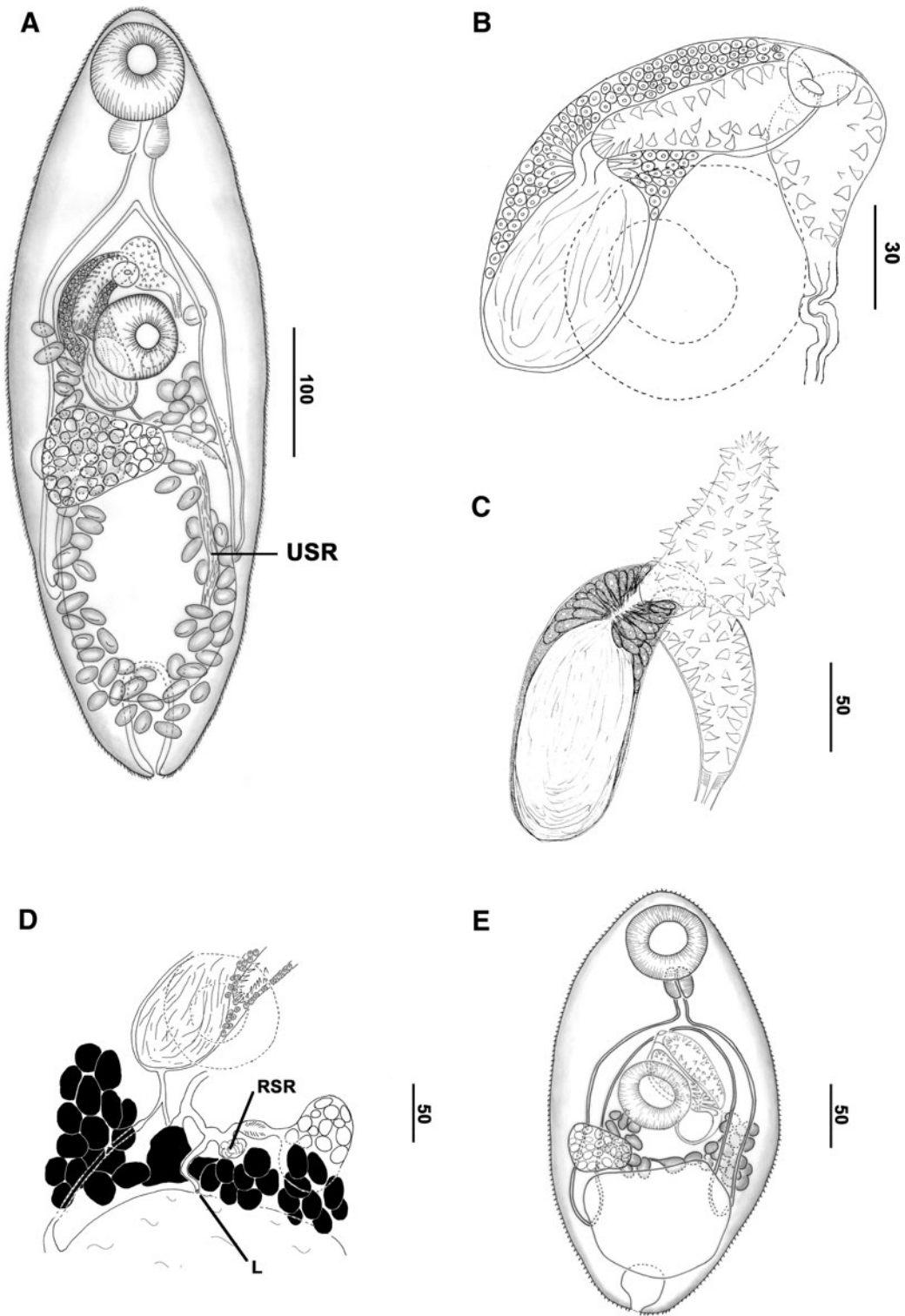
Prepharynx absent. Pharynx 36 (30–45) in length, 33 (25–40) in width (n = 4). Oesophagus short, 25 (20–30) in length (n = 2). Intestinal bifurcation anterior to ventral sucker. Caeca broad, thick-walled, pass lateral to ventral sucker, reaching to middle or posterior level of testis.

Testis single, longitudinally elongate, entirely in hindbody, occupying middle and posterior thirds of body, 168 (100–211) in length, 120 (90–140) in width (n = 5). Cirrus-sac dextral, 103 (70–140) in length, 40 in width (n = 4), overlapping ventral sucker and surpassing it. Seminal vesicle saccular, 58 (50–65) in length, 31 (25–38) in width (n = 3). Prostatic cells numerous. Cirrus tubular, 59 (40–70) in length (n = 3), armed with numerous conical spines 6–8 in length; spines at the tip of everted cirrus acicular, 5–7 in length (Fig. 1B–C). Genital atrium lacks spines. Genital pore median, slightly anterior to ventral sucker. (Figs. 1B, 2).

Ovary spherical to roughly subtriangular, 75 (70–80) in length, 83 (75–90) in width (n = 3), dextral, pretesticular or slightly overlapping anterior margin of testis. Both rudimentary and uterine seminal receptacles present. Rudimentary seminal receptacle close to internal side of ovary, small, filled with spermatozoa, visible only in live specimens (Fig. 1D). Uterine seminal receptacle observed on left side of testis, full of spermatozoa (Fig. 1A). Laurer's canal present (observed *in vivo*) (Fig. 1D). Uterine coils occupy most of hindbody, extending beyond posterior margin of testis, dorsal and ventral to testis and ovary. Terminal organ unipartite, elongate, 53 (50–55) in length, 30 (20–40) in width (n = 3), spinous; spines conical, 9–10 in length, more robust than those of cirrus. Metratrem enters distal part of terminal organ (Figs. 1B–C, 2). Vitellarium composed of 2 lateral groups of 12–16 follicles, extending from level of mid-ventral or posterior

Table 1 Prevalence (P) and mean intensity (MI) of *Proctotrema bartolii* n. sp.

Host	Nuevo Gulf Natural infection		San José Gulf Natural infection		Experimental infection	
	P	MI	P	MI	P	MI
<i>Odontesthes smitti</i>	7.7%	218	14.5%	321.6	25%	14
<i>O. nigricans</i>	3.8%	3	23.8%	12.4	–	–
<i>Eleginops maclovinus</i>	–	–	100%	46	90%	8.8



◀ **Fig. 1** *Proctotrema bartolii* n. sp. A. Gravid adult, ventral view (holotype No. 5881 (MLP) from *Odontesthes smitti* in San José Gulf, Argentina); B. Terminal genitalia, ventral view (for clarity, the ventral sucker is marked with a dashed line); C. Terminal genitalia, ventral view, detail of everted cirrus (for clarity, the ventral sucker is marked with a dashed line); D. Detail of the female reproductive organs, dorsal view (*in vivo*); E. Immature specimen, ventral view. *Abbreviations*: L, Laurer's canal; RSR, rudimentary seminal receptacle; USR, uterine seminal receptacle

margin of ventral sucker to anterior margin of testis; follicles 29.3 (25–38) in length, 23.2 (20–30) in width. Central vitelline reservoir prominent. Eggs large, elliptical, brownish in colour, operculate, unfilamented, 28.6 (25–31) in length, 16.5 (15–20) in width ($n = 20$).

Excretory vesicle saccular, 60 (40–70) in length, 32 (25–40) in width ($n = 3$). Excretory pore terminal.

Remarks

A combination of characters, the unipartite terminal organ of the terminal genitalia, with the metraterm joining its distal region, an unarmed genital atrium, a single testis, a follicular vitellarium lateral to the ovary and ventral sucker, and uterine coils occupying most of hindbody, place these specimens in *Proctotrema* Odhner, 1911 (see Madhavi, 2008).

The new species can be differentiated from the known local monorchiids, *Postmonorcheides maclovini* and *Monorcheides popovicii*, mainly by having a single testis (*vs* two testes) and a smooth (*vs* a trilobed) ovary (Szidat, 1950).

Proctotrema bartolii n. sp. differs from *P. bacilliovatum*, *P. amphitruncatum* and *P. guptai* by having: a round (*vs* funnel-shaped) oral sucker; a smooth (*vs* lobed) ovary, a saccular (*vs* tubular) excretory vesicle, a smaller body (305–650 *vs* 1,600–3,080, 1,500–1,800 and 2,150–2,670 μm , respectively), a greater number of vitelline follicles (12–16 *vs* 8–9, 9 and 6–8, respectively), and broader eggs (25–31 \times 15–20 *vs* 28–37 \times 9–12, 24–28 \times 7–10 and 24–30 \times 8–10 μm , respectively). Additionally, the new species differs from *P. bacilliovatum* and *P. amphitruncatum* by having a saccular rather than a coiled seminal vesicle, and from *P. bacilliovatum* and *P. guptai* by having a completely *vs* partly spined tegument. Table 2 shows the comparative measurements of the species of *Proctotrema*.

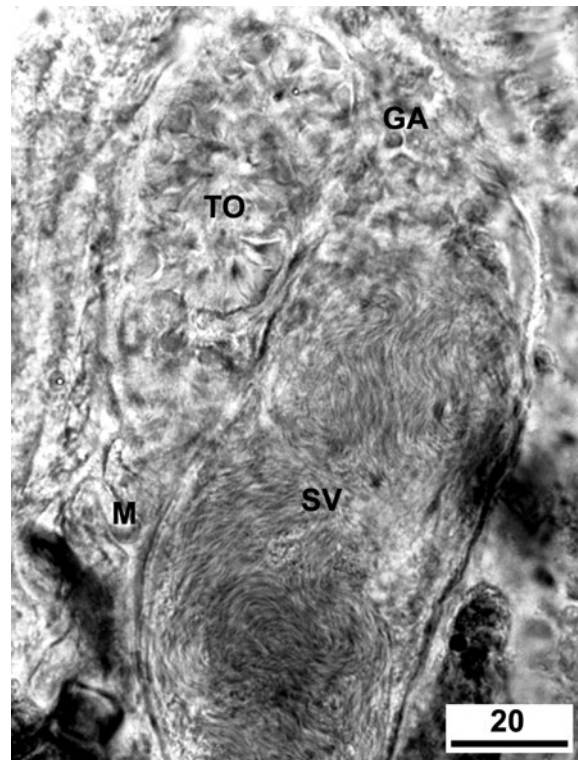


Fig. 2 Details of the terminal genitalia of *Proctotrema bartolii* n. sp. (dorsal view). *Abbreviations*: GA, genital atrium; M, metraterm; SV, seminal vesicle; TO, terminal organ

Discussion

In this paper we describe a new species of *Proctotrema* on the basis of specimens obtained from the silversides *Odontesthes smitti* and *O. nigricans* and from the Patagonian mullet *Eleginops maclovinus*, both naturally and experimentally infected, from Patagonian waters off Argentina. This finding represents the first record of *Proctotrema* from off the coast of South America.

Following the key of Madhavi (2008), the specimens studied here appear to belong to *Postmonorchis* Hopkins, 1941 in view of the posterior position of the testis. However, *Proctotrema* can be differentiated from *Postmonorchis* by the presence of a unipartite terminal organ, an unarmed genital atrium, uterine coils filling pre- and post-testicular regions, an elongate testis occupying the middle and posterior thirds of body and a vitellarium in the form of symmetrical bunches of follicles lateral to the ovary and ventral sucker.

Table 2 Comparative measurements of *Proctotrema* spp., with hosts, localities and data-sources. Measurements are given in micrometres except where otherwise stated

Species	<i>P. bacilliovatum</i> Odhner, 1911	<i>P. amphitruuncatum</i> & Thomas, 1969	Fischthal	<i>P. guptai</i> Ahmad & Dhar, 1987	<i>P. bartolii</i> n. sp.
Host(s)	<i>Mullus barbatus</i> and <i>M. surmuletus</i> (Mullidae)	<i>Pomadasyx jubelini</i> (Pomadasyidae)		<i>Pomadasyx maculatus</i> (Pomadasyidae)	<i>Eleginops maclovinus</i> (Eleginopsidae)
Locality(-ies)	Gulf of Marseille and off Trieste	Cape Coast, Ghana		Puri Coast, Bay of Bengal	Chubut, Argentina
Reference	Bartoli & Prévot (1966)	Fischthal & Thomas (1969)		Ahmad & Dhar (1987)	Natural specimens (Present study)
n	12	5	7	22	7
Body length	1.6–3.08 mm	1.5–1.8 mm	2.15–2.67 mm	411 (305–600)	505 (390–600)
Maximum width	360–800	240–315	470–560	171 (140–223)	246 (155–370)
Oral sucker length	220–296	226–235	280–330	86 (69–110)	90 (70–120)
Oral sucker width	114–220	182–252	200–230	94 (78–130)	91 (65–110)
Ventral sucker length	128–152	92–123	130–150	79 (70–100)	89 (50–115)
Ventral sucker width	90–164	111–121	130–150	77 (60–100)	84 (50–100)
Sucker-ratio	1:1.28–1:1.97	1:0.4–1:0.53	1:0.45–1:0.46	1:1 (1:0.9–1:1.3)	1:1 (1:0.7–1:1.2)
Pharynx length	70–110	53–62	60–70	43 (33–54)	43 (28–50)
Pharynx width	50–90	53–73	70–80	39 (30–45)	42 (30–40)
Oesophagus length	40–220	24–73	170–230	30 (20–42)	–
Forebody	–	445–592	760–830	164 (100–338)	141 (100–170)
Hindbody	–	0.96–1.1	1.4–1.7	253 (168–390)	364 (290–440)
Testis length	320–600	179–273	450–490	88 (60–130)	137 (110–190)
Testis width	160–384	116–138	210–225	75 (50–100)	108 (80–140)
Ovary length	208–320	121–145	175–220	49 (30–70)	66 (60–75)
Ovary width	208–296	85–109	175–210	40 (26–62)	50 (40–70)
Cirrus-sac length	240–380	196–242	440–495	122 (99–145)	123 (120–125)
Cirrus-sac width	100–160	105–136	120–130	49 (40–80)	85 (70–100)
Seminal vesicle length	–	98–135	160–190	82 (60–110)	101 (75–110)
Seminal vesicle width	–	55–110	90–95	44 (25–60)	80 (50–120)
Terminal organ length	–	–	120–140	70 (40–98)	90 (68–105)
Terminal organ width	–	–	70–80	30 (20–40)	43 (40–55)
Excretory vesicle length	–	–	–	50 (40–70)	–
Excretory vesicle width	–	–	–	39 (31–50)	–
Egg length	28–37	24–28	24–30	27.6 (25–30)	31 (28–35)

Table 2 Comparative measurements of *Proctotrema* spp., with hosts, localities and data-sources. Measurements are given in micrometres except where otherwise stated

Species	<i>P. bacilliovatum</i> Odhner, 1911	<i>P. amphitruncatum</i> & Thomas, 1969	Fischthal	<i>P. guptai</i> Ahmad & Dhar, 1987	<i>P. bartolii</i> n. sp.
Host(s)	<i>Mullus barbatus</i> and <i>M. surmuletus</i> (Mullidae)	<i>Pomadasyx jubelini</i> (Pomadasyidae)		<i>Pomadasyx maculatus</i> (Pomadasyidae)	<i>Eleginops maclovinus</i> (Eleginopidae)
Locality(-ies)	Gulf of Marseille and off Trieste	Cape Coast, Ghana		Puri Coast, Bay of Bengal	Chubut, Argentina
Reference	Bartoli & Prévot (1966)	Fischthal & Thomas (1969)	Ahmad & Dhar (1987)		Natural specimens (Present study)
n	12	5	7	22	11
Egg width	9–12	7–10	8–10	16.1 (15–18)	17(12–20)
Body length/width				1:2.4 (1:1.7–1:3.4)	1:2.3 (1:1.5–1:3)
Forebody % length				35.9 (28.6–39.7)	27.8 (24.8–31.5)
Oral sucker/pharynx ratio				1:2 (1:1.5–1:2.4)	1:2.1 (1.6–1:3.4)
Body length/forebody ratio				1:2.6 (1:1.6–1:3.5)	1:3.6 (1:3.2–1:4)
					17.6 (15–20)
					1:2 (1:1.8–1:3.2)
					33 (27.7–37.7)
					1:2.3 (1:2–1:2.6)
					1:3 (1:2.7–1:3.6)

The main controversies regarding *Proctotrema* involve the number of species included within this genus and its synonymy with *Lasiotocus* Looss, 1907. However, different approaches based on morphological characters have permitted the separation of these genera (e.g. Hopkins, 1941; Manter & Pritchard, 1961; Bartoli, 1965; Bartoli & Prévot, 1966; Durio & Manter, 1968; Dove & Cribb, 1998). Dove & Cribb (1998) proposed an amended diagnosis for *Lasiotocus*, focusing on the configuration of the terminal genitalia, particularly the terminal organ, and the number of testes as the best characters for distinguishing these genera. Accordingly, *Proctotrema* has a unipartite terminal organ, with the metraterm uniting with its distal region (Yamaguti, 1971; Madhavi, 2008), whereas members of *Lasiotocus* are distinguished by the presence of a bipartite terminal organ, consisting of a posterior vesicle and an anterior spiny part (Dove & Cribb, 1998; Madhavi, 2008).

Although five species have been previously included within *Proctotrema*, we accept only *P. bacilliovatum*, *P. amphitruncatum* and *P. guptai* as valid members of this genus. *P. pritchardae* and *P. odhneri* are not included because of the following reasons: *P. pritchardae*, described by Nahhas & Cable (1964), is now recognised as *Lasiotocus pritchardae* (Nahhas & Cable, 1964), as determined by El-Naffar et al. (1991) and supported by the specimens recently described by Kardousha (2003), because it possesses a bipartite terminal organ. Specimens of *P. odhneri*, described by Ramadan (1985), have a genital pore located posterior to the ventral sucker, an armed genital atrium and his figure depicts the terminal organ as bipartite. These characters do not agree with the generic diagnosis of *Proctotrema*. As we were not able to examine Ramadan's (1985) material, the systematic position and validity of this species remains uncertain, i.e. it is a *species inquirenda* and *incertae sedis*.

With regard to the life-cycle of *Proctotrema bartolii* n. sp., this species utilises the clam *Darina solenoides* as its first and second intermediate hosts (F. Cremonte, unpublished data). Clams from the SJG presented a higher prevalence of infection with this parasite compared with those from the NG (F. Cremonte, unpublished data). Since silversides and mullets prey on these clams (Escofet et al., 1979), they become infected more frequently in the SJG

(14.5–100%) than in the NG (3.8–7.7%). This finding explains the higher prevalence values found in fishes from the SJG.

Acknowledgements The authors wish to thank Juan Signorelli, Marco Calvetty, Germán Cheli, Florencia Grandi, Juan Guerra and Horacio for their help with the fishing, Juliana Notarnicola for help with the references, M. Cristina Estivariz for the drawings, Clelia Mosto for reviewing the English of the manuscript, and both anonymous reviewers for improving the manuscript. Special thanks go to Prof. Rokkam Madhavi for her critical comments and suggestions on an early version of the manuscript. The present study was funded by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PIP 5653), the Universidad Nacional de La Plata (N504) and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) (PICT 01374). Fieldwork was conducted in a Protected Natural Area of Chubut Province with permits from the Secretaría de Turismo y Áreas Protegidas. The authors are members of CONICET.

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