

A NEW GENUS AND TWO NEW SPECIES OF PROTEOCEPHALIDEAN TAPEWORMS (CESTODA) FROM CICHLID FISH (PERCIFORMES: CICHLIDAE) IN THE NEOTROPICS

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ABSTRACT: *Cichlidocestus* n. gen. is proposed to accommodate 2 new species of proteocephalidean cestodes, *Cichlidocestus gillesi* n. sp. from *Cichlasoma amazonarum* in Peru (type species) and *Cichlidocestus janikae* n. sp. from *Hypsophrys nicaraguensis* (all Perciformes: Cichlidae) in Costa Rica. The new genus is unique among all but 1 proteocephalidean genera in the position of the ovary that occupies the middle and posterior thirds of the median region of proglottids (vs. the ovary in the posterior third of proglottids near their posterior margin in all but 1 remaining taxa). In addition, *Cichlidocestus* is typified by the presence of a voluminous, spherical, internal seminal vesicle, several pairs of ventral excretory canals in the medulla, a pyramidal, quadrilobed scolex with an apical muscular sucker, and the posterior extent of the testes that may reach almost to the posterior margin of proglottids. The new genus shares the position of the ovary and its extension with *Sciadocephalus* (also a parasite of cichlids in the Neotropics as the new taxon); in all remaining proteocephalideans the ovary occupies the posterior third only. *Sciadocephalus* differs from *Cichlidocestus* by a different morphology of the scolex, which possesses an umbrella-like metascolex that is markedly wider than the strobila, the number of ventral osmoregulatory canals, and development of the uterus, which forms capsule-like formations filled with eggs in *Sciadocephalus megalodiscus* (vs. simple lateral diverticula in *Cichlidocestus* spp.). Both new species of *Cichlidocestus* can be easily distinguished from one another by the anterior extent of the poral vitelline follicles (anterior to the cirrus-sac, i.e., preporal, in *C. gillesi* vs. posterior, i.e., only postporal in *C. janikae*), size of the eggs (diameter of the external layer of the embryophore of *C. gillesi* 30–33 µm vs. 44–46 µm in *C. janikae*), and the number of uterine lateral diverticula (16–21 on 1 side in *C. gillesi* vs. only 8–12 in *C. janikae*) and the testes (37–46 in *C. gillesi* vs. 63–74 in *C. janikae*). Based on a phylogenetic analysis of the 28S rRNA gene (*lsr*DNA) sequences, *C. gillesi* forms a lineage sister to *S. megalodiscus*, thus supporting the morphological similarity of both genera that occur in fishes of the same family in the same zoogeographical region.

Cichlid fishes (Perciformes: Cichlidae) represent one of the more abundant and intensively studied groups of freshwater fishes in Africa and South and Middle America (Lévêque et al., 2008; Řičan et al., 2016). Cichlids have also been used as a suitable model for studies of host–parasite coevolution, especially African cichlids and their ectoparasitic monogeneans of the genus *Cichlidogyrus* Paperna, 1960 (see Vanhove et al., 2016 for review). In the Neotropical Region, cichlids serve as definitive and second intermediate hosts for a number of helminth parasites, especially trematodes and monogeneans (Vidal-Martínez et al., 2001; Thatcher, 2006; Kohn et al., 2007; Cohen et al., 2013).

In contrast, the cestode fauna of these fishes seems to be depauperate, especially compared with that of trematodes, nematodes, monogeneans, and acanthocephalans (Moravec, 1998; Vidal-Martínez et al., 2001; Pinacho-Pinacho et al., 2015). The following adult cestodes have been reported from native cichlids in the Neotropical Region: the proteocephalideans (based on molecular data, Caira et al. [2014] proposed a new cestode order,

Onchoproteocephalidea, which includes the previously recognized order Proteocephalidea Mola, 1928; the new order has to be better circumscribed using morphological traits; see Alves et al., 2015.) *Sciadocephalus megalodiscus* Diesing, 1850; *Proteocephalus macrophallus* Diesing, 1850; *Proteocephalus microscopicus* Woodland, 1935, all in *Cichla* spp.; *Proteocephalus gibsoni* Rego and Pavanelli, 1991 in *Astronotus ocellatus* (Agassiz, 1831) and *Geophagus brasiliensis* (Quoy and Gaimard, 1824) from South America, and the bothriocephalidean *Bothriocephalus pearsei* Scholz, Vargas-Vázquez and Moravec, 1996 in *Cichlasoma urophthalmum* (Günther, 1862) from the Yucatan Peninsula in southeastern Mexico (Scholz et al., 1996; Rego et al., 1999a; Rego, 2000).

During a large-scale survey of the helminth parasites of teleost fishes in the Peruvian Amazonia (see de Chambrier et al., 2006, 2015a; Mendoza-Palmero et al., 2015), proteocephalidean tapeworms with peculiar morphology that could not be assigned to any of the recognized genera were found in *Cichlasoma amazonarum* Kullander, 1983 from a fish farm in Peru. These tapeworms are described as a new species, and a new genus is proposed to accommodate them. In addition, congeneric tapeworms were found in another cichlid, *Hypsophrys nicaraguensis* (Günther, 1864), during a survey of helminth parasites of freshwater fishes from Guanacaste Province in Costa Rica. These cestodes are also described as a new, second species of the newly erected genus.

MATERIALS AND METHODS

A total of 31 *Cichlasoma amazonarum* from different localities around Iquitos, Peru, were examined in 2005, 2008, and 2009, but only 3 fish provided by an exporter of ornamental fish, Edgard Panduro in Iquitos, were infected with proteocephalideans.

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phalidean tapeworms. Cestodes found were gently washed in saline and fixed immediately with hot, almost boiling 4% formaldehyde solution (= 4% formalin) and subsequently stored in 70% ethanol. A few posterior proglottids (a piece about 2 mm long) of the holotype were cut off and placed in molecular-grade ethanol for DNA sequencing before fixation of this worm (= hologenophore according to Pleijel et al., 2008) with formalin.

In Costa Rica (Guanacaste Province), a total of 3 *H. nicaraguensis* (Perciformes: Cichlidae) from Lake Arenal were examined for tapeworms. Live worms found in 1 *H. nicaraguensis* were removed from the intestine, washed in saline, fixed in hot water, and preserved in formalin.

Specimens used for morphological study were stained either with Mayer's hydrochloric carmine solution or with acetocarmine, dehydrated in an ethanol series, cleared with eugenol (clove oil) or methylsalicylate, and mounted in Canada balsam. Pieces of the strobila were embedded in paraffin wax, cross-sectioned at 12–15 µm, stained with Weigert's hematoxylin, and counterstained with 1% eosin B following the protocol outlined by de Chambrier (2001). For comparison, voucher specimens of *S. megalodiscus* Diesing, 1850 from *Cichla monoculus* Agassiz, 1831 (Perciformes: Cichlidae), Amazon River at Iquitos, Peru (host field No. PI 185a) collected by T. Scholz on 21 April 2005 (Natural History Museum, Geneva, Switzerland – MHNG-PLAT 37332), and Paraná River at Porto Rico, Brazil, collected by A. A. Rego on May 1998 (MHNG-PLAT 24621) were studied.

For scanning electron microscope (SEM) observations, 2 scoleces of each new species were prepared by the procedure outlined by Kuchta and Cairn (2010) and examined using a Zeiss 940A electron microscope (Carl Zeiss, Oberkochen, Germany) at the Natural History Museum in Geneva and a JEOL JSM 6700F scanning electron microscope (Jeol Ltd., Tokyo, Japan) at the Institute of Parasitology, BC AS CR, České Budějovice, Czech Republic. Microthrix terminology follows Chervy (2009).

Total genomic DNA (gDNA) from a few posterior (gravid) proglottids of the holotype of *C. gillesi* was extracted using a QIAamp DNA Blood kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The protocol for PCR amplification of the large subunit nuclear ribosomal RNA gene (*lsrDNA*, D1–D3 domains) and sequencing were done as outlined in Brabec et al. (2012). Contiguous sequences were assembled using Geneious version R8 (<http://www.geneious.com/>; Kearse et al., 2012) and submitted to GenBank. The new sequence of the *lsrDNA* obtained for *C. gillesi* was aligned with those of Clade A of de Chambrier et al. (2015b) using the E-INS-i algorithm of the program MAFFT (Katoh and Standley, 2013) implemented in Geneious. The alignment (1,129 base pairs [bp]) was trimmed to match the shortest sequence prior to phylogenetic analyses.

The model of evolution Tamura and Nei (TrN + G + I) was chosen using the Akaike Information Criterion implemented in jModelTest v0.1.1 (Posada, 2008). Phylogenetic trees were estimated by maximum-likelihood (ML) and Bayesian inference (BI) analysis. For ML analyses, the program RAxML v7.0.4 (Stamatakis, 2006) was used. A GTRGAMMAI substitution model was used for ML analyses, and 1,000 bootstrap replicates were run to assess nodal support; models less complex than the GTR model are not implemented in RAxML. For the BI analyses,

the implemented model was GTR + G + I because the less-complex TrN model (substitution rates = 3) is not implemented in MrBayes. BI analysis was performed using MrBayes v3.2 (Ronquist et al., 2012), with 2 runs and 4 chains (one cold, three heated) per run. The Metropolis-coupled Markov chain Monte Carlo (MC3) was run for 10 million generations, sampled every 1,000 generations, and the first 2,500 samples were discarded as burn-in (25%). The outputs of MrBayes were examined with Tracer v1.4 (Rambaut and Drummond, 2007) to check for convergence of different parameters, to determine the approximate number of generations at which log-likelihood values stabilized, and to identify the effective sample size (EES > 200) for each parameter and the estimated magnitude of model parameters in individual and combined runs. Posterior probabilities (PPs) of clades were obtained from the 50% majority rule consensus of sampled trees after excluding the initial 25% as burn-in.

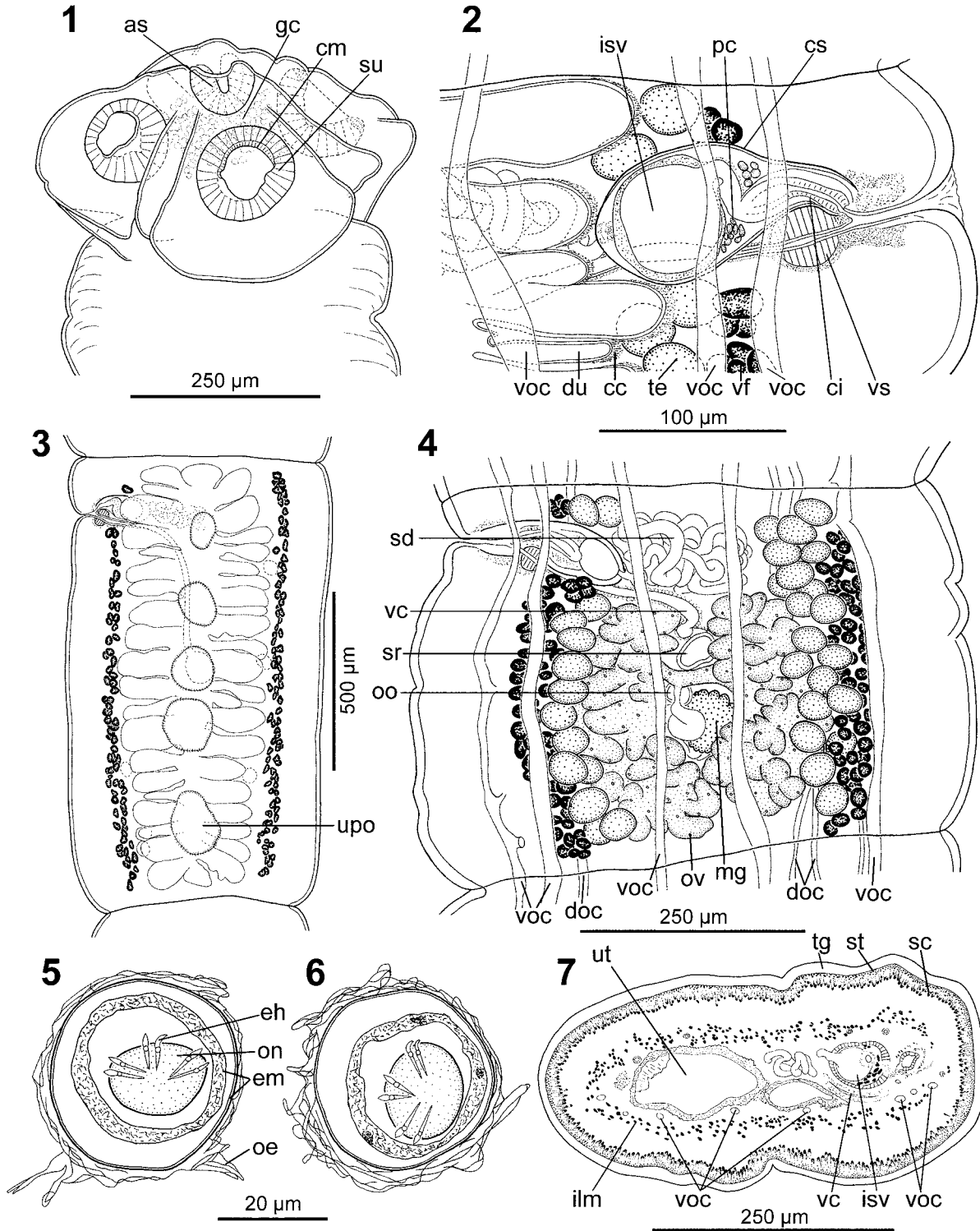
Acronyms of collections: CNHE – National Helminthological Collection of Mexico, Institute of Biology, National Autonomous University of Mexico, Mexico City, Mexico; IPCAS – Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic; MHNG-PLAT – Natural History Museum, Geneva, Switzerland.

DESCRIPTION

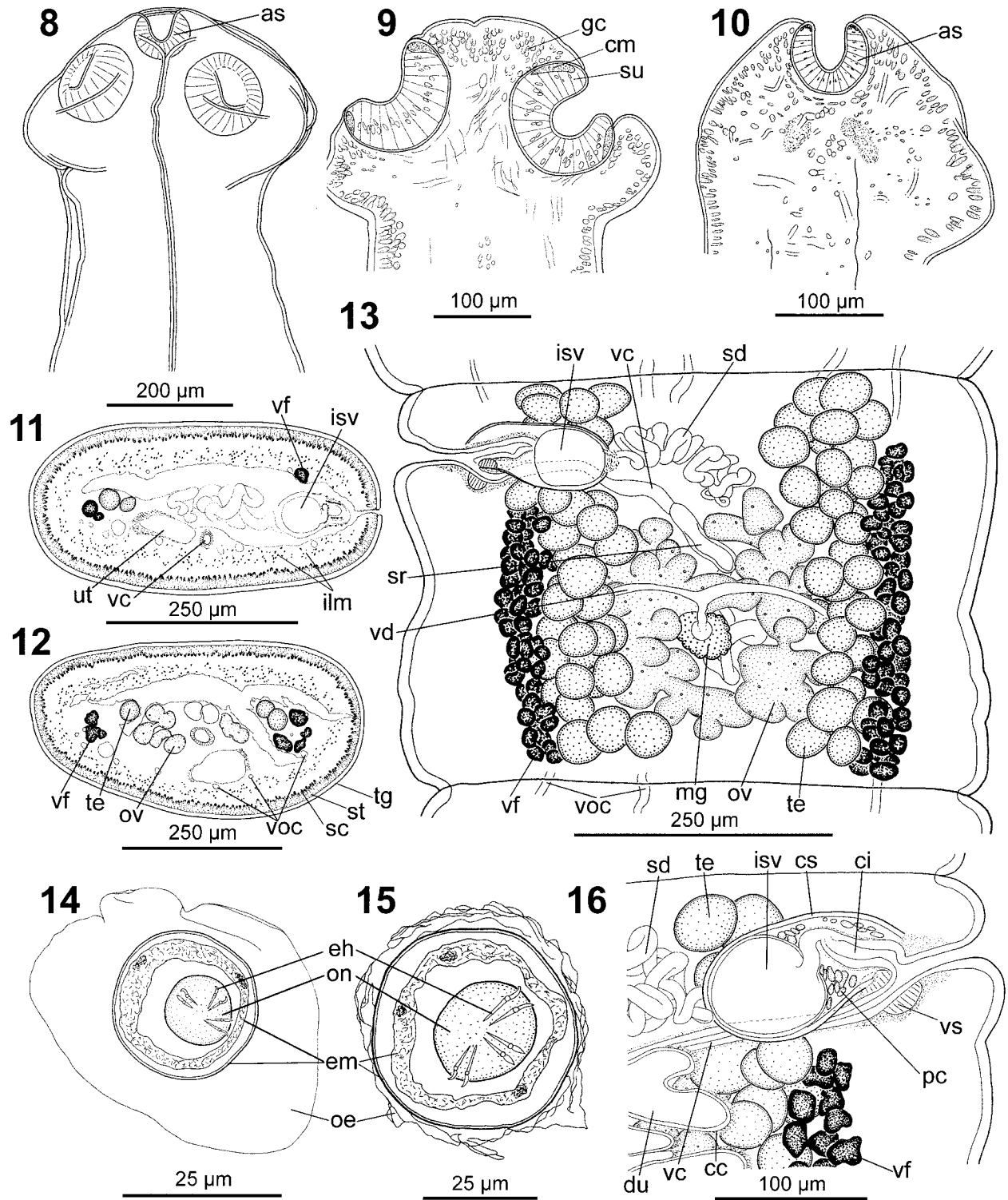
Cichlidocestus n. gen.

(Figs. 1–21)

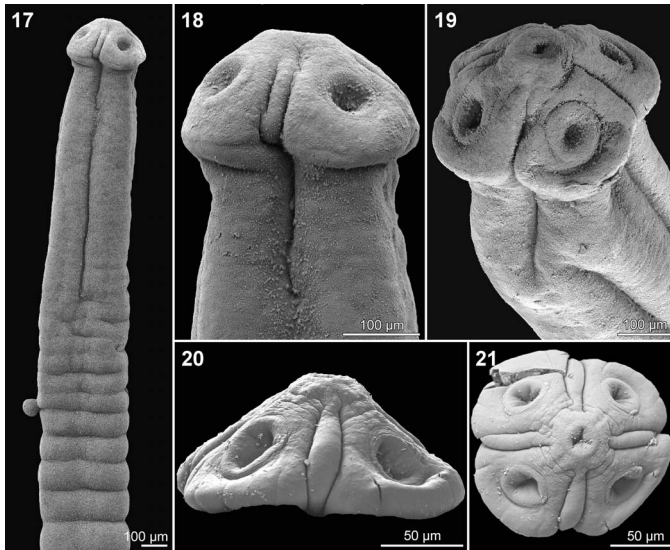
Diagnosis: Proteocephalidae, Proteocephalinae. Testes, ovary, vitellarium, and uterus medullary. Small-sized worms. Strobila acraspedote, with immature and mature proglottids wider than long, pregravid proglottids wider than long to longer than wide, and gravid proglottids longer than wide. Scolex without metascolex, rosette-like, with 4 prominent lobes posteriorly, separated from one another by longitudinal grooves, each lobe bearing central, small uniloculate sucker. Apex of scolex with muscular sucker. Inner longitudinal musculature well-developed, formed by small bundles of muscle fibers. Ventral osmoregulatory canals numerous (8–10), in medulla just beneath fibers of inner longitudinal musculature; 2–4 dorsal canals in medulla. Testes medullary, in 3 to 4 layers and 2 separate lateral fields, with some testes reaching posterior margin of proglottids, thus being posterior to ovary or even vitelline follicles. Vas deferens (internal sperm duct) enlarged in cirrus-sac to form voluminous, spherical internal seminal vesicle filling almost entirely proximal half of cirrus-sac. Vaginal canal short, opening posterior to cirrus-sac, with circular vaginal sphincter in its terminal (distal) part. Genital atrium deep and narrow, irregularly alternating, near anterior margin of proglottids. Ovary large, medullary, bilobed, with narrow ovarian isthmus almost in center of proglottid and folliculate (grape-like) lateral wings that may reach to vitelline follicles; ovary occupies middle and posterior thirds of median portion of mature proglottids. Vitelline follicles forming 2 lateral bands, not reaching anterior margin of proglottids. Uterus ventral, with lateral diverticula, with Type 2 of uterine development of de Chambrier et al. (2004a). Parasites of Neotropical cichlids (Perciformes: Cichlidae). Type species: *Cichlidocestus gillesi* n. sp. (Figs. 1–7, 20, 21). Another species: *Cichlidocestus janikae* n. sp. (Figs. 8–19).



FIGURES 1–7. *Cichlidocestus gillesi* n. g., n. sp. from *Cichlasoma amazonarum*, Peru, line drawings. (1) Scolex, dorsal view. (2) Terminal genitalia, ventral. (3) Gravid proglottid, ventral; note ventral uterine openings. (4) Pregravid proglottid, ventral; the uterus is not drawn and the ventral osmoregulatory canals are not all figured. (5, 6) Eggs. (7) Cross section at level of the cirrus-sac. (1, 2, 4–7 = holotype – MHNG-PLAT 63132; 3 = paratype – IPCAS C-733). Abbreviations: as – apical sucker; cc – chromophilic cells lining uterine diverticula; ci – cirrus; cm – circular musculature; cs – cirrus-sac; doc – dorsal osmoregulatory canal; du – diverticula of uterus; eh – embryonic hooks; em – bilayered embryophore; gc – gland cells; ilm – internal longitudinal musculature; isv – internal seminal vesicle; mg – Mehlis' gland; oe – outer envelope; on – oncosphere; oo – oocapt; ov – ovary; pc – prostatic cells; sc – subtegumental cells; sd – sperm duct; sr – seminal receptacle; st – subtegumental muscle fibers; su – sucker; te – testes; tg – tegument; upo – uterine pore-like opening; ut – uterus; vc – vaginal canal; vf – vitelline follicles; voc – ventral osmoregulatory canal; vs – vaginal sphincter.



FIGURES 8–16. *Cichlidocestus janikae* n. g., n. sp. from *Hypsophrys nicaraguensis*, Costa Rica, line drawings. (8) Scolex, dorsal view (paratype 3 – CNHE No. 10044). (9) Frontal sections of scolex, showing the anterior circular musculature of suckers (paratype 4 – MHNG-PLAT 94086). (10) Frontal sections of the scolex, showing an apical sucker (paratype 4 – MHNG-PLAT 94086). (11, 12) Cross sections at level of the cirrus-sac and ovary, respectively (holotype – IPCAS C-734). (13) Pregravid proglottid, ventral (holotype – MHNG-PLAT 94085); the uterus is not drawn and the ventral osmoregulatory canals are not all figured. (14, 15) Eggs (paratype 4 – MHNG-PLAT 94086). (16) Terminal genitalia, ventral (holotype – MHNG-PLAT 94085). Abbreviations: as – apical sucker; cc – chromophilic cells lining uterine diverticula; ci – cirrus; cm – circular musculature; cs – cirrus-sac; du – diverticula of uterus; eh – embryonic hooks; em – bilayered embryophore; gc – gland cells; ilm – internal longitudinal musculature; isv – internal seminal vesicle; mg – Mehlis’ gland; oe – outer envelope; on – oncosphere; ov – ovary; pc – prostatic cells; sc – subtegumental cells; sd – sperm duct; sr – seminal receptacle; st – subtegumental muscle fibers; su – sucker; te – testes; tg – tegument; ut – uterus; vc – vaginal canal; vd – vitelline duct; vf – vitelline follicles; voc – ventral osmoregulatory canal; vs – vaginal sphincter.



FIGURES 17–21. Scanning electron micrographs of the scoleces of *Cichlidocestus janikae* n. g., n. sp. from *Hypsophrys nicaraguensis*, Costa Rica (17–19), and *Cichlidocestus gillesi* n. g., n. sp. from *Cichlasoma amazonarum*, Peru (20, 21).

Taxonomic summary

Etymology: The new genus is named according to the family of fish hosts (Cichlidae) and suffix “-cestus”, which means a tapeworm. The name is treated as masculine.

Remarks

The new genus is placed in the subfamily Proteocephalinae as defined by Rego (1994) because of the medullary position of the testes, ovary, vitelline follicles, and uterus, and the absence of a metascolex (which is, however, present in a recently erected *Frezella* Alves, de Chambrier, Scholz and Luque, 2015; see Alves et al., 2015).

Cichlidocestus n. gen. differs from the genera of the Proteocephalinae and also from all but one remaining proteocephalidean genera by the unique appearance and position of the ovary, which occupies the middle and posterior thirds of the median region of proglottids with the ovarian isthmus situated almost equatorially, i.e., at the half-length of mature proglottids. All other proteocephalideans have the ovary in the posterior third or fourth of proglottids, usually near their posterior margin (Rego, 1994), except for *S. megalodiscus* Diesing, 1850. This species differs from both species of *Cichlidocestus* most conspicuously by a different morphology of the scolex, which possesses an umbrella-like metascolex which is markedly wider than the strobila (see Rego et al., 1999b), development of the uterus which forms capsule-like formations filled with eggs in gravid proglottids (vs. simple lateral diverticula in species of *Cichlidocestus*), and number of ventral osmoregulatory canals (only 2 vs. 8–10) (A. de Chambrier and T. Scholz, unpubl. data).

Cichlidocestus can also be distinguished from all proteocephalidean genera including *Sciadocephalus* Diesing, 1850 by the presence of a spherical internal seminal vesicle that occupies the proximal half of the cirrus-sac. The new genus is also characterized by morphology of its scolex, which is tetralobed and possesses

a muscular apical sucker with a deep cavity, and the presence of several (8–10) ventral osmoregulatory canals in the medulla just beneath fibers of the inner longitudinal musculature.

Cichlidocestus gillesi n. sp.

(Figs. 1–7, 20–22)

Diagnosis (based on 4 specimens from *Cichlasoma amazonarum* in Peru including 1 scolex studied using SEM; measurements in micrometers unless otherwise stated; \bar{x} = mean, n = number of measurements): Proteocephalidae, Proteocephalinae. Total body length 11–13 mm ($n = 2$), maximum width up to 760 ($n = 3$). Strobila acraspedote, anapolytic, consisting of about 35–38 proglottids: 25–30 immature (up to appearance of spermatozoa in vas deferens), 1–2 mature (up to appearance of eggs in uterus), 6–7 pregravid (up to appearance of hooks in oncospheres), 1 gravid proglottid. Immature and mature proglottids wider than long (length:width ratio 0.32–0.66), pregravid and gravid proglottids wider than long to longer than wide (length:width ratio 0.71–2.21).

Scolex 240–245 long and 360–505 wide ($n = 3$), wider than neck (proliferation zone), 570–590 long and 410–470 wide (Figs. 1, 20, 21). Suckers spherical, 120–140 ($n = 8$) in diameter, with anterior distal margin lined with circular musculature (Fig. 1). Apical part of scolex with a muscular apical sucker, 75–80 long \times 85–90 wide, and numerous cells with granular content between suckers (Fig. 1). Apex of scolex, sucker cavities, anterior part of scolex, and proliferation zone covered with capilliform filitriches of similar appearance and density; proglottids covered with acicular filitriches (data not shown).

Inner longitudinal musculature well-developed, formed by 1 irregular row of numerous small bundles of muscle fibers (Fig. 7). Eight to 10 anastomosed ventral osmoregulatory canals situated in medulla, just beneath fibers of inner longitudinal musculature 10–20 wide (Fig. 4). Two to 3 dorsal osmoregulatory canals thick-walled, narrow, about 5 in diameter.

Testes ovoid, small, 35–45 long and 25–35 wide, in 3–4 irregular layers, 37–46 in number ($\bar{x} = 42$, $n = 5$). Testes form 2 well-separated groups, with some testes dorsal to cirrus-sac and vagina (Fig. 4); aporal group with 17–22 testes, poral group with 19–26 testes. Posteriormost testes approach, but do not reach, posterior margin of proglottids, being posterior to ovary, but not posterior to posteriormost vitelline follicles (Fig. 4). Testes disappear in first pregravid proglottids.

Vas deferens strongly coiled, with loops forming elongate field crossing median line of proglottid (Fig. 4). Cirrus-sac pyriform to elongate, thin-walled, widened towards proximal part (Fig. 2), 125–140 long and 40–70 wide ($n = 6$), cirrus-sac length:width ratio 1.95–3.5, length of cirrus-sac represents 21–28% ($\bar{x} = 25\%$, $n = 6$) of proglottid width. Internal sperm duct enlarged to form voluminous, spherical internal seminal vesicle occupying proximal 1/3–1/2 of cirrus-sac (Fig. 2). Cirrus short, muscular, reaching up to 40% of cirrus-sac length ($n = 6$). Common genital atrium narrow, deep (Fig. 2), alternating irregularly, situated near anterior margin of proglottids, at 10–18% ($\bar{x} = 12$, $n = 6$) of its length from anterior margin (Figs. 3, 4).

Ovary medullary, bilobed, with narrow isthmus situated almost equatorially, and 2 follicular (grape-like) lateral wings (Fig. 4), occupying two-thirds of median space of proglottids. Length of ovary represents 62–70% ($\bar{x} = 65\%$, $n = 11$) of proglottid length,

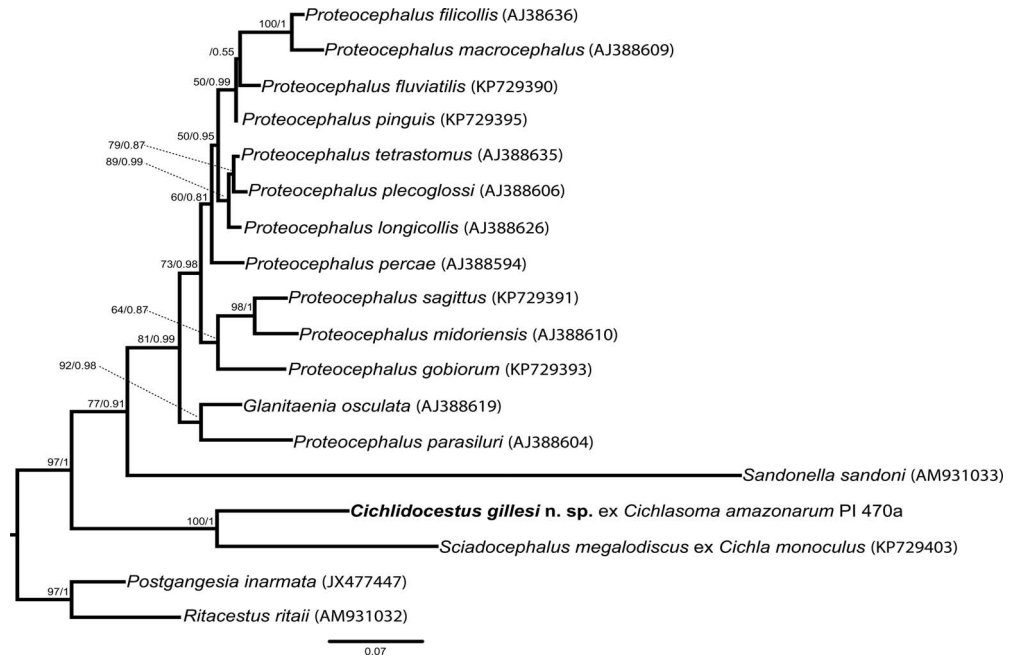


FIGURE 22. Tree resulting from Bayesian inference analysis of 28S rDNA data. Nodal support is given as bootstrap/posterior probabilities. Branch length scale bar indicate number of substitutions per site. *Cichlidocestus gillesi* n. sp. in bold.

its width representing 45–52% (\bar{x} = 48%, n = 6) of proglottid width (Fig. 4); ovary may reach laterally up to vitelline follicles. Mehlis' gland about 65–90 in diameter, representing 11–17% of proglottid width (n = 5). Relative ovarian size, i.e., percentage of ovary surface to total surface of mature or pregravid proglottids (see de Chambrier et al., 2012), about 22%. Ovary disintegrates soon after appearance of first eggs (in fourth to fifth pregravid proglottid).

Vaginal canal slightly sinuous; seminal receptacle ovoid, thick-walled, situated anterior to ovarian isthmus (Fig. 4), full of spermatozooids in mature and first pregravid proglottids; terminal (distal) part of vaginal canal (pars copulatrix vaginae) surrounded by chromophilic cells and large, spherical vaginal sphincter (Fig. 2). Vagina posterior to cirrus-sac (n = 30).

Vitelline follicles medullary, forming 2 long, narrow lateral bands, absent anterior to cirrus-sac on poral side (Figs. 3, 4). Length of bands represents 89–93% (\bar{x} = 91%, n = 7) and 83–93% (\bar{x} = 88%, n = 7) of length of proglottid on poral and aporal side, respectively, width of vitelline bands presents 11–13% of proglottid width. Uteroduct passing dorsal to ovarian isthmus, entering uterus anteriorly, at about 25% of length of proglottid from anterior margin.

Uterus medullary, with development of type 2 (see de Chambrier et al., 2004a), i.e., uterine stem present as elongated longitudinal median concentration of chromophilic cells in immature proglottids, with lumen appearing in last immature proglottids. In mature proglottids, uterine stem thin-walled, with median lumen and almost invisible long and narrow digitate diverticula. In pregravid proglottids, uterine diverticula (lateral uterine branches) thin-walled, digitate, lined with few chromophilic cells. Uterus with 16–21 lateral diverticula on each side (Fig. 3).

Eggs spherical, with hyaline outer envelope (collapsed in whole mounts); embryophore bilayered, with external layer 30–33 in diameter and internal nucleate envelope 24–25 in diameter; oncosphere 14–15 in diameter, with 6 embryonic hooks 7–8 long (Figs. 5, 6). Ripe eggs released through 4–5 pore-like uterine openings on ventral side (Fig. 3).

Taxonomic summary

Type host: *Cichlasoma amazonarum* Kullander, 1983 (Perciformes: Cichlidae), vernacular name 'bujurqui.'

Type locality: A private aquarium in Iquitos, Region of Loreto, Peru (3°12'S, 52°12'W); precise origin of fish is unknown, but they were captured near Iquitos (E. Panduro, pers. comm.).

Site of infection: Anterior intestine.

Infection rate: Three of 11 fish from a private aquarium of Edgard Panduro in Iquitos, Peru, examined by T. Scholz on 1 October 2008, were infected with 4 tapeworms (prevalence 27%; total length of infected fish 9, 12, and 12.5 cm; mean intensity 1.3; range 1–2); the remaining 20 fish from other localities around Iquitos examined by T. Scholz and A. de Chambrier in 2005 and 2009 were negative.

Deposition of specimens: Holotype (MHNG-PLAT 63202; 1 whole mount with a complete specimen from the host of the field number PI 477a), 1 paratype (MHNG-PLAT 63208; whole mount with proglottids of a specimen from the host field number PI 474d), 1 paratype (IPCAS C-733; whole mount of the anterior part and several proglottids of a complete specimen from the host field numbers PI 470); all specimens were collected on 1 October 2008 by T. Scholz in Iquitos, Peru.

Etymology: Specific name refers to Gilles Roth from the Natural History Museum in Geneva for his excellent illustrations (line drawings) of proteocephalidean cestodes made more than 20 yr ago.

Molecular data: A fragment of 1,085 bp of the 28S rRNA gene of 1 specimen of *C. gillesi* was amplified. The nucleotide sequence is available in the GenBank database (Accession No. KY403889).

Remarks

The new species is designated as the type species of the new genus because its material is of a better quality compared to specimens of the second new species from Costa Rica (see below), and molecular data are available for *C. gillesi*. The fact that hosts

of this species were found in a private aquarium in Iquitos does not enable us to locate their precise origin, but they were captured near Iquitos according to the provider of these fish (E. Panduro, Iquitos, pers. comm.). This species is typified by the morphological characteristics listed in the diagnosis of the new genus (see above) such as the peculiar appearance, size and position of the ovary with follicular, large lateral wings, which occupy middle and posterior thirds of the median region of proglottids (Fig. 4), presence of a voluminous internal seminal vesicle (Fig. 2) and several narrow, thin-walled ventral osmoregulatory canals in the medulla (Fig. 7), morphology of the scolex, which is quadrilobed and bears a muscular apical sucker with a deep cavity (Figs. 1, 20, 21), and the testes disappearing in the first pregravid proglottids. The new species was found only in 3 of 31 cichlids (overall prevalence less than 10%) examined.

A comparative analysis of partial 28S rRNA gene sequences of 1 sample of *C. gillesi* (a piece of the holotype) with available sequences of proteocephalideans has revealed the closest relationship with another parasite of Neotropical cichlids, *S. megalodiscus* (Fig. 22). This species appeared at a very basal position compared to all other Neotropical proteocephalideans in a comprehensive molecular phylogenetic analysis of de Chambrier et al. (2015b), but its position was unstable. Interestingly, species of both genera from Neotropical cichlids share some peculiar morphological traits, namely (1) a large-sized ovary that occupies more than one-half of the proglottid lengths, but disappears soon after the eggs appear in the first pregravid proglottids; (2) internal sperm duct is enlarged in the proximal part of the cirrus-sac to form spherical internal seminal vesicle; (3) the anterior rim of suckers comprises a narrow band of circular musculature, thus forming a small sphincter near sucker opening; and (4) a functional apical sucker is present.

***Cichlidocestus janikae* n. sp.**

(Figs. 8–19)

Diagnosis (based on 6 specimens and anterior parts of 2 specimens studied using SEM from H. nicaraguensis in Costa Rica; measurements in micrometers unless otherwise stated; \bar{x} = mean, n = number of measurements): Proteocephalidae, Proteocephalinae. Total body length up to 10 mm, maximum width 675 ($n = 2$). Strobila acraspedote (Fig. 17), anapolytic, consisting of about 30–32 proglottids: 23–24 immature, 1–2 mature, 4–6 pregravid, 1 gravid proglottid. Immature and mature proglottids wider than long (length:width ratio 0.28–0.69), pregravid and gravid proglottids wider than long to longer than wide (length:width ratio 0.69–1.54).

Scolex 240–260 long and 440–520 wide ($n = 3$), wider than neck (proliferation zone), 730–790 long and 330–440 wide. Scolex rosette-like, with 4 prominent lobes posteriorly, separated from one another by longitudinal grooves, each lobe bearing in center small uniloculate sucker, with internal circular musculature on its distal margin (Figs. 8–10); diameter of suckers 120–140 ($n = 12$). Apical part slightly conical, with a muscular sucker 70–75 long and 90–95 wide (Figs. 8, 10). Numerous cells with granular content situated between suckers. Apex of scolex, sucker cavities, anterior part of scolex, and proliferation zone covered with capilliform filitriches of similar appearance and density; proglottids covered with acicular spinitriches (data not shown).

Inner longitudinal musculature well-developed, formed by 1 irregular row of numerous small bundles of muscle fibers (Figs. 11, 12). Eight to 10 anastomosed ventral osmoregulatory canals situated in medulla beneath fibers of inner longitudinal musculature (Figs. 11, 12). Dorsal osmoregulatory canals paired, thick-walled, narrow.

Testes ovoid, small, 40–70 long and 35–45 wide, in 3–4 irregular layers, 63–74 ($\bar{x} = 70$, $n = 3$) in number. Testes form 2 well separated groups (poral and aporal – Fig. 13), present also dorsal to cirrus-sac and vagina (Fig. 16): aporal group with 33–42 testes, and poral group with 27–38 testes. Posteriormost testes approach, but do not reach, to posterior margin of proglottids, being posterior to ovary (Fig. 13). Testes disappear in first pregravid proglottids.

Vas deferens strongly coiled, with loops forming elongated field crossing median line of proglottid (Fig. 13). Cirrus-sac pyriform to elongate, thin-walled, widened towards proximal part (Fig. 16), 110–135 long and 55–80 wide ($n = 6$), cirrus-sac length:width ratio 1.2–2.3, length of cirrus-sac represents 17–23% ($\bar{x} = 20\%$, $n = 6$) of proglottid width. Internal seminal vesicle voluminous, spherical, occupies almost entirely proximal half of cirrus-sac (Fig. 16). Cirrus short, muscular, reaching up to 40% of cirrus-sac length ($n = 6$). Common genital atrium narrow, deep (Figs. 13, 16), alternating irregularly, situated near anterior margin of proglottids, at 10–15% ($\bar{x} = 12$, $n = 6$) of their length (Figs. 13, 16).

Ovary medullary, bilobed, with narrow, equatorially situated isthmus and 2 follicular (grape-like) lateral wings, occupying middle and posterior third of median space of proglottids (Fig. 13); length of ovary represents 43–57% of proglottid length ($\bar{x} = 50\%$, $n = 12$) and its width 35–46% of proglottid width ($\bar{x} = 41\%$, $n = 7$). Mehlis' gland about 65–90 in diameter, representing 10–14% of proglottid width ($n = 5$). Relative ovarian size (see de Chambrier et al., 2012) about 18% of size of mature and pregravid proglottids. Ovary disintegrates soon after appearance of first eggs (in third or fourth pregravid proglottid).

Vaginal canal almost straight in proximal part, slightly sinuous in distal part, with an elongated thick-walled seminal receptacle full of sperms in mature and first pregravid proglottids situated anterior to ovary isthmus (Fig. 13); terminal (distal) part of vaginal canal (pars copulatrix vaginae) surrounded by chromophilic cells and large, spherical vaginal sphincter (Fig. 16). Vagina posterior to cirrus-sac ($n = 26$).

Vitelline follicles medullary, forming 2 long, narrow, lateral bands not reaching to anterior margin of proglottids; follicles absent preporally, i.e., anterior to cirrus-sac on poral side (Figs. 13, 16). Length of bands represents 63–69% ($\bar{x} = 66\%$, $n = 6$) and 68–69% ($\bar{x} = 69\%$, $n = 6$) of length of proglottid on poral and aporal side, respectively, width of vitelline bands represent 8–11% of proglottid width.

Uterus medullary, with development of type 2 of de Chambrier et al. (2004a), i.e., uterine stem present as elongated longitudinal median concentration of chromophilic cells in immature proglottids, with lumen appearing in last immature proglottids. In mature proglottids, uterine stem thin-walled, with median lumen and almost invisible, long, and digitate diverticula. In pregravid proglottids, uterine diverticula (lateral uterine branches) thin-walled, digitate, lined with few chromophilic cells. Uterus with 8–12 lateral diverticula on each side.

Eggs become progressively larger as they mature, considerably larger in gravid proglottids compared to those in most pregravid

ones. Eggs spherical, hyaline outer envelope up to 100 in diameter (measured in eggs released from unmounted proglottids to water); embryophore bilayered, with external layer 44–46 in diameter and internal nucleate envelope 36–40 in diameter; oncosphere subspherical to spherical, 22–25 long and 20–22 wide, with 6 embryonic hooks 10–11 long (Figs. 14, 15). Uterine pore-like openings not observed.

Taxonomic summary

Type and only host: *Hypsophrys nicaraguensis* (Günther, 1864) (Perciformes: Cichlidae), vernacular name ‘moga amarilla’ (host field No. 844).

Type locality: Lake Arenal, Guanacaste Province, Costa Rica (10°29'18''N, 84°50'29''W).

Site of infection: Intestine.

Infection rate: One of 3 *H. nicaraguensis* from Lake Arenal examined on 19 February 2015 was infected with a hundred tapeworms (prevalence 33%; total length of infected fish 8 cm); the remaining 37 fish from other rivers in Guanacaste Province examined in February 2015 were negative.

Deposition of specimens: Holotype MHNG-PLAT 94085 (1 whole mount with a complete specimen and cross sections) and IPCAS C-734 (cross sections); 4 paratypes (IPCAS C-734; whole mounts of 1 complete and 2 incomplete specimens) and cross sections, 1 paratype (CNHE 10044; whole mount of 1 incomplete specimen), 1 paratype MHNG-PLAT 94086 (whole mount of a complete specimen and cross sections), 1 voucher (MHNG-PLAT 94087; anterior ends of 2 specimens and cross sections); all specimens from *H. nicaraguensis* (host field No. 844).

Etymology: Specific name refers to Janik Pralong from the Natural History Museum in Geneva for her excellent technical assistance over the past 10 yr.

Remarks

Cichlidocestus janikae shares with *C. gillesi*, type species of the genus, the characteristics unique or rare among the Proteocephalidea such as the possession of a large ovary occupying two-thirds of the medial region of proglottids, with grape-like (follicular) lateral wings (Fig. 13), a peculiar morphology of the cirrus-sac, which contains a spherical internal seminal vesicle in its proximal part (Fig. 16), a quadrilobed scolex with a well-developed muscular apical sucker (Figs. 8–10, 18, 19), several anastomosed ventral osmoregulatory canals, and the testes disappearing in the first pregravid proglottids.

The new species from Costa Rica can be distinguished from *C. gillesi* by the extent of vitelline follicles on the poral side, with follicles absent anterior to the cirrus-sac and vagina (Figs. 13, 16; vs. present preporally in *P. gillesi*; see Figs. 2–4), larger eggs (diameter of the external layer of the embryophore 44–46 µm in *C. janikae* vs. 30–33 µm in *C. gillesi*) that increase during their development in the uterus in the former species (in *C. gillesi*, they do not increase markedly during their intrauterine development), fewer uterine diverticula (8–12 in *C. janikae* vs. 16–21 in the latter species), and more testes (63–74 in *C. janikae* vs. 37–46 in *C. gillesi*).

DISCUSSION

The new genus of proteocephalidean cestodes erected here is so markedly distinct from all known genera of the order that its

differentiation is easy and straightforward. The most peculiar characteristic of this genus, which has never been observed in the Proteocephalidea except for *S. megalodiscus*, is the position of the ovary, which is situated almost in the center of the proglottid and occupies as much as one-half to two-thirds of proglottid length, with the ovarian isthmus situated in the middle of the proglottid (equatorial). Within the Proteocephalidea, the ovary is always situated near the posterior margin of proglottids. Only 2 species, *Margaritaella gracilis* Arredondo and Gil de Pertierra, 2012 from the catfish *Callichthys callichthys* Linnaeus, 1758 in Argentina, and *Tejidotaenia appendiculata* (Baylis, 1947), a parasite of *Tupinambis teguixin* Linnaeus, 1758 (Reptilia) in Brazil, have the ovary situated at a distance from the posterior margin of proglottids, but it never reaches more than over the posterior third of the proglottids (Rego and de Chambrier, 2000; Arredondo and Gil de Pertierra, 2012). Since no molecular data on these two species are available, it is not clear whether this anterior position of the ovary is a homoplastic feature or if it indicates close relatedness of these three taxa from distantly related hosts (cichlids, catfishes, and a lizard). The only proteocephalidean species that has the ovary even larger in relation to the length of proglottids is *S. megalodiscus*, whose ovary may span over almost the entire length of premature and mature proglottids (A. de Chambrier and T. Scholz, unpubl. data). In species of both genera, i.e., *Cichlidocestus* and *Sciadocephalus*, the ovary disappears soon after the eggs are formed in the first pregravid proglottids.

The ovary of both species of *Cichlidocestus* is also peculiar in its structure, being composed of a narrow ovarian isthmus and large lateral wings formed by numerous follicles on the ventral and dorsal side of proglottids (grape-like). Surface of the ovary of the new species is extraordinarily large, representing as many as 18–22% of the proglottid surface, which considerably exceeds corresponding values in proteocephalideans parasitizing fishes, i.e., 5.4–20.2% (in average 12.3%) (de Chambrier et al., 2012; the extreme value 28.8% given for *Proteocephalus midoriensis* Shimazu, 1990 was found to be incorrect and it is only about 16.5%; A. de Chambrier, unpubl. data).

A follicular (grape-cluster-like) ovary is not very common among proteocephalideans, but it is present in 3 species from Neotropical catfishes (Siluriformes), namely *Brooksiella praeputialis* (Rego, Santos and Silva, 1974) from the cetopsid *Cetopsis coecutiens* (Lichtenstein, 1819), *Manaosia bracodemoca* Woodland, 1935 from the pimelodid *Sorubim lima* (Bloch & Schneider, 1801), and *Rudolphiella szidati* Gil de Pertierra and de Chambrier, 2000 from the pimelodid *Luciopimelodus pati* (Valenciennes, 1836) (Gil de Pertierra and de Chambrier, 2000; de Chambrier, 2003; de Chambrier et al., 2004b). The presence of the follicular ovary thus seems to be a result of convergent evolution in the above-mentioned taxa from catfishes and both species of *Cichlidocestus* from cichlids.

Another unique characteristic, which has never been observed in proteocephalidean cestodes but which is common in cyclophyllideans (Khalil et al., 1994), is the presence of a spherical internal seminal vesicle that fills completely the proximal part of the cirrus-sac in both new species. A somewhat similar structure, i.e., a voluminous, tightly sinuous internal sperm duct, which resembles an internal seminal vesicle, was observed by de Chambrier and Scholz (2008) in species of *Chambriella* Rego, Chubb and Pavanelli, 1999, parasites of catfishes in the

Neotropical Region, but this enlarged sperm duct is divided into several well-separated compartments (see figs. 43 and 47 in de Chambrier and Scholz, 2008). *Sciadocephalus megalodiscus* has also an enlarged internal sperm duct, but its internal seminal vesicle is much smaller and thick-walled compared to that in *Cichlidocestus* spp.

Unlike most proteocephalideans, including *S. megalodiscus* with only 2 pairs of dorsal and ventral osmoregulatory canals, both species of *Cichlidocestus* possess more canals, with 8–10 ventral canals and 2–4 dorsal ones. *Cairaella henrii* Coquille and de Chambrier, 2008, a parasite of *Norops trachyderma* (Cope, 1876) in Ecuador, and *Nomimoscolex touzeti* de Chambrier and Vaucher, 1992 from the frog *Ceratophrys cornuta* Linnaeus, 1758 in South America, have several osmoregulatory canals, but they are cortical unlike those of *C. gillesi* and *C. janikae*, which are medullary, just beneath fibers of the inner longitudinal musculature (de Chambrier and Vaucher, 1992; Coquille and de Chambrier, 2008; Figs. 7, 11, 12 in the present paper). As discussed above for the presence of a follicular ovary, the higher number of osmoregulatory canals in apparently distantly related taxa, yet from the same zoogeographical region (Neotropics), is most probably a result of convergence, i.e., this character is homoplastic.

The testes in the species of *Cichlidocestus*, especially in *C. janikae*, may reach near the posterior margin of proglottids, even posterior to the ovary and vitelline follicles, which is quite unusual among proteocephalidean cestodes. A similar posterior position of the testes was observed only in 2 species of *Rudolphiella* Fuhrmann, 1916, namely *Rudolphiella lobosa* (Riggenbach, 1895) (see fig. 95 in de Chambrier and Vaucher, 1999), and *R. szidati* Gil de Pertierra and de Chambrier, 2000 (see figs. 1 and 2 in Gil de Pertierra and de Chambrier, 2000). Also in this case, the posterior extent of the testes is most probably a homoplastic character, as it is present in distantly related taxa (see de Chambrier et al., 2015b, and Fig. 22 in the present paper for phylogenetic position of the taxa discussed above).

The scolex of both new species of *Cichlidocestus* is quadrilobed and possesses a muscular apical sucker with a deep cavity. Such a well-developed apical sucker is not common among proteocephalideans. It is present in distantly related taxa that belong to distinct lineages (see fig. 1 in de Chambrier et al., 2015b) and occurs in different fish hosts from distant zoogeographic region, such as *S. megalodiscus* from cichlids in the Neotropical region and *Glanitaenia siluri* (Goeze, 1782) from European wels *Silurus glanis* Linnaeus, 1758 (Siluriformes: Siluridae) in the Palearctic region (Rego et al., 1999a; de Chambrier and Scholz, 2016).

Both new species from cichlids also possess suckers with well-developed circular musculature on their anterior rim, and the anterior part of the scolex contains numerous gland cells. Circular musculature in suckers that may form strong sphincters is present in relatively few, but not closely related, proteocephalideans such as *Barsonella* de Chambrier, Scholz, Beletew and Mariaux, 2009 from clariid catfishes in Africa, *Megathylacoides* Jones, Kerly & Sneed, 1956 from channel catfishes (Ictaluridae) in North America, and *Megathylacus* Woodland, 1934 and *Mariauxiella* de Chambrier and Rego, 1995, both from pimelodid catfishes in South America (Rego, 1994; de Chambrier and Rego, 1995; de Chambrier et al., 2009). A narrow band of circular musculature at the anterior rim of the suckers is also present in closely related *S. megalodiscus* (unpubl. data), which possesses, however, a well-

developed, umbrella-shaped metascolex (Rego et al., 1999a). The presence of the circular musculature among distantly related proteocephalidean taxa is another example of convergent evolution of some morphological traits and indicates that the degree of homoplasy is rather common within this group of cestodes.

Similarly, gland cells were observed in the scoleces of numerous unrelated proteocephalideans, but relatively little attention has been paid to their more-precise description. It seems that at least 3 types can be recognized on the basis of their size, distribution, and position within the scolex: (1) small unicellular gland cells beneath the tegument of the scolex (see, e.g., *Proteocephalus cernuae* (Gmelin, 1790) from ruff *Gymnocephalus cernua* (Linnaeus, 1758) [Perciformes: Percidae] in Europe; see Scholz et al., 1998); (2) gland cells accumulated in the scolex apex (e.g., *Cichlidocestus* spp. described herein; Figs. 1, 8–10); and (3) gland cells of different size and density between suckers or in the posterior region of the scolex (e.g., *Ophiotaenia hylae* Johnston, 1912 [now *Australotaenia hylae*] in *Litoria aurea* (Lesson, 1827) (Anura: Hylidae); see de Chambrier, 2004).

Phylogenetic analysis of the nuclear ribosomal RNA gene (*18S*DNA) of *C. janikae* reveals that this species is not closely related to any other Neotropical proteocephalidean except *S. megalodiscus*, thus supporting erection of a new genus to accommodate these cestodes of peculiar morphology. However, the precise phylogenetic position of the new genus among proteocephalidean genera remains unclear, partly because of a long branch of *S. megalodiscus* (see de Chambrier et al., 2015b). The host of *C. janikae*, *H. nicaraguensis*, belongs to the clade amphiphophines sensu Řičan et al. (2008), i.e., 1 of the 3 main clades of Middle American cichlids, whereas *Cichlasoma amazonarum*, the host of *C. gillesi*, is a South American cichlid, and it is not closely related to any Middle American cichlids (Řičan et al., 2013, 2016).

Interestingly, cichlid fishes in Middle America have received widespread attention, and studies have focused on understanding the processes of colonization, diversification, and phylogenetic relationships of this group (Concheiro Pérez et al., 2007; Řičan et al., 2008). There are different hypotheses about the colonization of cichlids to Middle America. For example, Řičan et al. (2013) suggest that 2 colonizations of Middle-America and one of the Greater Antilles occurred from South America within a narrow time window during the Oligocene. Cichlid colonization of Central America proceeded from the north in the Early-Middle Miocene. Central America later became repeatedly fragmented during the latter half of the Miocene, which led to the formation of the present ichthyological provinces prior to the final closure of the Panama Isthmus. Choudhury et al. (2016) examined the extent to which adult helminths of freshwater fishes have been part of the Great American Biotic Interchange (GABI) by integrating information in published studies and new data from Panama with fish biogeography and Earth history of Middle America. Their review illustrated, among others, that “the helminth fauna south of the Trans-Mexican Volcanic Belt, and especially south of the Isthmus of Tehuantepec, shows strong Neotropical affinities, and that host–parasite associations follow principles of the ‘biogeographic core fauna’ in which host-lineage specificity is pronounced.” (Choudhury et al., 2016).

The present proposal of a new proteocephalidean genus with 2 new species of peculiar morphological characteristics represents

another evidence that the diversity of proteocephalidean cestodes in freshwater fishes of the Neotropical Region is still poorly known. Interestingly, the species of the new genus have patchy (disjunct) distribution. One species occurs in the Amazon River in South America (even though the precise origin of the hosts of this species could not be identified, they undoubtedly originated from the upper reach of the Amazon River around Iquitos in Peru) and the other in Central America. Future research is needed to map the actual species composition of this genus, host associations of its species, and their distribution and interrelations.

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