Margaritaella gracilis gen. n. et sp. n. (Eucestoda: Proteocephalidea), a parasite of Callichthys callichthys (Pisces: Siluriformes) from the Paraná River basin, Argentina

Nathalia J. Arredondo and Alicia A. Gil de Pertierra


Abstract: Margaritaella gracilis gen. n. et sp. n. (Proteocephalidea: Proteocephalinae) found in the intestine of Callichthys callichthys (Linnaeus) from the Paraná River basin is described. The new genus is placed in the Proteocephalinae because of the medullary position of the genital organs. It differs from all known genera included in the Proteocephalinae by the following combination of characters: 1) scolex with a conspicuous cluster of drop-shaped gland cells posterior to suckers; 2) strobila with a low number of proglottides, all much longer than wide; 3) testes arranged in one field, composed of two parallel rows of testes separated by the uterus; 4) ovary delicate, H-shaped, with branches slender and deeply folliculate at the edges, located at 25–35% from the posterior end; 5) uterus largely extended posterior to the ovary but not reaching the end of proglottis; and 6) vitelline follicles in two narrow lateral bands, largely extended posterior to the ovary. Scanning electron microscopy revealed three types of microtriches on the tegument surface: acicular and capilliform filiformes and gladiate sminitrices. The relative size of the ovary, a character recently used in the systematics of the proteocephalidean cestodes, was calculated for the new species and compared with other species of the group. M. gracilis is the first record of a proteocephalidean cestode parasitizing a callichthyid catfish.

Keywords: Proteocephalinae, taxonomy, morphology, catfish, Callichthyidae, Neotropical region

Cestodes of the order Proteocephalidea Mola, 1928 are widely distributed in the Neotropical region. They are mainly parasites of siluriform fishes inhabiting the great river basins of South America (de Chambrier and Vaucher 1999, Rego et al. 1999, de Chambrier et al. 2006a).

During a survey of the parasite fauna from freshwater fishes of the Paraná River basin (Entre Rios Province, Argentina), Margarita Ostrowski de Núñez (Universidad de Buenos Aires) found specimens of an undescribed proteocephalidean species in the intestine of the catfish Callichthys callichthys (Linnaeus). These tapeworms, described herein, were assigned to the Proteocephalinae La Rue, 1911, but they could not be allocated to any of the known genera. A new genus is proposed to accommodate this new species.

MATERIALS AND METHODS

Two specimens of Callichthys callichthys (Siluriformes: Callichthyidae) were caught in the Paraná-Guazú River (Paraná River basin) (33°54’S, 58°52’W), Entre Rios Province, Argentina, during December 2008 and January 2009. All worms found in the intestines were removed, fixed in hot 4% formaldehyde solution and subsequently stored in 70% ethanol. Entire tape-worms were stained with Langeron’s alcoholic chlorhydric carmine (Langeron 1949), differentiated in acid ethanol, dehydrated through a graded ethanol series, cleared in beechwood creosote and mounted in Canada balsam. Details of the internal anatomy were determined from thick, hand-cut, transverse serial sections of proglottides stained with Langeron’s alcoholic chlorhydric carmine.

Two specimens of the new species were prepared for scanning electron microscopy (SEM) as follows: worms were post-fixed in 1% osmium tetroxide, dried with hexamethyldisilazane (Riedel-De Haën®), mounted on stubs with adhesive tape, sputter coated with gold in a Thermo VG Scientific Polaron SC 7630 and examined with a Philips XL 30 scanning electron microscope. The types and distribution of microtriches were studied on the scolex, proliferation zone and proglottides of the specimens. Measurements of the microtriches were made from photomicrographs. Terminology used for describing the microtriches follows Chervy (2009).

Unless otherwise stated, all measurements are given in micrometres. Measurements include the range followed by the mean in parentheses, standard deviation, the coefficient of variability (CV) and number of observations (n). For two-dimensional measurements, length is given before width. The relative size of the ovary was calculated according to de Chambrier et al. (2012). Illustrations were made with the aid of a camera lu-
cida attached to a Zeiss Axioscope microscope equipped with differential interference contrast optics. Fish classification and authorities follow FishBase (Froese and Pauly 2012).

Holotype and paratypes were deposited in the Parasitological Collection of the Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Buenos Aires, Argentina (MACN-Pa) and in the Institute of Parasitology, České Budějovice, Czech Republic (IPCAS).

RESULTS

*Margaritaella* gen. n.

Proteocephalidae, Proteocephalidaeae, Proteocephalinae. Testes, ovary, vitelline follicles, and uterus medullary. Small-sized worms, flattened dorsoventrally. Strobila acraspedote, with all proglottides much longer than wide. Scolex with conspicuous cluster of drop-shaped gland cells posterior to suckers, not surrounded by membrane and without retractor muscle bundles. Suckers uniloculate, almost spherical. Metascolex absent. Testes distributed in one layer, arranged in one field composed of two parallel rows separated by preformed uterus. Genital pore irregularly alternating, almost equatorial. Ovary delicate, H-shaped, with branches slender and deeply folliculate at edges, located at one-third from posterior end. Vagina anterior or posterior to cirrus-sac, without vaginal sphincter. Vitelline follicles scarce, arranged in two lateral bands, with few follicles paramuscular, largely extending posterior to ovary, not reaching end of mature and gravid proglottides. Uterine development of type 1 (sensu de Chambrier et al. 2004), uterus preformed, extending largely posterior to ovary in mature and gravid proglottides, not reaching end of proglottis. Parasites of Calli- chthyiidae. Type and only species: *Margaritaella gracilis* sp. n.

**Etymology:** The generic name is given in honour of Dr. Margarita C. Ostrowski de Núñez, who generously donated material for this study and greatly contributed to the knowledge of trematodes (Digenea); the name should be treated as feminine.


The new genus differs from all the genera mentioned above by combination of the following characters: 1) scolex with a conspicuous cluster of drop-shaped gland cells posterior to suckers; 2) all proglottides much longer than wide; 3) testes arranged almost in one field, formed by two parallel rows of testes separated by the uterus; 4) ovary delicate, H-shaped, with branches slender and deeply folliculate at the edges, situated at one-third from the posterior margin of the proglottis; 5) uterus largely extending posterior to the ovary; and 6) vitelline follicles in two narrow lateral bands, largely extending posterior to the ovary.

*Margaritaella gracilis* sp. n. Figs. 1–3

**Description** (based on 12 specimens and pieces of strobila as transverse sections. Measurements made on 4 complete and 2 incomplete worms): Proteocephalidaeae, Proteocephalinae. Small to medium-sized worms 26–29 mm (28) (n = 4) in total length. Strobila acraspedote, flattened dorsoventrally, with a low number of proglottides, 28–31 (29) (n = 4), which are much longer than wide, 21–26 (23) (n = 4) immature proglottides (up to appearance of spermatozoa in vas deferens), 2–4 (3) (n = 4) mature proglottides (up to appearance of eggs in uterus), and 3–5 (4) (n = 4) gravid proglottides. Mature proglottides (up to appearance of eggs in uterus), and 3–5 (4) (n = 4) gravid proglottides. Mature proglottides (up to appearance of eggs in uterus), and 3–5 (4) (n = 4) gravid proglottides. Immature proglottides 150–1 720 (600 ± 390, CV = 65%) × 100–310 (190 ± 50, CV = 25%) (n = 42); length/width ratio 1–9 : 1; mature proglottides 1 740–2 220 (2 060) × 190–425 (255) (n = 9); length/width ratio 5–11 : 1; gravid proglottides 1 520–2 740 (2 280) × 180–320 (260) (n = 12), length/width ratio 7–12 : 1 (Fig. 2A).

Scolex small, quadrangular, formed by four lobes separated by grooves, without metascolex, slightly wider than proliferation zone, 75–105 (90) (n = 6) wide (Fig. 3A–C), with conspicuous, deeply embedded cluster of drop-shaped gland cells posterior to suckers, not surrounded by membrane and without retractor muscle bundles, 85–125 (105) × 40–65 (55) (n = 6). Numerous small canals filled with granular content emerge from gland cells, ending beneath tegument surface (Fig. 1A). Suckers spherical, uniloculate, 30–50 (40) (n = 22). Proliferation zone 1 090–1 620 (1 300) × 65–100 (90) (n = 6) (Fig. 3A–C).

Internal longitudinal musculature weakly developed, formed by small bundles of muscle fibres (Fig. 1B,C). Osmoregulatory canals situated between testes and vitelline follicles, sometimes overlapping testes and ovary. Ventral canal 5–20 (10) (n = 7) in diameter, dorsal canal 3–5 (3) (n = 7) (Fig. 1B,C,E,F).

Testes medullary, spherical to oval 25–65 (40 ± 10, CV = 24%) × 20–50 (30 ± 10, CV = 22%) (n = 30); 40–55 (47) (n = 9) in total number per mature proglottis, 18–28 (24) aporal testes, 3–10 (5) postporal testes, and 14–21 (18) preporal testes; distributed in one layer, arranged in almost one field forming two parallel rows separated by preformed uterus. Testicular field occupying 57–68% (62%) (n = 9) of proglottis length in mature proglottides.
Testes not overlapping cirrus-sac, sometimes extending beyond osmoregulatory canals; never overlapping ovary (Figs. 1C,F, 2B). Cirrus-sac pyriform, with thin muscular wall, 75–130 (90) × 40–50 (45) (n = 9), occupying 30–40% (37%) (n = 9) of proglottis width in mature proglottides. Cirrus long, occupying 60–80% (70%) (n = 9) of cirrus-sac length in mature proglottides (Fig. 1F). Length of evaginated cirrus 95–145 (n = 2) × 30–35 (Fig. 2B). Vas deferens coiled 15–25 (20) (n = 9) in diameter, directed anteriorly, surpassing body mid-line in mature and gravid proglottides. Genital pore equatorial, irregularly alternating, situated at 45–55% (30%) (n = 9) from ante-
Ovary medullary, delicate, H-shaped, with branches slender and deeply folliculate at edges, located at one-third from posterior end, 100–180 (130) (n = 8) wide, occupying 40–70% (50%) (n = 8) of proglottis width and 0.6–1.8% (n = 3) of proglottis surface in mature proglottides, situated at 25–35% (30%) (n = 8) from posterior end of mature proglottis. Mehlis’ gland 25–55 (40) × 25–50 (35) (n = 5) (Figs. 1B,E, 2B,C). Vagina thick-walled, 10–25 (15) (n = 8) in diameter, with chromophilic cells in terminal part; vaginal sphincter not observed. Vagina anterior (43%) or posterior (57%) (n = 23) to cirrus-sac; when vaginal canal anterior, it overlaps cirrus-sac and does not surround vas deferens (Figs. 1E,F, 2B).

Fig. 2. Margaritaella gracilis gen. n. et sp. n. from Callichthys callichthys. A – entire worm, dash lines indicate portions of the strobila that are not shown; ventral view, holotype (MACN-Pa 522/1); B – mature proglottis; dorsal view, paratype (IPCAS C-621/1); C – gravid proglottis; ventral view, paratype (IPCAS C-621/1). Scale-bars: A = 250 µm; B, C = 200 µm.
Vitelline follicles medullary, scarce, with few follicles in paramuscular position in transverse section; arranged in two narrow lateral bands occupying about 90% of total proglottis length, not reaching posterior margin of proglottis, largely surpassing ovary. Follicles interrupted at level of cirrus-sac, rarely overlapping testes (Figs. 1B,C, 2B,C).

Uterine stem medullary but frequently partly cortical. Uterine development of type 1 (sensu de Chambrier et al. 2004). Uterus preformed in mature proglottides, largely...
surpassing ovary posteriorly. Uterus occupying 20–80% (50%) (n = 12) of proglottis width and 80–95% (90%) (n = 12) of proglottis length; with 35–60 (45) poral branches and 35–60 (50) (n = 9) aporal branches. Uteroduct 95–225 (165) × 15–25 (20) (n = 3) wide, occupies 5–10% (8%) (n = 3) of proglottis length (Figs. 1B,C,E).

Intraterine eggs elliptical, with collapsed outer envelope; embryophore thick, 27–31 (29) × 17–23 (19) (n = 4) consisting of two layers (outer transparent, inner containing nuclei), oncosphere 12–15 (12) × 9–11 (10) (n = 4), with hooks 4–6 (5) (n = 3) long (Fig. 1D).

Type host: *Callichthys callichthys* (Linnaeus, 1758) (Siluriformes: Callichthyidae). Vernacular name in Argentina “cascarudo”.

Type locality: Paraná-Guazú river (tributary of Paraná River) (33°54'S, 58°52'W), Entre Ríos Province, Argentina.

Type material: Holotype MANc-Pa No. 522/1 (entire worm and transverse sections, on one slide), paratypes MANc-Pa 522/2 (entire worm and transverse sections, on one slide), MANc-Pa 522/3A–B (entire worm and transverse sections, on two slides), MANc-Pa 522/4–8 (three scolecis, pieces of strobila and transverse sections, on five slides) and paratypes IPCAS C-621/1 (entire worm and transverse sections, on one slide), IPCAS C-621/2 (scolex and piece of strobila with immature proglottides, on one slide), IPCAS C-621/3 (piece of strobila with mature proglottides and transverse sections, on one slide), IPCAS C-621/4 (piece of strobila with gravid proglottides and transverse sections, on one slide).

Site: Intestine.

Infection rate: Prevalence: 50% (1/2); intensity 12 worms per host.

Etymology: From the Latin “gracilis” meaning “gracile”, feminine in gender.

Microthrix pattern

SEM observation of the tegument surface revealed the presence of acicular, capilliform and gladiate microtriches distributed on the scolex, proliferation zone and immature proglottides. The microtriches of each type have fairly similar sizes: acicular filitriches 0.25–0.50 (0.40) × 0.10–0.15 (0.10) (n = 25); capilliform filitriches 0.60–1.0 (0.80) × 0.05–0.10 (0.08) (n = 22); and gladiate spinitriches 0.60–1.20 (0.80) × 0.20–0.60 (0.40) (n = 20).

Microtriches on the scolex are distributed as follows: only acicular filitriches on the apical surface of the scolex (Fig. 3D); sparse gladiate spinitriches interspersed with capilliform filitriches on the luminal surface of suckers (Fig. 3F); dense gladiate spinitriches interspersed with capilliform filitriches on the marginal ring surface of suckers (Fig. 3E); gladiate spinitriches interspersed with acicular filitriches on the non-adherent surface of suckers (Fig. 3G). The proliferation zone is covered with gladiate spinitriches interspersed with acicular filitriches on its anterior surface (Fig. 3G), very sparse gladiate spinitriches (decreasing in density posteriorly) interspersed with acicular filitriches on its mid-surface (transitional surface) (Fig. 3H), and only acicular filitriches on its posterior surface (Fig. 3I). Immature proglottides only have densely packed acicular filitriches (Fig. 3J). The central apical surface of the scolex is covered with scarce tumuli exhibiting a pinched-off appearance, probably due to the released content from the cluster of gland cells.

**DISCUSSION**

*Margaritaela gracilis* gen. n. et sp. n. belongs to the Proteocephalinae as defined by Schmidt (1986) and Rego (1994), based on the following characters: medullary position of testes, ovary, uterus and vitelline follicles. The new species is allocated to a new genus because it possesses a scolex with four spherical uniloculate suckers, a conspicuous cluster of drop-shaped gland cells posterior to the suckers; all proglottides much longer than wide; testes almost arranged in one field (composed of two parallel rows of testes not overlapping ovary); an H-shaped ovary located at one-third from the posterior end of the proglottis; vitelline follicles arranged in two narrow lateral bands, which do not reach the posterior margin of the proglottis but extend far beyond the ovary; and a uterus largely surpassing the ovary posteriorly (Fig. 2A–C). The fact that some of these characters are rare in Proteocephalidea makes *M. gracilis* a particularly interesting fish parasite.

Notwithstanding the unique morphology of *M. gracilis*, it shares some characters with other proteocephalidean cestodes from different fish hosts. For example, the scolecis of *Proteocephalus cernuae* (Gmelin, 1790), *P. longicolis* (Zeder, 1800) and *P. soniae* de Chambrier et Vaucher, 1994 also have a cluster of gland cells located posterior to the suckers (see de Chambrier and Vaucher 1994, Scholz et al. 1998, Scholz and Hanzelová 1998). However, *P. cernuae* and *P. longicolis* possess a fifth sucker at the apex of the scolex, and *P. soniae* has an apical organ, whereas in *M. gracilis* they are absent.

*Vaucheriella bicheti* de Chambrier, 1987 and *Ophioforma habanensis* Freze et Ryžavý, 1976, both from reptiles, are similar to *M. gracilis* in the morphology of the proglottides (all much longer than wide) and the arrangement of testes (in one field distributed in two parallel rows separated by the uterus) (see fig. 5 of Freze and Ryžavý 1976, and figs. 1A and B of de Chambrier 1987). *Margaritaela gracilis* differs from *V. bicheti* because the latter, as a member of the Zygobothriinae, has cortical vitelline follicles located ventrally between the genital pore and the ovary, and a small and bilobed ovary. On the other hand, *O. habanensis* has a compact and bilobed ovary located near the posterior end of the proglottis, and both the vitelline follicles and uterus do not extend posteriorly to the ovary.

Recently, Ammann and de Chambrier (2008) and de Chambrier et al. (2012) proposed the relative ovarian size as a useful character to distinguish between two
similar genera, namely *Proteocephalus* Weinland, 1858, parasites of teleost fishes, and *Ophiotrema* La Rue, 1911, parasites of reptiles. It is interesting to note that the ovary of *M. gracilis*, a parasite of Neotropical fishes, is smaller (0.6–1.8%) than those of *Proteocephalus* species found in fishes from all over the world (5.4–28.8%) (Table 2 in de Chambrier et al. 2012) and those of *Ophiotrema* species occurring in reptiles from the New World (1.9–5.5%) (Table 2 in Ammann and de Chambrier 2008). In addition, the relative size of the ovary of the Neotropical proteocephalidean *Tejidotaenia appendiculata* (Baylis, 1947) from South American lizards (8.4% according to Fig. 5 of Rego and de Chambrier 2000), is similar to that of the Neotropical genus *Proteocephalus*, the members of which parasitize fishes (6.1–18.2%, sensu de Chambrier et al. 2012). In turn, the ovaries of *T. appendiculata* and *M. gracilis* show a similar location (28–30% and 25–35%, respectively).

In most of the known proteocephalideans, the uterus usually does not extend beyond or slightly overlaps the ovary, except for a few species in which the uterus occupies almost the entire length of gravid proglottides, such as *Cairarella henrii* Coquille et de Chambrier, 2008, *Cangatiella arandasi* Pavanelli et Machado dos Santos, 1991, *C. macdonaghi* (Szidat et Nani, 1951), *Proteocephalus sophiae* de Chambrier et Rego, 1994, *Pseudocrepidobothrium eirasi* (Rego and de Chambrier, 1995), and *T. appendiculata* (see de Chambrier and Rego 1994, Rego and de Chambrier 1995, 2000, Gil del Pertierra and Viozzi 1999, Coquille and de Chambrier 2008). In contrast, the uterus of *M. gracilis* extends largely posterior to the ovary in mature (preformed uterus) and gravid proglottides, but it does not reach the end of the proglottis.

On the apical surface of the scolex, capilliform filitriches are the most common microthrix type, this being the only one found in, for example, *Lenhataenia megacephala* (Woodland, 1934); *Monticellia lenha* Woodland, 1933; *M. magna* (Rego, dos Santos et Silva, 1974); *Nomimoscolex chubbi* (Pavanelli et Takemoto, 1995); *N. sudobim* Woodland, 1935; *Proteocephalus hemioliopteri* de Chambrier et Vaucher, 1997; *P. sulcatus* (Klaptocz, 1906); *Spatulifer maringaensis* Pavanelli et Rego, 1989 and *S. rugosa* (Woodland, 1935) (see Gil de Pertierra 2004, 2005, de Chambrier et al. 2005, 2006b, 2007, de Chambrier and Scholz 2008, Arredondo and Gil de Pertierra 2008). In contrast, acicular filitriches are the only type present on the apical surface of the scolex in *M. gracilis* (present paper), *Monticellia belavistensis* Pavanelli, Machado, Takemoto et dos Santos, 1994 and *M. ventrei* de Chambrier et Vaucher, 1999 (see sizes of microtriches in Gil de Pertierra 2005). On the proliferation zone, gladiate spinitriches are the most common microthrix type and it is the only one present in *M. ventrei*, *N. chubbi*, *N. lopesi* Rego, 1989 and *Scholzia emarginata* (Diesing, 1850) (see de Chambrier et al. 2005, Gil de Pertierra 2005), whereas two types of microtriches are found on the proliferation zone of *M. gracilis* (gladiate spinitriches and acicular filitriches, see above for microthrix distribution) and *M. belavistensis* (gladiate spinitriches and capilliform filitriches, see Gil de Pertierra 2005).

However, in some of these species, the microtriches were reported but their measurements were not provided (e.g., *L. megacephala*, *M. lenha*, *N. sudobim* and *P. sulcatus*), despite the relevance of size for their correct classification (Chervy 2009). This fact indicates the need to confirm the actual identity of the microthrix types found in some previously described species, as well as to provide a more complete set of data for new species descriptions. Another important issue for a more accurate taxonomic classification of proteocephalideans is to describe the microtriches present on all the tegumental surfaces, which may show specific differences (e.g., apex of the scolex, luminal and marginal surface of suckers, non-adherent surface, metascolecom surface, when present, proliferation zone, proglottides) (see Gil de Pertierra 2005).

*Margaritaella gracilis* is the first proteocephalidean to be described from *Callichthytes callichthys*, a small fish usually used to keep the aquarium clean or as bait for large siluriforms (Almirón et al. 2008, Froese and Pauly 2012). Pimelodids of commercial value are the most frequently reported freshwater fish hosts of proteocephalideans in South America (de Chambrier and Vaucher 1999, Rego et al. 1999, de Chambrier et al. 2006a). However, new parasite species are continually being found in fishes of other orders or families. For example, *Nomimoscolex chubbi*, *N. dechambrieri* Gil de Pertierra 2003 and *N. guillermoi* Gil de Pertierra, 2003 are parasites of *Gymnotus carapo*, usually used as bait fish like *C. callichthys* (see Pavanelli and Takemoto 1995, Gil de Pertierra 2003). This reflects the importance of studying fishes of minor commercial value for discovering new proteocephalidean cestodes.

**Acknowledgements.** Special thanks are due to the two reviewers for their valuable comments that helped improve this manuscript. Research was supported by Universidad de Buenos Aires (Grants UBACYt – 20020090200511 and 20020090200529).

**REFERENCES**


105
de Parques Nacionales, Ciudad Autónoma de Buenos Aires, 216 pp.


Received 16 February 2012

Accepted 30 March 2012

Coquelle S.C., de Chambrier A. 2008: Caira ella henrii gen. n., sp. n., a parasite of Norops trachyderma (Polychrotidae), and Ophiotaenia nicoleae sp. n. (Eucestoda: Proteocephalidea), a parasite of Thracodes sphenicus (Gekkonidae), in Ecuador. Folia Parasitol. 55: 197–206.


Rego A.A., de Chambrier A. 1995: Crepidobothrium eirasi n. sp. (Cestoda: Proteocephalidea), a parasite of the silurid fish Phractocephalus hemilampus (Schneider, 1801) (Pisces: Pimelodidae) from the Brazilian Amazon. Rev. Suisse Zool. 102: 3–11.


