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Tapeworms (Cestoda: Proteocephalidea) of *Hoplias malabaricus* (Pisces: Characiformes, Erythrinidae) in Paraguay: description of *Proteocephalus regoi* sp. n., and redescription of *Nomimoscolex matogrossensis*

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Abstract. The erythrinid fish *Hoplias malabaricus* in Paraguay harbour two species of proteocephalid cestodes, *Proteocephalus regoi* sp. n. and *Nomimoscolex matogrossensis* Rego et Pavanelli, 1990. The former species differs from most South American members of *Proteocephalus* from fishes by: 1) presence of a small apical organ, 2) distribution of vitellaria, which do not reach to posterior margin of segment and 3) posterior position of vagina. *Nomimoscolex matogrossensis* is redescribed on the basis of type and recent material, with emphasis given to the morphology of genital organs and scolex, which possesses an apical organ. Both species possess a dense network of osmoregulatory canals in the postacetabular region of both scolex and neck. *Proteocephalus regoi* sp. n. is the first representative of the subfamily Proteocephalinae parasitizing erythrinid fish.

Proteocephalan tapeworms are among the most frequent platyhelminth parasites of freshwater fishes in South America. The highest number of proteocephalid species occurs in siluriform fishes (see Freze 1965, Rego and Pavanelli 1992). In the erythrinid fish, *Hoplias malabaricus* (Bloch, 1794), only one species of monticelliid, *Nomimoscolex matogrossensis* Rego et Pavanelli, 1990, has been hitherto reported (Rego and Pavanelli 1992). The examination of *H. malabaricus* from Paraguay revealed two species of proteocephalid cestodes. Taxonomic evaluation of these parasites showed one new species of *Proteocephalus* Weinland, 1858 described herein. The other taxon appeared conspecific with *N. matogrossensis*, recently described by Rego and Pavanelli (1990) from Brazil. As the original description of *N. matogrossensis* did not contain data on some morphologically useful structures, the concerned species is redescribed in this paper.

MATERIALS AND METHODS

Fifty two *Hoplias malabaricus* were examined for helminths by two of us (A. de C. and C. V.) in Paraguay. Worms found in the intestine were isolated and fixed in hot 4 % formaldehyde solution, then stored in 75 % ethanol. Thereafter, they were stained with Mayer's hydrochloric carmine, dehydrated in ethanol, cleared with Eugenol and mounted in Canada balsam. Pieces of strobila were embedded in paraffin, transversely sectioned at 12–15 μm, stained with Weigert's haematoxylin and counterstained with eosin. The material studied has been registered at the Natural History Museum Geneva (INVE) and at the Institute of Parasitology, Academy of Sciences of the Czech Republic (IPASCR), České Budějovice. All measurements are given in micrometres (μm) unless otherwise stated. Abbreviations: m = mean; n = number of measurements; CV = coefficient of variation; desc. = original description; t = own measurements of the type material.

RESULTS

Proteocephalus regoi sp. n. Figs. 1–9

Host: *Hoplias malabaricus* (Bloch, 1794) (Characiformes: Erythrinidae).

Localities: Road San Juan Bautista – San Juan Neembucu, km. 55, Neembucu Province, Paraguay, 16.10.1989 (holotype INVE 19671, paratype IPASCR No. C- 245), and 3 km North of Carapegua, Paraguari Province, Paraguay, 09.10.1982 (paratype INVE 19672.)

Site of infection: intestine.

Prevalence: 6 %

Intensity: 1 worm/fish.

Description: Proteocephalidae, Proteocephalinae. Testes, ovary, uterus and vitellaria medullar. Strobila acraspedote, 75–265 mm long and up to 2.2 mm wide. About 130 immature proglottides (to appearance of spermatozoa in vas deferens), about 5–6 mature proglottides (to appearance of eggs in uterus) and about 240 proglottides in total. Immature and mature proglottides wider than long. Gravid proglottides wider than long to longer than wide. Tegument thick, about 5 in scolex and 10–13 in strobila. Scolex wider than neck, width of scolex 525–875 (Fig. 1). Apical organ present, small, sucker-like, with narrow and deep central pit (Fig. 2), 25–30 × 25–40. Apical part of scolex with numerous lanceolate or elongate cells with granular content (?unicellular glands) opening by narrow ducts to surface (Fig. 2). Suckers uniloculate, strongly muscular, 275–340 in diameter. Growth zone very long. Ventral osmoregulatory canals densely anastomosed in posterior part of scolex and in neck region, with 12–17 small canals ending beneath tegument (Fig. 3). In strobila, osmoregulatory canals situated ventrally or mediolaterally to vitellaria (Figs. 6, 8). In mature segments, osmoregulatory canals crossing cirrus sac in its basal part (Fig. 4); ventral canals anastomosed, provided with secondary canals ending beneath tegument (Figs. 4, 5, 7, 8). Longitudinal muscles strongly developed, anastomosed.

Testes one-layered, in two lateral fields, connected by several testes in anterior part of segments, even in immature proglottides, reaching vitellaria and not crossing osmoregulatory canals (Figs. 4, 5). Testis number 88–144 (m = 119, n = 20, CV = 13.41), 40–75 in diameter. Genital pore situated anteriorly, 26–34 % (m = 30 %, n = 25, CV = 9.84) of proglottis length. Genital atrium present. Cirrus sac elongate, pyriform, with wider proximal (basal) part, 195–335 (x= 265, n = 16) × 85–125 (m = 100, n = 16); Cirrus sac length representing 14–21 % (m = 18 %, n = 22 %, CV = 11.26) of segment width (Fig. 7). Cirrus 125–205 long, representing 55–68 % of cirrus sac length. Sperm duct and ejaculatory canal long and coiled (Fig. 7). Vagina always posterior to cirrus sac, with well-developed, ring-shaped sphincter. Mehlis' gland obliquely elongate, about 150 in diameter, representing about 10 % of ovary width. Ovary medullar, bilobed, follicular, representing 60–66 % (m = 63 %, n = 22, CV = 3.06) of proglottis width (Figs. 4–5). Vitellaria in two longitudinal bands, representing 81–90 % of segment length, situated dorsally to cirrus pouch. Vitellaria anteriorly reaching almost segment margin, posteriorly reaching only the anterior half of ovarian lobes (Figs. 4, 5).

Uterus medullar, preformed; uterine primordium already visible in immature proglottides as medio-ventral, cylindrical, longitudinal concentration of chromophil cells. In first mature proglottides, uterine stem with thick irregular wall of chromophil cells (Fig. 4). Uterine stem developes thin-walled diverticles on each side immediately before first eggs appear in uterus. From 9 to 25 (m = 15, n = 50) uterine branches on each side, representing up to 85 % of proglottis width. Ripe eggs, containing hooklets in oncosphere, released by several ventral longitudinal apertures in sites where uterine stem penetrated ventrally across longitudinal internal musculature ending on tegument surface. Eggs with thin external shell, about 70 in diameter; thick hyaline outer envelope; separate spherical membrane 28–30; embryophore 21–27 and oncosphere 14–15 in diameter with central hooks 7.4–8.8 long and lateral hooks 8–8.8 long (Fig. 9). According to Swiderski (1994), we interprete the described structures as: 1) shell; 2) outer envelope; 3) inner envelope consisting in bilayered embryophore with external layer much bigger than nucleate envelope; 4) oncospherical membrane rarely visible, not illustrated; 5) oncosphere.

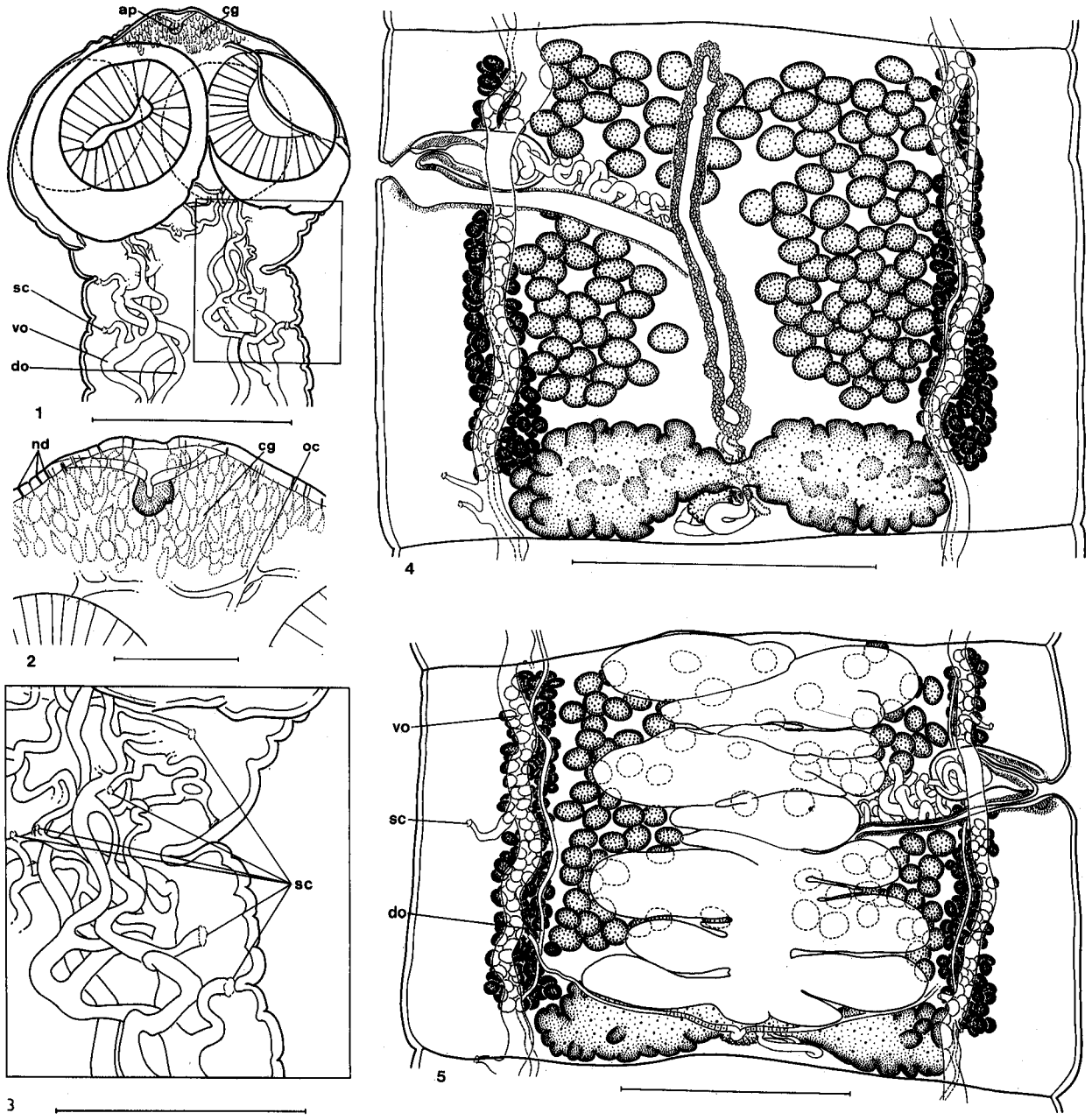
Etymology: the species has been named after Professor Amilcar Arandas Rego, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, for his contributions to the taxonomy of the Proteocephalidea.

Differential diagnosis: The geographical repartition of the host is restricted to South America (Berra 1981). Consequently, we compare the new taxa with those occuring in South American freshwater fishes. Fifteen *Proteocephalus* species have been described from freshwater fishes in South America (Diesing 1850, Riggenbach 1896, Woodland 1933, 1934, 1935, Vigueras 1936, Szidat and Nani 1951, Lynsdale 1959, Freze 1965, Rego et al. 1974, de Chambrier and Vaucher 1984, 1994, Rego and Pavanelli 1990, 1991, de Chambrier and Rego 1994).

Proteocephalus regoi sp. n. is characterized especially by the presence of an apical organ, which is lacking in most *Proteocephalus* species occurring in South American freshwater fish. *Proteocephalus regoi* sp. n. can be differentiated from species possessing an apical organ by the following features:

Proteocephalus gibsoni Rego et Pavanelli, 1990 (syn. *Proteocephalus ocellatus* Rego et Pavanelli, 1990, nec Rudolphi, 1802), a parasite of *Geophagus brasiliensis* (Quoy et Gaimard, 1824), *Astronotus ocellatus* (Agassiz in Spix et Agassiz, 1829) and *Astronotus* sp. in Brazil, is lacking a vaginal sphincter and possesses a sucker-like apical organ, with well-developed musculature.

Proteocephalus soniae de Chambrier et Vaucher, 1994 from *Platydoras costatus* (Linnaeus, 1766) in Paraguay can be differentiated by the distribution of vitellaria, occupying almost total proglottid length, reaching the posterior margin of the ovary. In addition, *P. soniae* differ by the number of testes (139–371; mean



Figs. 1–5. *Proteocephalus regoi* sp. n. **Fig. 1.** Scolex. Bar = 500 µm. **Fig. 2.** Scolex, detail of apical part. Bar = 250 µm. **Fig. 3.** Detail of osmoregulatory system in neck region, ventral view. Bar = 100 µm. **Fig. 4.** Mature proglottis, ventral view. Bar = 500 µm. **Fig. 5.** Gravid proglottis, ventral view. Bar = 500 µm. Abbreviations – see p. 139.

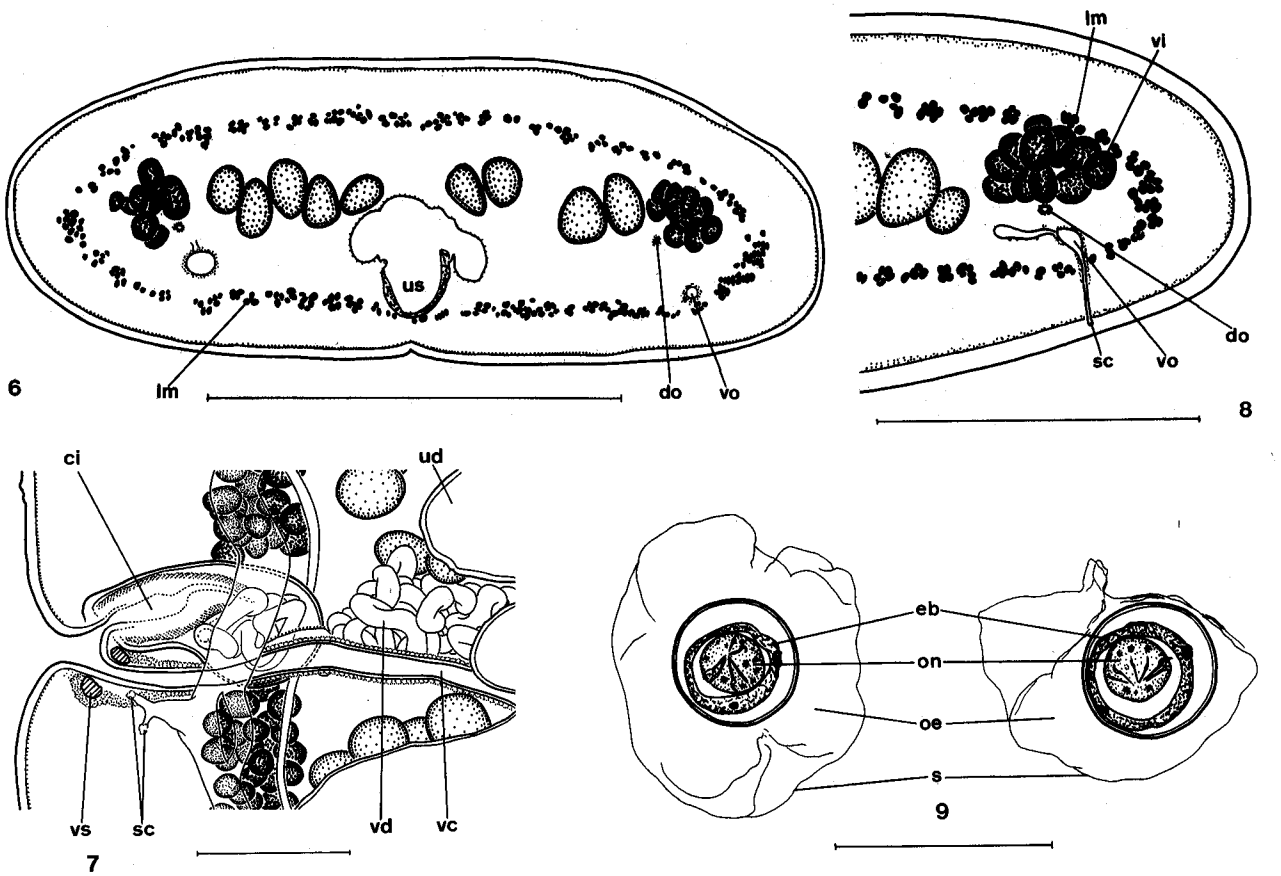
222) and by different structure of the distal part of vagina.

Proteocephalus sophiae de Chambrier et Rego, 1994 from *Paulicea luetkeni* (Steindachner, 1875) in Brazil is distinguishable by possessing vitellaria not reaching the anterior margin of the proglottides, by the structure of the apical organ (spherical and larger) and by the structure of the vaginal sphincter, which is elongate, strongly muscular in *P. sophiae*.

Proteocephalus fossatus (Riggenbach, 1896), from *Luciopimelodus pati* (Valenciennes, 1840) in Paraguay is easily distinguishable by the distribution of testes in one continuous field, by the relative size of the cirrus sac, which represents 33 % of segment width (estimated from the original description – Fig. 11), and by the different position of the vagina (irregularly alternating, both anterior and posterior) in *P. fossatus*.

On the basis of the above differences, specimens found in *H. malabaricus* are considered to represent a new species and the name *Proteocephalus regoi* sp. n. is proposed.

Remarks: *Proteocephalus regoi* sp. n. is the first known representative of the subfamily Proteocephalinae parasitizing erythrinid fish. Hitherto, only



Figs. 6–9. *Proteocephalus regoi* sp. n. **Fig. 6.** Transverse section of anterior part of segment. Bar = 500 µm. **Fig. 7.** Cirrus pouch and vagina, ventral view. Bar = 250 µm. **Fig. 8.** Transverse section of posterior part of segment with osmoregulatory canal ending under surface. Bar = 250 µm. **Fig. 9.** Eggs drawn in distilled water. Bar = 50 µm. Abbreviations – see p. 139.

Nomimoscolex matogrossensis has been reported in *Hoplias malabaricus*. *Proteocephalus regoi* sp. n. possesses several morphological features different from most other proteocephalans parasitizing freshwater fishes in South America. In particular, the number of mature proglottides is low (n = 6). Such a low number has been recorded in only a few proteocephalans, e. g. in *Proteocephalus cernuae* (Gmelin, 1790); this feature was also reported for *Nomimoscolex touzeti* de Chambrier and Vaucher, 1992 (de Chambrier and Vaucher 1992).

The presence of a densely anastomosed osmoregulatory system in the posterior part of the scolex and in the neck region is remarkable. Such a dense network has been rarely reported in *Proteocephalus*: in *P. torulosus* (Batsch, 1786), a parasite of cyprinid fishes in the Palearctic region (Wagner 1917), and *P. renaudi* de Chambrier et Vaucher, 1994, parasitizing the catfish *Platydoras costatus* in Paraguay (de Chambrier et Vaucher 1994). In addition, osmoregulatory canals forming a dense network were found in the scolex of *Mariauxiella pimelodi* de Chambrier et Rego, 1995, a monticellid from South American siluroid fishes (de Chambrier and Rego 1995).

The apical part of the scolex in *Proteocephalus regoi* sp. n. (Fig. 2) shows numerous cells with granular content, opening to the surface by narrow ducts. The function of these cells remains unclear. However, it seems that these cells (?unicellular glands) may play a role during the attachment of the worms to the host intestine (see Befus et Freeman 1973, p. 256). Similar cells were observed only in a few tapeworms of the genus *Proteocephalus*. One specimen of *P. regoi* was infected by one encysted cysticercoids.

Nomimoscolex matogrossensis Rego et Pavanelli, 1990 Figs. 10–17

Host : *Hoplias malabaricus* (Bloch, 1794)

Localities : Type material: Salobra, Mato Grosso, Brazil, IOC No 32.512 (holotype), IOC No. 32513 (paratype) and IOC No. 32514 a–c (transverse sections); Paraguayan material: Estancia Santa Sofia, 18 km South East San Carlos, Province Concepcion, Paraguay, 29.10.1987 (INVE 17912), and Arroyo Tapicuaray, 8 km West San Estanislao, San Pedro Province, Paraguay, 24.10.1989 (INVE 17913).

Site of location: intestine.

Prevalence: 4 %

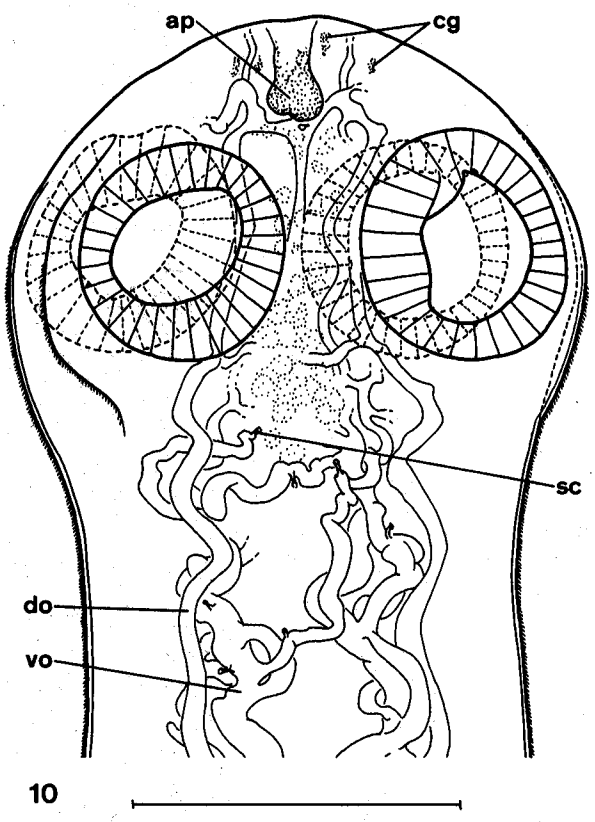


Fig. 10. *Nomimoscolex matogrossensis* Rego et Pavanelli, 1990. Scolex. Bar = 250 µm. Abbreviations – see p. 139.

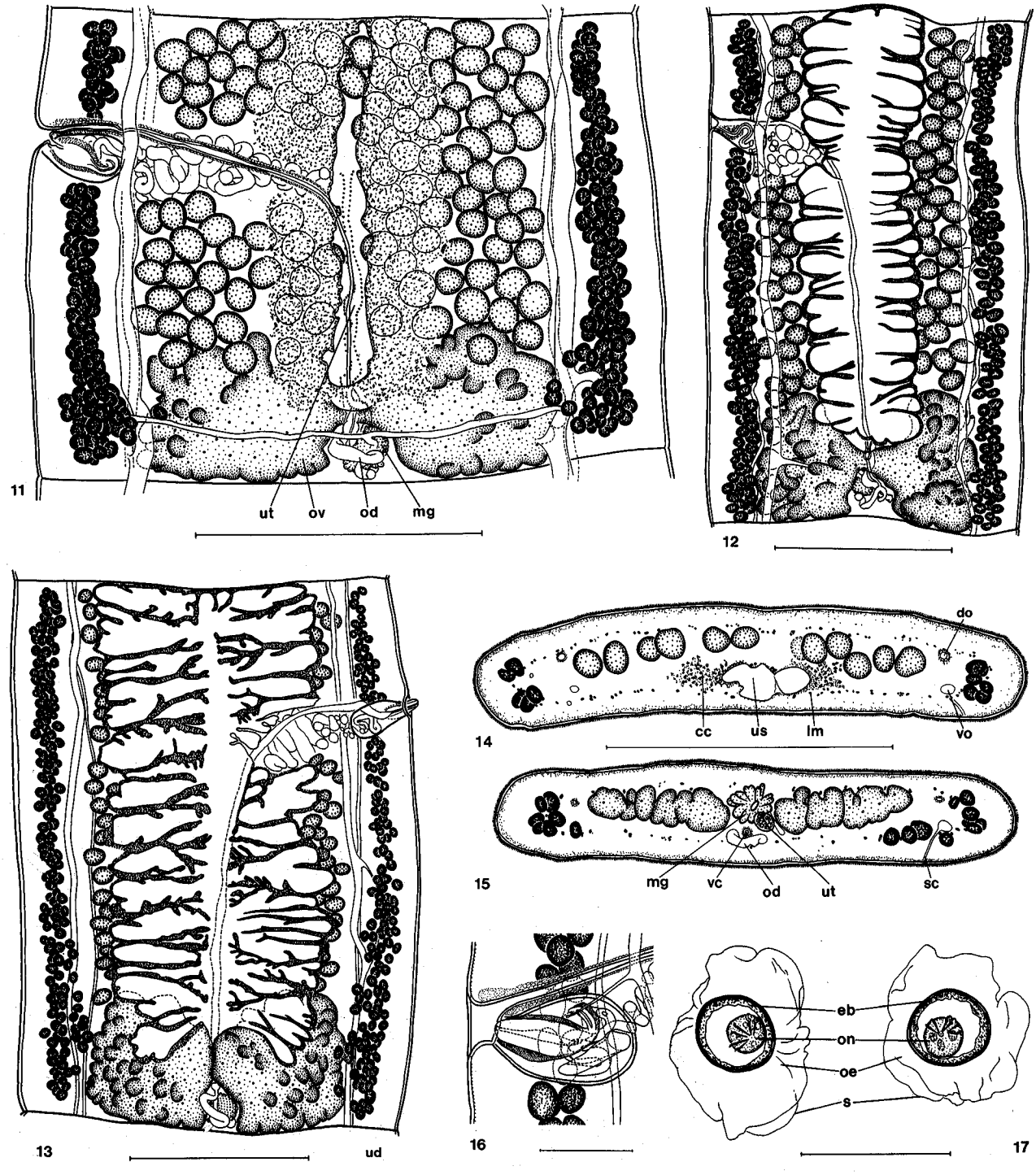
Intensity: 1–3 worms/fish.

Description: Monticelliidae, Zygobothriinae. Testes and ovary medullar; uterine stem cortical with diverticula growing in medulla; vitellaria cortical, sometimes paramuscular (see de Chambrier 1990, p. 92). Strobila acraspedote, 40–70 mm long (t = 23–27 mm). Tegument thin, covered with very dense and long microtriches, visible with optical microscope. Immature proglottides wider than long. Mature and gravid proglottides wider than long to longer than wide. Scolex 390–430 (t = 270–290) in diameter (Fig. 10). Apical organ thin-walled, funnel-shaped, with narrowed medial part and widened terminal portion, 70 long and 35–40 wide, containing in its basal part cells with granular content (Fig. 10). No parenchyme difference observed between internal and external part of apical organ (Fig. 10). Four uniloculate suckers, 145–185 (t = 105–120) in diameter. Neck present. Ventral osmoregulatory canals forming very dense network in neck region, with 4–7 small canals ending beneath the tegument. Osmoregulatory canals crossing cirrus pouch in its basal part; canals situated between testes and vitelline follicles; ventral canals anastomosed, with canals ending beneath tegument surface, near proglottid lateral margins (Figs. 11–13). Internal longitudinal muscles poorly developed, in form of slim bundles (Figs. 14,15).

Testes medullar, in one or two layers, lying in one field, interrupted only along medial line of body and around vas deferens. Testes 105–135 (m = 121, n = 9; t = 97–131, desc. = less than 200) in number, only rarely overlapping osmoregulatory canals and exceptionally reaching vitellaria (Figs. 11, 12). Genital pore anterior, situated in 24–32 % (n = 9; t = 14–27 %) of proglottis length. Genital atrium present. Cirrus sac ovoid, 135–165 (t = 100–150) long by 60–100 (t = 60–80) wide, representing 12–16 % (n = 13, t = 11–17 %) of proglottis width. Cirrus 85–110 (t = 95–100) long, representing about 65–75 % (t = 60–80 %) of cirrus sac length (Fig. 16). Ejaculatory duct and sperm duct coiled. Vagina anterior to cirrus sac, without sphincter (in transverse sections, circular muscle fibres observable in terminal part of vagina). Ovary medullar, bilobed, follicular, representing 66–73 % (t = 59–69 %) of proglottis width (Figs. 11–13, 15). In gravid proglottis, ovary crosses internal longitudinal muscles dorsally.

Vitellaria in two longitudinal bands, occupying almost total proglottis length, interrupted ventrally at level of cirrus pouch. Near posterior margin of proglottides, ventral vitelline follicles more numerous, with a few (mostly 1–3) follicles lying ventrally to ovarian lobes (Figs. 11–13). Uterine primordium as cortical cylindrical mass of chromophil cells. In immature proglottides, growing uterus penetrating into medullar parenchyme through longitudinal internal musculature and forming wide medullar band (about 30 % of proglottis width) (Figs. 11, 14). Lumen appearing in first mature proglottides. Lateral diverticles forming in this band before appearance of first eggs in uterus. In more developed proglottides, diverticles becoming lobate, with walls lined by thick layer of chromophil cells. In gravid proglottides, uterine branches representing up to 75 % of proglottis width, numbering 18–29 (t = 22–28) on each side. Eggs with thin external shell, about 50–60 in diameter; thick hyaline outer envelope; embryophore bilayered, 24–26 and oncosphere 12–14 in diameter with lateral hooks 7.2–8 long and central hooks 7.6–8.4 long (Fig. 17).

Remarks: Rego and Pavanelli (1990) described the species *N. matogrossensis* on the basis of 2 specimens from *H. malabaricus* from Brazil. They provided only a brief description and rather schematic drawings. The present study include the evaluation of the type material and new specimens found in the same host but from a different geographical region (Paraguay). Thus we are able to improve on the original description with new data, including the formation of the uterus, the existence of an apical organ and the presence of dense osmoregulatory canals in the neck region. Despite some differences between the material from Paraguay and the type specimens from Brazil, which include differences in body length, we consider all specimens to be



Figs. 11–17. *Nomimoscolex matogrossensis* Rego et Pavanelli, 1990. Scolex. Bar = 250 µm. **Fig. 11.** Mature proglottis, ventral view. Bar = 500 µm. **Figs. 12–13.** Gravid proglottides, ventral view. Bar = 500 µm. **Figs. 14–15.** Transverse sections at level of uterus and ovary, respectively. Bar = 500 µm. **Fig. 16.** Cirrus pouch and vagina, ventral view. Bar = 100 µm. **Fig. 17.** Eggs drawn in distilled water. Bar = 50 µm. Abbreviations – see p. 139.

conspecific. One specimen of *N. matogrossensis* was infected by two encysted cysticercoids.

The present study revealed that *N. matogrossensis* has several characteristic features which were not used by Rego and Pavanelli (1990) for differentiation from related species: the presence of a thin-walled apical organ containing granulated cells; the distribution of the vitelline follicles, which are more numerous posteriorly; the presence of strongly anastomosed osmoregulatory canals in the neck region; and the presence of long, very dense microtriches, which are readily visible under an optical microscope. The taxonomic value of these above mentioned features in relation to the systematics of the genus *Nomimoscolex* and other proteocephalid genera from South American freshwater fishes remains to be confirmed.

DISCUSSION

In *Hoplias malabaricus* from Paraguay, two proteocephalan tapeworms were found. However, no simultaneous infection was recorded. Both parasites were rare, with both prevalence and intensity values very low, 6 and 4 %, respectively.

It remains unclear whether *H. malabaricus* serves as a principal (typical) host of these tapeworms or if this species is only an accidental host. Seasonality in the occurrence, which is common in many fish proteocephalans from the Holarctic Region (see Chubb 1982), may also be a reason for the low infection levels observed in the present case.

Both cestode species found in *H. malabaricus* from Paraguay possess in the anterior part of their body dense network of osmoregulatory canals with secondary canals ending beneath the tegument. Such a network is not common in proteocephalan tapeworms and it has been reported in two species of *Proteocephalus* (*P. torulosus* and *P. renaudi*) and in *Mariauxiella pimelodi*.

As demonstrated in the present study, taxonomic work on this cestode group should include a thorough description of the morphology, including for example the evaluation of the osmoregulatory system, the distribution of the vitelline follicles, the formation of the uterus and the structure of the apical organ.

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Abbreviations: ap = apical organ, cc = mass of chromophil cells, cg = cells with granular content, ci = cirrus, do = dorsal osmoregulatory canal, eb = embryophore, lm = internal longitudinal musculature, mg = Mehlis glands, nd = narrow duct, oc = osmoregulatory canals, od = oviduct, oe = outer envelope, on = oncosphere, ov = ovary, s = shell, sc = secondary canals extremity in external tegumental layer, ud = uterine diverticles, us = uterine stem, ut = uteroduct, vc = vaginal canal, vd = vas deferens, vi = vitellaria, vo = ventral osmoregulatory canal, vs = vaginal sphincter.

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