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A novel microhabitat for parasitic copepods: A new genus of Ergasilidae (Copepoda: Cyclopoida) from the urinary bladder of a freshwater fish

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ABSTRACT

An endoparasitic copepod is reported from the urinary bladder of a fish for the first time. Endoparasitic copepods on fish hosts are extremely rare and the impact of colonization of this novel microhabitat on the biology of the parasite is discussed. This curious association was reported from two different host families of Neotropical freshwater fishes, Erythrinidae and Cichlidae, collected from the Cristalino River, a tributary of the Araguaia River, in Brazil. The copepod is fully described using light and scanning electron microscopy. *Urogasilus brasiliensis* n. g., n. sp. represents a new genus and species of the family Ergasilidae and can be distinguished from other genera by its unique tagmosis, in which the fourth and fifth pedigerous somites and the genital double-somite are all fused to form an elongate trunk. The anal somite is the only free abdominal somite present. The pattern of leg segmentation is also unique, with legs 1 to 3 each having a 2-segmented endopod and leg 4 reduced to a single seta. The discovery of ovigerous female ergasilids in the urinary bladder of a fish is novel and this discovery represents a good model for further studies on the adaptations to an endoparasitic lifestyle.

1. Introduction

More than 2000 species of copepods parasitize marine and freshwater fishes and most are ectoparasitic: they are found all over the external body surface of the host as well as in more sheltered microhabitats that are permanently directly connected to the external environment, including the external nares, the eyes, the oral and branchial cavities, the gills and the cloaca [1]. Some fish-parasitic copepod genera are mesoparasitic including four genera of the family Ergasilidae von Nordmann, 1832: *Therodamas* Krøyer, 1864, *Paeonodes* Wilson, 1944, *Mugilicola* Tripathi, 1960 and *Majalincola* Tang & Kalman, 2008. They live with their anterior (cephalothoracic) end embedded in host tissues and their posterior trunk protruding from the host's body surface [2,3]. A few are internal parasites: some members of the family Philichthyidae Vogt, 1877, for example, inhabit the subcutaneous spaces associated with the lateral line system and skull bones of marine teleosts, while others produce invaginations by burrowing into the walls of the alimentary canal in the rectal areas [1]. However, philichthyids differ from true endoparasites by permanently retaining contact with their external environment via their pore of entry, and have thus been considered as ectoparasitic despite their virtually endoparasitic lifestyle [4]. True endoparasitic copepods live entirely within the host body cavity and, although they are known widely from invertebrate hosts, are extremely rare on fish hosts.

Here we report the first endoparasitic copepod ever found in the urinary bladder of a fish. The urinary bladder of teleosts is an accessory osmoregulatory organ where urine is retained for a period during which its ionic composition is modified. Examination of the bladder for parasites is included in standard fish necropsy protocols (for example, Kabata [5]) and a great diversity of parasites has been found in the urinary bladder of fishes, including unicellular eukaryotes from the phyla Amoebozoa and Ciliophora, myxozoans [6] (currently regarded as basal metazoans with affinities to the Cnidaria; see Holland et al. [7] for details), and flatworms from both the Monogenea and Digenea [6]. Many species are exclusively parasitic in this particular microhabitat. Copepods are overwhelmingly ectoparasitic and it might be significant that the host was a freshwater fish, given that this organ has a low osmotic permeability in freshwater fishes and the fish excretes very dilute urine compared to marine teleosts [8].

Preliminary studies showed that the copepod parasite from the bladder belonged to the family Ergasilidae. There are currently 23 genera in the Ergasilidae that utilise fish as hosts: 19 of these are ectoparasitic on the body surface, gills and in the nasal cavities of their hosts [1], and the other four are mesoparasitic [2,3]. Sixteen ergasilid genera occur in South America, 13 of them are endemic, and 63 endemic species are known from the Neotropics [9]. In Brazil, 60 species had been reported representing 15 genera of Ergasilidae, 46 of these are parasites of freshwater fishes [10,11]. Here we extend

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our knowledge of this spectacular Neotropical diversity by the description of a new genus and species from the bladder, a new microhabitat.

2. Materials and methods

We examined the urinary bladders of three non-migratory species of freshwater fish: 22 specimens of *Hoplias malabaricus* (Bloch, 1794) (Characiformes: Erythrinidae), 10 specimens of *Cichla* spp. (mix of *Cichla piquiti* Kullander & Ferreira, 2006 and *Cichla temensis* Humboldt, 1821) (Perciformes: Cichlidae), and five specimens of *Osteoglossum bicirrhosum* (Cuvier, 1829) (Osteoglossiformes: Osteoglossidae). The fish were caught in a 42-hectare permanent lagoon (13° 22′ 20.2″ S and 50° 52′ 8.5″ W) that was connected to the Cristalino River, a tributary of the Araguaia River, in the area of Bananal Island, in the state of Mato Grosso, Brazil (Fig. 1). Collections were made during October 2007 and fish were individually frozen and stored whole at minus 20 °C until examination. After thawing, the bladder was removed from the fish, dissected and examined for the presence of internal parasites using a dissecting microscope. The urinary bladder of teleosts lies dorsal to the posterior end of the gonads and can be difficult to see: we recommend locating it immediately after the visceral cavity is exposed, before the dissection of any visceral structures, to avoid injuring the bladder and losing parasites. Most copepods were found attached to the inner wall of the bladder, but since fish hosts were stored frozen, after thawing the copepods were easily detached by applying a fine jet of water to the internal wall of the bladder and by pulling the specimens off using acupuncture needles (0.25 mm of average thickness). The copepods were transferred to a vial containing absolute or 70% ethanol.

The copepods were dissected and examined in lactophenol as temporary slide preparations. Measurements were made with an ocular micrometre and drawings were made with the aid of drawing tube on a Leitz Diaplan microscope equipped with differential interference contrast. Specimens were found to be extremely fragile and often broke during recovery from the host: only entire specimens in good condition were measured. Morphological terminology follows Huys & Boxshall [12].

Material for SEM was washed in distilled water, dehydrated through a graded acetone series, critical point dried using liquid carbon dioxide as the exchange medium, mounted on aluminium stubs and sputter coated with gold–palladium. Coated material was examined on a Phillips XL30 FE Scanning Electron microscope at 5 Kv. The type material was deposited in the Coleção Carcinológica do Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, Brazil. Other representative specimens (vouchers) were deposited in the collections of the Natural History Museum, London, United Kingdom.

3. Results

3.1. Urogasilus n. g.

3.1.1. Diagnosis

Adult female body comprising cephalothorax, partly incorporating modified first pedigerous somite, free second and third pedigerous somites, long trunk derived from fusion of fourth pedigerous, fifth pedigerous, and genital-double somites. Anal somite free. Antennule 5-segmented. Antenna robust, with single terminal claw. Mandible reduced, with single distal blade. Maxillule with 1 medial and 2 distal setae. Maxilla with spinule row on syncoxa, basis tapering to trifid apex. Maxilliped absent in female. Legs 1 to 3 biramous, each with indistinctly 3-segmented exopod and 2-segmented endopod. Leg 4 represented by single seta on surface of trunk. Leg 5 absent. Egg sacs uniseriate.

3.1.2. Taxonomic summary

Type species: *Urogasilus brasiliensis* n. g., n. sp., by original designation.

Etymology: The name of the new genus, *Urogasilus*, combines the prefix *uro-* alluding to its novel microhabitat, and *-gasilus*, which is derived from *Ergasilus* von Nordmann, 1832, the type genus of the family.

3.1.3. Remarks

The adult female of the new genus has a modified body characterised by fusion of the fourth pedigerous, fifth pedigerous and genital-double somites to form a trunk which carries the free anal somite posteriorly. Ancestral abdominal somites 2 and 3 are lacking in the adult female; they may be fused, either to the trunk or to the anal somite, or they may simply not be expressed. Without information on the developmental stages we lack the evidence to conclude which of these anatomical interpretations is correct. The boundary between the first pedigerous somite and the cephalosome is modified so the female appears to have a cephalothorax incorporating the first pedigerous somite, and the second and third pedigerous somites are free. No other ergasilid shares this tagmosis. The four mesoparasitic genera of Ergasilidae all have modified bodies exhibiting fusions

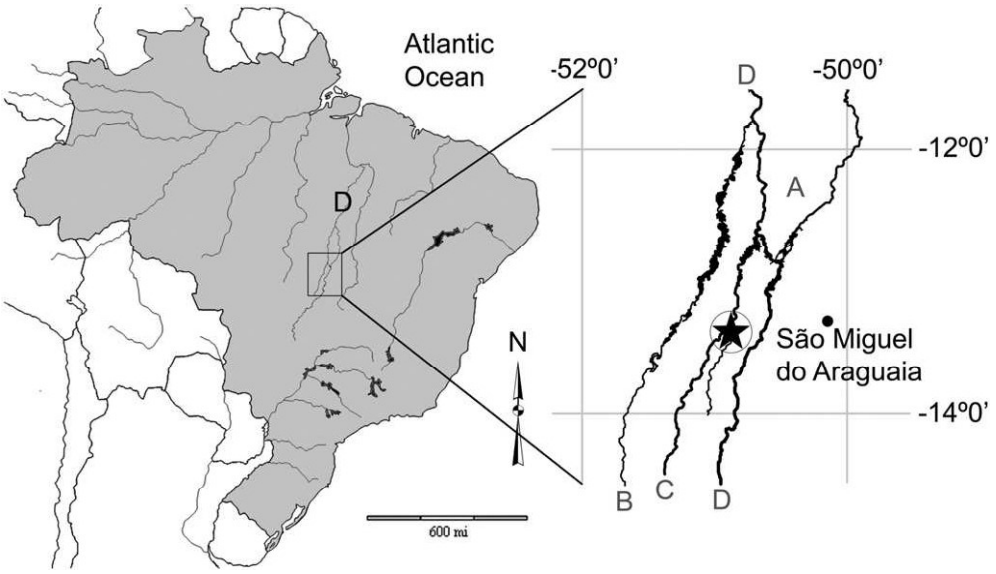

Fig. 1. Main river basins of Brazil (shaded) showing the position of the sampling locality at Araguaia River Basin (squared). Enlargement showing sampling locality on the Cristalino River. Key: star = collecting site, A = Bananal Island, B = Mortes River, C = Cristalino River, D = Araguaia River.

which result in a reduction in the number of expressed somites (see El-Rashidy & Boxshall [13] and Tang & Kalman [3]). However, the tagmosis patterns differ. Both *Therodamas* and *Majalincola* have a "neck" formed within the cephalosome, between the antennal region and mouth. In contrast, both *Mugilicola* and *Paeonodes* have a "neck" formed posterior to the mouth and anterior to the first pair of legs. In the new genus the second and third pedigerous somites are narrower than the cephalosome and the trunk, and thus appear to form a "neck", but this is located posterior to the first pedigerous somite. The term "neck" is not useful when comparing the morphology of these genera since the three types of neck are not homologous. The new genus is unique in the incorporation of the genital double-somite into a larger complex together with the fourth and fifth pedigerous somites. In all other ergasilid genera the genital double-somite remains separate from the fifth pedigerous somite.

Almost all ergasilids retain the plesiomorphic mandible with three blades [1]. Species of the mesoparasitic genus *Therodamas* and three species of *Mugilicola* have only two blades (see Kruger et al. [14], El-Rashidy & Boxshall [13] and Thatcher [10]), where known, but the mandible of the new genus is armed with just a single blade. This reduced state has been reported for a small number of *Ergasilus* species [15], but in each case the quality of the original description is questionable.

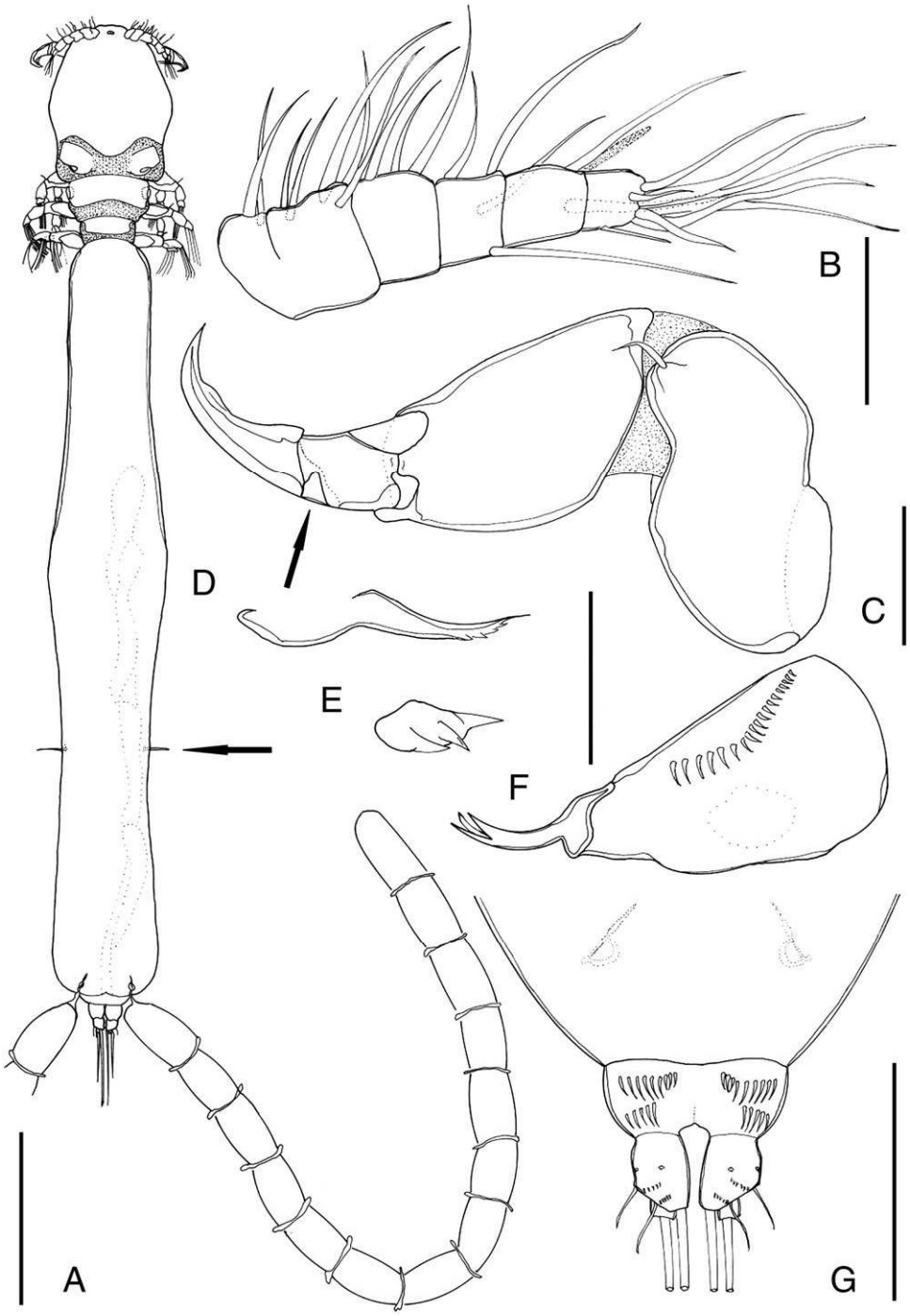

Fig. 2. *Urogasilus brasiliensis* n. g., n. sp., adult female. A — habitus, dorsal. B — antennule. C — antenna. D — mandible. E — maxillule. F — maxilla. G — anal somite and caudal rami, ventral. Scale bars: A = 200 μm, B–G = 20 μm.

Urogasilus n. g. has the fourth pair of legs reduced to a single seta. It shares the extreme reduction or loss of leg 4 with four other genera: *Mugilicola*, *Abergasilus* Hewitt, 1978, *Brasergasilus* Thatcher & Boeger, 1983 and *Rhinergasilus* Boeger & Thatcher, 1988. *Abergasilus* was originally described as with fourth legs lacking [16] but Jones [17] subsequently confirmed that leg 4 was represented by a seta. The phylogenetic analysis of ergasilid genera by Tang & Kalman [3] clustered these four genera into a clade, together with *Paeonodes* (which has a biramous leg 4). Inspection of their data matrix suggests that this clade appears to be characterized by the reduction of the fourth legs. However undue weighting is afforded to this character in the Tang & Kalman [3] analysis. In those genera in which leg 4 is represented by a seta or is absent (i.e. *Abergasilus*, *Brasergasilus*, *Rhinergasilus* and *Mugilicola*) the characters relating to the form of the exopod and endopod of leg 4 should have been coded as inapplicable. The double scoring effectively weights the derived states of leg 4, creating bias by giving additional importance to the characters. The new genus differs from *Abergasilus*, *Brasergasilus* and *Rhinergasilus* in tagmosis and in having a 2-segmented endopod in legs 2 and 3.

The lack of shared similarities either in tagmosis or in leg segmentation patterns serves to emphasise the isolated position of this parasite and justifies the establishment of the new genus.

3.2. *U. brasiliensis* n. g., n. sp.

3.2.1. Description of adult female

Body elongate (Figs. 2A, 4A), consisting of cephalosome and partially incorporated first pedigerous somite, 2 free pedigerous somites (bearing legs 2 and 3), long trunk region, and free anal somite. Cephalothorax covered by dorsal shield apparently fused with first pedigerous somite but with transverse zone of thinner, flexible cuticle separating shield from paired dorso-lateral areas of smooth cuticle representing subdivided tergite of first pedigerous somite (Figs. 2A, 4B). Dorsal shield sparsely ornamented with sensillae. Mid-frontal part of dorsal shield produced anteriorly between bases of antennules. Rostrum (Fig. 4B) weakly developed, ornamented with median pore and paired sensillae. Ventral surface of cephalosome ornamented with spinules in broad area just anterior to intercoxal sclerite of first legs (Figs. 3A, 4C). Second pedigerous somite narrower than cephalothorax, bearing paired organs (integumental windows) laterally on tergite (Fig. 2A, arrowed in 4D). Third pedigerous somite narrower than second, separated from it by wide zone of flexible cuticle (Fig. 2A), and bearing small tergite surrounded laterally and posteriorly by broad stretches of flexible cuticle (Figs. 2A, 4A). Trunk about 7.6 times longer than broad: comprising fourth and fifth pedigerous somites plus genital double-somite and bearing paired, slit-like genital apertures near posterolateral corners (Figs. 2A, 4G). Cuticle of trunk with longitudinal striations especially marked in posterior half (Figs. 2A, 4A, G), cuticle smoother and less strongly striated in anterior half. Anal somite free, deeply incised in midline; about 1.1 times broader than long: surface cuticle non-striated, ornamented with 2 spinule rows on ventral surface (Fig. 2G, 4F) and row of tiny denticles along dorsal part of posterior margin (arrowed in Fig. 4G). Caudal rami about 1.5 times longer than wide: ornamented with 2 oblique rows of spinules and 2 pores ventrally; armed with 4 setae, all naked (Fig. 2A). Mean body length from anterior margin of cephalothorax to posterior margin of caudal rami 1.19 mm (range 0.98 to 1.45 mm, based on 5 specimens). Egg sacs uniseriate, up to 1.9 mm in length, containing up to 18 cylindrical eggs: mean egg length 84.9 μm (range 57.7–149.4), width 45.8 μm (range 29.2–59.6). Detached egg sacs found in bladder contained up to 16 cylindrical eggs, mean length 102.3 μm (93.5–121.2), width 73.5 μm (64.9–77.9), or up to 38 rounded eggs, mean length 54.5 μm (48.5–65.8), width 87.7 μm (84.9–91.8).

Antennule 5-segmented (Fig. 2B), first segment with incomplete suture line; setal formula 7: 3: 4: 2 + ae: 7. Antenna 4-segmented (Fig. 2C) comprising robust coxobasis armed with naked seta distally, 3-segmented endopod and curved terminal claw. Second segment (first endopodal segment) unarmed. Third segment (second endopodal segment) shorter and narrower than preceding segment, unarmed. Fourth segment (third endopodal segment) reduced to incomplete hoop of sclerotized cuticle (arrowed in Fig. 2C). Claw curved, with fossa located just distal to middle of concave margin.

Mandible (Fig. 2D) tapering into long distal blade, ornamented with 4 small teeth along posterior margin. Maxillule (Fig. 2E) lobate, armed with 2 distal setae and 1 medial seta. Maxilla (Fig. 2F) comprising large tapering syncoxa and basis: syncoxa ornamented with long row of conspicuous spinules; basis with broad base tapering to claw, trifid at tip. Maxilliped absent, as in all female ergasilids.

Legs 1–3 (Fig. 3A–C) biramous with indistinctly 3-segmented exopods and 2-segmented endopods. Intercoxal sclerites slender,

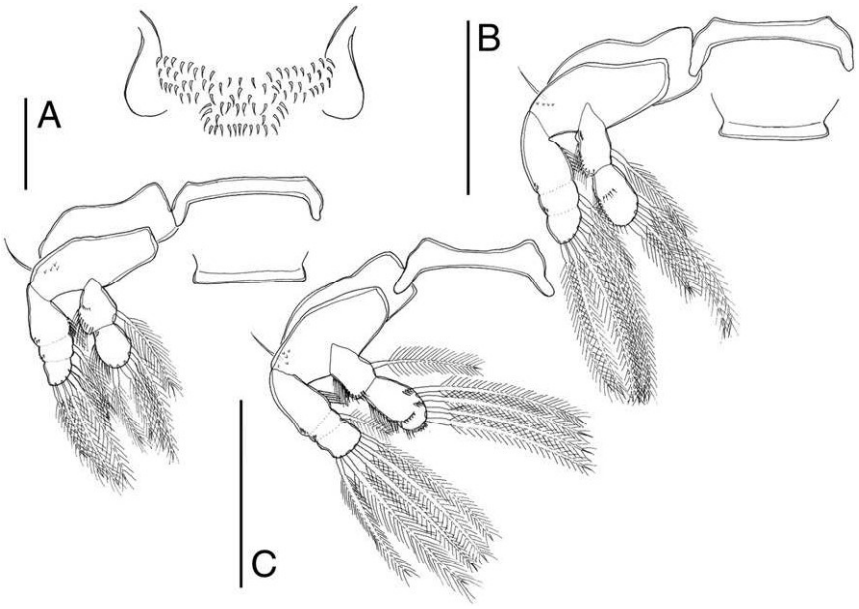

Fig. 3. *U. brasiliensis* n. g., n. sp., adult female. A — Leg 1. B — Leg 2. C — Leg 3. Scale bars = 50 μm.

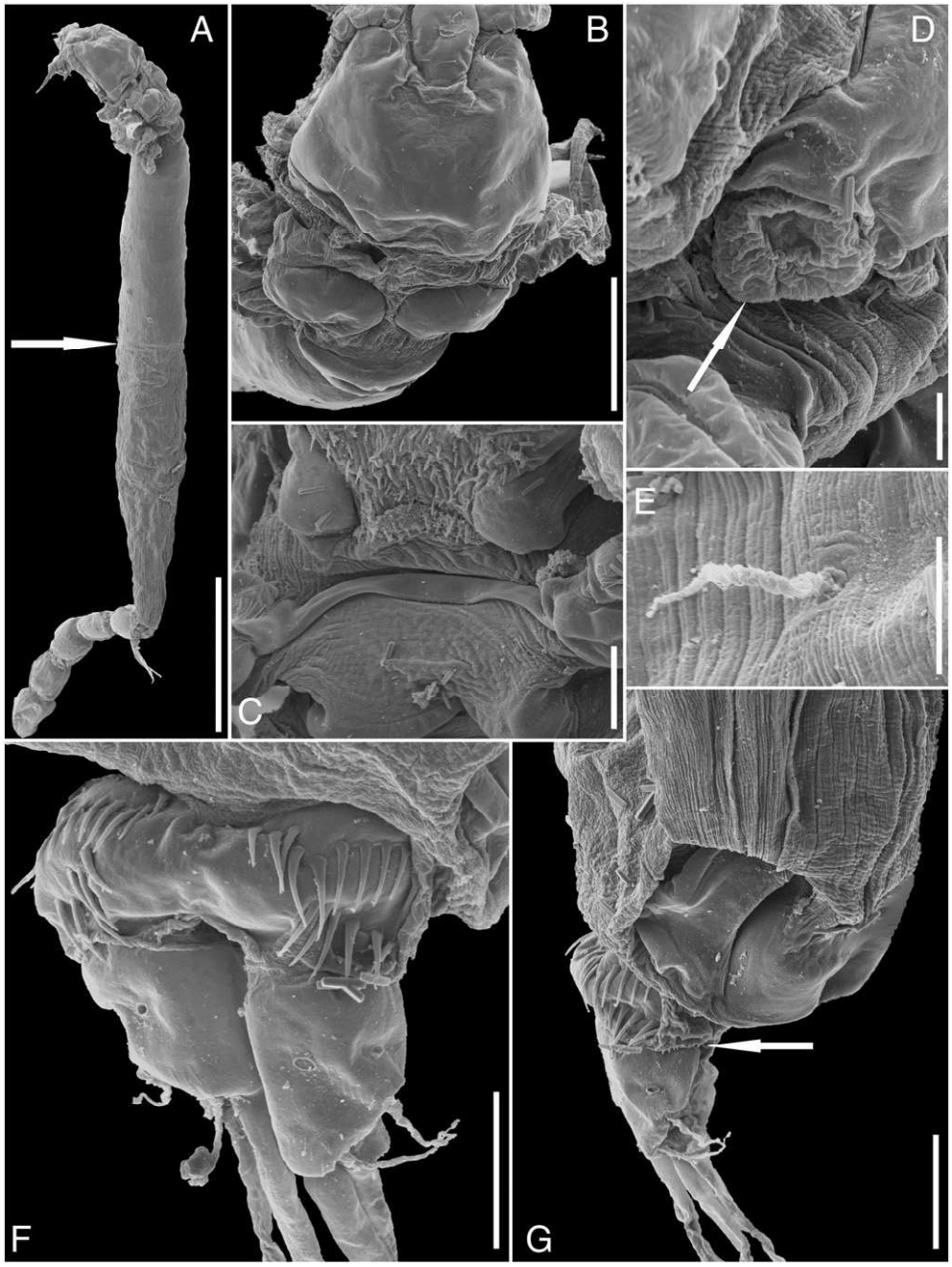

Fig. 4. *U. brasiliensis* n. g., n. sp., adult female, scanning electron micrographs: A — whole animal, lateral view showing change in nature of integument in mid-trunk region (arrowed). B — dorso-frontal view of cephalothorax showing rostral region and subdivided tergite of first pedigerous somite. C — ventral ornamentation of cephalothorax and first interpodal plate. D — lateral organ on tergite of second pedigerous somite (arrowed). E — seta representing leg 4. F — ventral spinular ornamentation of anal somite. G — dorsal ornamentation of anal somite (arrowed) and left genital aperture on trunk. Scale bars: A = 200 μm, B = 50 μm, C, F = 10 μm, D = 5 μm, E = 5 μm, G = 10 μm.

interpodal plates weakly developed, unornamented (Fig. 4C). Spine and seta formula as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0–0	1–0	0–0; 0–1; 5	0–1; 4
Leg 2	0–0	1–0	0–0; 0–1; 6	0–1; 4
Leg 3	0–0	1–0	0–0; 0–1; 5	0–1; 4

Leg 1 with outer margin of first endopodal segment ornamented with rows of long and short spinules; second endopodal segment with long spinules along outer margin. Legs 2 and 3 with outer margin of first endopodal segment ornamented with rows of long and short spinules; second segment with outer row of spinules becoming stronger distally and with double row distally in leg 2. Spinules present on second segment in legs 2 and 3. Legs 1 to 3 with inner margin of first exopodal segment with row of long spinules.

Leg 4 represented by isolated seta (Fig. 4E) on ventrolateral surface of trunk at about 70% of trunk length (arrowed in Fig. 2A).

Fifth leg absent.

3.2.2. Taxonomic summary

3.2.2.1. Type host. Hoplias malabaricus (Bloch, 1794).

3.2.2.2. Type locality. A permanent lagoon located at 13° 22′ 20.2″ S and 50° 52′ 8.5″ W which is connected to the Cristalino River, a

Table 1
Morphometric comparison of *Urogasilus brasiliensis* n. g., n. sp. from different hosts collected from the Cristalino River. Values given are mean (range) in micrometres, except for number of eggs in attached sac (L = length; W = width; max = maximum value observed).

Characters	*Hoplias malabaricus* (type host; N = 5)	*Cichla* spp. (additional host; N = 2)
Body (L)	1192.4 (978.8–1454.6)	769.4 (701.2–837.7)
Trunk (L)	912.4 (743.5–1157.7)	550.6 (512.9–588.2)
Trunk (W)	138.1 (84.8–119.9)	91.8 (89.4–94.1)
Anal somite (L)	15.5 (13.5–17.0)	10.9 (10.7–11.1)
Anal somite (W)	17.2 (14.7–18.6)	13.2 (13.0–13.4)
Caudal rami (L)	16.9 (15.1–17.9)	13.5 (11.11–14.8)
Caudal rami (W)	11.6 (9.2–12.8)	10.5 (10.2–11.1)
Egg (L)	84.9 (57.7–149.4)	76.5 (56.5–89.4)
Egg (W)	45.8 (39.2–59.6)	56.9 (51.8–61.2)
Eggs in sac (min–max)	5–18 eggs	12–13 eggs
Egg sac (max L)	1900	1500

tributary of the Araguaia River, in the area of Bananal Island, Mato Grosso, Brazil.

3.2.2.3. Site on host. Urinary bladder.

3.2.2.4. Type material. Holotype female taken from urinary bladder of *H. malabaricus*, stored in the collections of MZUSP, Registration No. MZUSP 25080; paratype material comprising 29 intact females and 22 incomplete specimens from urinary bladder of *H. malabaricus*: 14 intact paratypes stored in the collections of MZUSP, Registration No. MZUSP 25081; 11 intact paratypes stored in collection of Natural History Museum, London, Registration No. BMNH 2011.1241-1251; 1 intact paratype mounted on aluminium stub and sputter coated with gold–palladium stored in collection of Natural History Museum, London, Registration No. BMNH 2012.262. Incomplete specimens also stored in collections; 8 incomplete specimens in MZUSP, Registration No. MZUSP 25082; and 12 incomplete specimens in the Natural History Museum, London, Registration No. BMNH 2011.1252-1262. Remaining material – 3 intact females and 2 incomplete specimens – stored in collection of CEPTA/ICMBio, Pirassununga, São Paulo, Brazil.

3.2.2.5. Additional material. 3 Complete and 3 incomplete females from urinary bladder of 10 specimens of *Cichla* spp. (a mix of *Cichla piquiti* Kullander & Ferreira, 2006 and *Cichla temensis* Humboldt, 1821) caught at the same locality. Two intact females stored in MZUSP, Registration No. MZUSP 25084; 1 intact female stored in the Natural History Museum, London, Registration No. BMNH 2011.1264. Three incomplete specimens were stored in MZUSP, Registration No. MZUSP 25085. Vials with detached egg sacs also stored in collections as additional material. Two vials with detached egg sacs of *U. brasiliensis* n. g., n. sp. from the urinary bladder of *H. malabaricus* were stored: one in MZUSP, Registration No. MZUSP 25083, the other in the Natural History Museum, London, Registration No. BMNH 2011.1263. One vial with detached egg sacs of *U. brasiliensis* n. g., n. sp. from the urinary bladder of *Cichla* spp. stored in MZUSP, Registration No. MZUSP 25086.

3.2.2.6. Etymology. The species name, *brasiliensis*, refers to the country where this species was collected.

3.2.3. Remarks

There are some minor differences between the material from *H. malabaricus* and that from the two *Cichla* species. The females from *H. malabaricus* are larger (see Table 1) than those from *Cichla* spp. and there is no overlap between the two sets. In addition there are slight differences in body proportions: the trunk in females from *H. malabaricus* is 6.6 times longer than wide compared to only 6.0 times longer in females from *Cichla* spp., and the dorsal cephalothoracic shield has angular lateral margins in females from *H. malabaricus* but more evenly convex margins in females from *Cichla* species. No morphological differences were observed in limb setation patterns or in the surface ornamentation of spinules on the limbs and anal somite.

3.3. Novel microhabitat and host–parasite relationship

The adult females of *U. brasiliensis* n. g., n. sp. were found to use the urinary bladder as a specific habitat in their freshwater fish hosts. The bladder is a completely new microhabitat for parasitic copepods. The new copepod causes macroscopic lesions in the wall of the urinary bladder which appear as multifocal petechial haemorrhages. These are associated with changes in the shape of the distal part of the parasitized bladder from rounded to diamond-shaped, and with thickening of the internal bladder wall (Fig. 5A–C). The lesions may be caused by the feeding activity of the parasites and by its attachment to the wall of the bladder. The prevalence of *U. brasiliensis* n. g., n. sp. was 86% (19 of 22 fishes) in *H. malabaricus* (an erythrinid), considerably higher than the 30% (3 from 10) found in the *Cichla* spp. (cichlids). The urinary

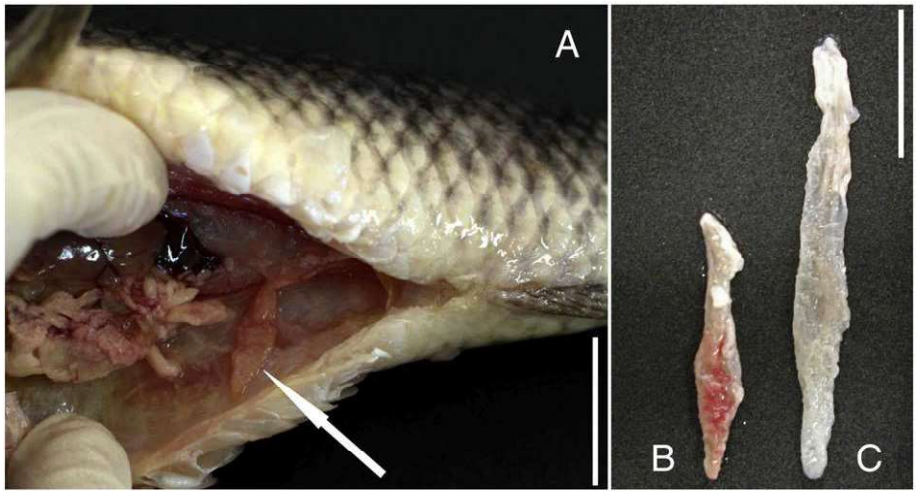

Fig. 5. A — urinary bladder of *Hoplias malabaricus in situ* (arrowed). B — appearance of bladder infected by *U. brasiliensis* n. g., n. sp., (total length of fish = 26.2 cm). C — appearance of uninfected bladder (total length of fish = 27.0 cm). Scale bars = 20 mm.

bladders of all specimens examined of *O. bicirrhosum* (an osteoglossid) were uninfected.

4. Discussion

The life history of the Ergasilidae is unique amongst the Copepoda and two basic feeding patterns by adult females have been proposed [18]. The first pattern is the typical one found in most ergasilids: early development from hatching through the naupliar phase takes place in the plankton, copepodid stages have never been found on the fish and it is likely that development through to sexual maturity takes place away from the fish [1]. After mating, the adult females only locate and infect a suitable host and begin the final, parasitic phase of the life cycle, continuing to produce eggs while attached to the host until they die [18]. The second pattern points to the possibility that ergasilid females feed and reproduce without permanent attachment on their host and has been proposed to explain the presence of ovigerous female ergasilids swimming freely in the plankton [18]. We infer, from the highly modified body morphology of females *U. brasiliensis* n. g., n. sp., that it exhibits the typical life cycle pattern and that the mated adult female is probably the infective stage. This must enter the bladder of its host by penetrating the urinary or urogenital aperture. In most teleosts the outlet of urine is guarded by one or more sphincters allowing periodic urination [19] and it seems likely that the gradient of urine concentration could serve as an attractant to the mated female copepods.

Most females collected were ovigerous and the eggs they carried were relatively large, but no nauplii were found. Ergasilid nauplii hatch from the egg sac and the first stage nauplius larva survives on its yolk reserves and does not feed (see Alston et al. [20]). According to Cameron & Wood [19], the urine of *H. malabaricus* was found to be steady-state acid excretion and may be stored in the bladder for some time prior to periodic natural discharge. It is likely that nauplii of *U. brasiliensis* n. g., n. sp. hatch and are retained in the bladder until being eliminated with the urine of the host.

Rohde [21] regarded that physiological and morphological factors are involved in microhabitat selection by parasites, and that the selection of narrow microhabitats is shaped mainly by increased mating opportunities, rather than by interspecific competition. However, since mating takes place prior to infection in ergasilids, we infer that colonization of the bladder as a novel microhabitat by *U. brasiliensis* n. g., n. sp., was driven by other factors. The unusual microhabitat by *U. brasiliensis* n. g., n. sp. may impose physiological challenges, particularly with regard to osmoregulation in this copepod. Such challenges are analogous to those experienced by copepods that move between marine and freshwater salinity regimes. Experimental evaluation of salinity tolerance in the free-living copepod *Eurytemora affinis* (Poppe, 1880) has shown that tolerance of low salinity stress is genetically determined while tolerance of higher salinities is not [22]. *U. brasiliensis* n. g., n. sp. might be a suitable model for comparative studies of such physiological adaptations in copepods, since it experiences opposing osmotic stresses at different stages of development.

The paired lateral organs on the tergite of the second pedigerous somite present in females of the new genus are integumental windows. Integumental windows are non-sensory organs that have been considered to be sites of ion exchange, aiding in the osmoregulation of brackish and freshwater copepods [23]. We have unpublished evidence showing that such organs are present in many South American ergasilids belonging to several other genera, and we consider that their presence in *U. brasiliensis* n. g., n. sp. is unlikely to be related to the unique microhabitat exploited by this parasite.

Urogasilus n. g. is the only known genus with endoparasitic adults amongst the ergasilids parasitic on vertebrates. Despite its remarkable modifications for permanent attachment to the host, the morphology of the adult female appears to be little modified for its endoparasitic existence. This phenomenon was previously noted in *Trebius shiinoi* Nagasawa, Tanaka & Benz, 1998, an unusual siphonostomatoid copepod regarded as an ectoparasitic-endoparasite, which was reported from the uterine lining and on the embryos within the uteri of Japanese angelshark and on embryos of the clouded angelshark [24].

U. brasiliensis n. g., n. sp. appears to have restricted habitat requirements, expressed as site (the urinary bladder) but not host specificity (parasitic on fish hosts from Erythrinidae and Cichlidae). Despite their smaller size the parasites from *Cichla* spp. do not differ significantly from the type material of *U. brasiliensis* n. g., n. sp. as described above based on material from *H. malabaricus*. In the absence of supporting characters from limb setation or ornamentation, we interpret the variation in body size and head shape as probably host related, although further investigation is recommended. Future studies of these endoparasitic ergasilids will help us to better understand how this novel host–parasite system functions.

Endoparasitic copepods on fish hosts are rare and the discovery of ovigerous female ergasilids in the urinary bladder is new to science. Study of the morphological and physiological adaptations of the adult females may help us better understand the evolution of an endoparasitic lifestyle on fish hosts, and provides an interesting parallel with the colonization of freshwater by copepods with marine origins. We recommend that fish parasitologists include examination of the bladder of the host for the presence of copepods in their routine protocol.

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