



Monogeneans of the catfish *Pimelodus blochii* Valenciennes (Siluriformes: Pimelodidae) from the Brazilian Amazon, with a description of a new species of *Ameloblastella* Kritsky, Mendoza-Franco & Scholz, 2000 (Monogenea: Dactylogyridae)

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Abstract *Ameloblastella amazonica* n. sp. (Monogenea: Dactylogyridae) collected on the gills of *Pimelodus blochii* Valenciennes (Siluriformes: Pimelodidae) from Rivers Acre and Iaco, State of Acre (Brazil) is described. The new species differs from the congeners mainly by the morphology of the dorsal bar, i.e. slightly U-shaped bearing a posteriorly directed flap, arising from the anterior edge of its middle third. The most morphologically similar congeners, *A. paranaensis* (França, Isaac, Pavanelli &

Takemoto, 2003) and *A. satoi* Monteiro, Kritsky & Brasil-Sato, 2010, resemble *A. amazonica* n. sp. regarding the general structure of the copulatory complex; however, besides other features, they also differ from the new species because their hooks are similar in size (vs hooks with three different sizes). In addition to the dorsal bar lacking a flap, *A. amazonica* n. sp. can be differentiated from all congeners based on the combination of the following features: size of hooks (one or two different sizes), shape of the dorsal bar (other than slightly U-shaped), anchor points largely passing the level of superficial root (vs not or slightly passing it), number of MCO rings (other than 3–4) and structure of the articulation between the MCO base and the accessory piece (other than thick, medium-sized, smooth and tubular). *Demidospermus leptosynophallus* Kritsky & Gutierrez, 1998 and *Scleroductus yuncensi* Jara & Cone, 1989 were also found infesting *P. blochii*, both representing new host and locality records.

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Introduction

The Amazon basin represents one of the most diverse biomes for fish species in the world (Nelson et al., 2016). In this sense, these organisms considered as hosts, may harbour a rich fauna of parasites including several taxa (Hechinger & Lafferty, 2005; Poulin, 2014). However, the biodiversity of these parasites is underestimated as is the case of the Monogenea (see Mendoza-Palmero et al., 2015).

Dactylogyridae Bychowsky, 1933 represents a large and speciose family within the Monogenea and is composed by highly host-specific parasites (Boeger & Vianna, 2006; Cohen et al., 2013). The dactylogyrid genera *Amelloblastella* Kritsky, Mendoza-Franco & Scholz, 2000, *Demidospermus* Suriano, 1983 and *Scleroductus* Jara & Cone, 1989 include parasites mostly infesting catfish (Siluriformes) from South and Central America (Mendoza-Franco & Scholz, 2009; Kritsky et al., 2013; Mendoza-Franco et al., 2016). Currently, *Amelloblastella* and *Demidospermus*, both parasitic on the gills, comprise 10 and 30 valid species, respectively (Mendoza-Franco & Scholz, 2009; Mendoza-Franco et al., 2016; Acosta et al., 2017; Franceschini et al., 2018). *Scleroductus*, which parasitises the body surface mainly from pimelodid fishes (Pimelodidae) in South America, allocates only three valid species (Kritsky et al., 2013).

Although a large number of taxonomic studies have been dedicated to the above-mentioned genera, their biodiversity potential is enormous (Mendoza-Palmero et al., 2015) and should be explored, since the taxonomic knowledge represents the basis for understanding further biological issues (Padial et al., 2010; Poulin, 2014). During parasitological surveys on *Pimelodus blochii* Valenciennes (Pimelodidae), collected in two rivers from the Brazilian Amazon, State of Acre, specimens of monogeneans were found on the gills of the fish. Detailed morphological examination revealed that these parasites belong to three different species of three different genera, one of which we assigned to *Amelloblastella*, as a new species described herein.

Materials and methods

One hundred and sixty specimens of *P. blochii* were collected with the aid of local fisherman in the upper

River Acre, near the municipality of Rio Branco (10°1'59.53"S, 67°52'4.93"W), and in the River Iaco, near the municipality of Sena Madureira (9°04'18.35"S, 68°39'12.56"W), State of Acre (Brazil), from June 2015 to May 2017. Fish were kept alive in sterile water tanks until necropsy in the Laboratory of Aquiculture of the Instituto Federal de Educação, Ciências e Tecnologia do Acre (acronym IFAC). Host nomenclature and classification follow Froese & Pauly (2019).

Gills were removed, immediately placed in vials containing hot water (60°C), gently shaken and transferred to Petri dishes containing formaldehyde solution to a final concentration of 5%. Parasites were collected with the aid of a thin probe under a stereomicroscope. In order, to study the sclerotised structures, some specimens were mounted in Gray and Wess medium and others were stained with Gomori's trichrome stain and mounted in Canada balsam for observation of the internal anatomy (Boeger & Vianna, 2006). Observations using light microscopy, drawings, and measurements were made with the aid of a Leica DM 5500B microscope equipped with Nomarski interference contrast, a drawing tube attached and a camera Leica DFC495 with the software Leica LAS (version 4.0). Measurements, all in micrometers, represent straight-line distances, except for the male copulatory organ (MCO), between extreme points of the structures and are expressed as the range followed by the mean and number (n) of measured structures in parentheses. Numbering and distribution of hook pairs follow Mizelle & Price (1963); orientation of the MCO coils, (i.e. counter-clockwise or clockwise) was determined as suggested by Kritsky et al. (1985). Base on the results of Mendoza-Palmero et al. (2015), we refrain from using the subfamily hierarchy within the Dactylogyridae.

For comparative purposes the following specimens, deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), were examined: *Amelloblastella paranaensis* (França, Isaac, Pavanelli & Takemoto, 2003) (referred to as *Pseudovancleaveus paranaensis*) (CHIOC 34588a, b and 34598b) and *Demidospermus leptosynophallus* Kritsky & Gutierrez, 1998 (referred to as *D. mandi*) (CHIOC 34586b, 34587c, 34589, 34590, 34591, 34592, 34593a-b).

Type- and voucher specimens were deposited in the CHIOC and in the Coleção Zoológica da Universidade Federal de Mato Grosso do Sul (acronym ZUFMS).

Subclass Polyonchoinea Bychowsky, 1937
Order Dactylogyridea Bychowsky, 1937
Family Dactylogyridae Bychowsky, 1933
Genus *Ameloblastella* Kritsky, Mendoza-Franco & Scholz, 2000

***Ameloblastella amazonica* n. sp.**

Type-host: *Pimelodus blochii* Valenciennes (Siluriformes: Pimelodidae).

Type-locality: River Iaco (9°04'18.35"S, 68°09'12.56"W), municipality of Sena Madureira, State of Acre, Brazil.

Other locality: River Acre (10°01'59.53"S, 67°52'4.93"W), municipality of Rio Branco, State of Acre, Brazil.

Type-material: The holotype (CHIOC 40020a) and 4 paratypes (CHIOC 40020b–e) were submitted to the Coleção Helminológica do Instituto Oswaldo Cruz. One paratype (ZUFMS-PLA00030) was submitted to the Coleção Zoológica da Universidade Federal de Mato Grosso do Sul.

Site on host: Gills.

Prevalence and abundance: Prevalence: 3.7% (3 infected fish/80 examined in River Iaco); 32.5% (26 infected fish/80 examined in River Acre). Mean abundance: 0.05 ± 0.3 (Iaco River); 1.1 ± 3.8 (River Acre).

Etymology: The specific epithet refers the macrorregion of the type locality.

Description (Fig. 1)

[Based on 14 specimens: 10 mounted in Gray and Wess medium and 4 stained with Gomori's trichrome.] Body 229–288 (265; $n = 10$) long; greatest width 82–98 (93; $n = 10$) at mid-level of germarium (Fig. 1A). Cephalic region rounded, with indistinct cephalic lobes and 3 pairs of head organs; cephalic glands not observed (Fig. 1A). Eye-spots absent. Pharynx subspherical, $23\text{--}30 \times 15\text{--}20$; (27×18) ($n = 9$), followed by oesophagus, $c.50$ long (Fig. 1A). Haptor sub-hexagonal, $32\text{--}61$ (38; $n = 5$) long, $41\text{--}81$ (65; $n = 5$) wide (Fig. 1A). Anchors dissimilar in size and shape (Fig. 1B, C). Ventral anchors composed of well-developed superficial root, short deep root, straight shaft and point not passing level of superficial root tip, $31\text{--}33$ (32; $n = 10$) long (Fig. 1B). Dorsal anchors with well-developed superficial and deep

roots, straight shaft and point slightly passing level of superficial root tip, $32\text{--}35$ (33; $n = 10$) long (Fig. 1C). Ventral bar $42\text{--}52$ (47; $n = 9$) long, slightly curved, rounded in lateral ends, bearing posteromedial projection (Fig. 1D). Dorsal bar $47\text{--}63$ (57; $n = 9$) long, slightly U-shaped, rounded in lateral ends, with posteriorly directed flap arising from its anterior edge of middle third (Fig. 1E). Hooks dissimilar in size and similar in shape, composed of 2 subunits, shank with rounded proximal end, erect thumb and curved shaft ending in distal point (Fig. 1F–H). Hook pair 1, $13\text{--}15$ (14; $n = 4$) long (Fig. 1G); pairs 5, $8\text{--}13$ (9; $n = 3$) long (Fig. 1F); pairs 2–4, 6 and 7, $18\text{--}22$ (20; $n = 9$) long (Fig. 1H). FH loop in all hooks about 10% of shank length (Figs. 1F–H). Male copulatory organ (MCO) coiled, with 3–4 counterclockwise rings, its small base articulated with accessory piece by medium-sized, thick, smooth, tubular structure; diameter of proximal ring $9\text{--}11$ (10; $n = 9$) (Fig. 1I). Accessory piece $15\text{--}18$ (16; $n = 9$) long, with ventral median groove serving as guide for part of MCO (Fig. 1I). Germarium, $100\text{--}125$ (111; $n = 3$) long, $20\text{--}50$ (31; $n = 3$) wide, ventrally overlapping testis (difficult to observe). Seminal receptacle subspherical, median, dorsally superposed to anterior end of germarium (Fig. 1A). Vagina opening on sinister margin of body at about its mid-length, consisting of sinuous and poorly sclerotised thin tube (Fig. 1A). Seminal vesicle well-developed, subspherical and adjacent to pair of subspherical prostatic reservoirs dissimilar in size; all posterior to MCO. Vas deferens, oviduct, oötype and uterus not observed. Vitelline follicles dense, coextensive with caeca (Fig. 1A).

Remarks

The present specimens were allocated in the genus *Ameloblastella* mainly because of the absence of eye-spots, the presence of a medial process in the ventral bar, the MCO is composed by counterclockwise rings and its base being articulated with the accessory piece (Kritsky et al., 2000). Although the testis was difficult to observe, it was possible to confirm that the organ dorsally overlaps the germarium, consequently agreeing with the generic diagnosis of *Ameloblastella* (see Kritsky et al., 2000).

The dorsal bar bearing one median flap is present only in the new species and in the most closely related congener *A. paranaensis*, a parasite of pimelodid

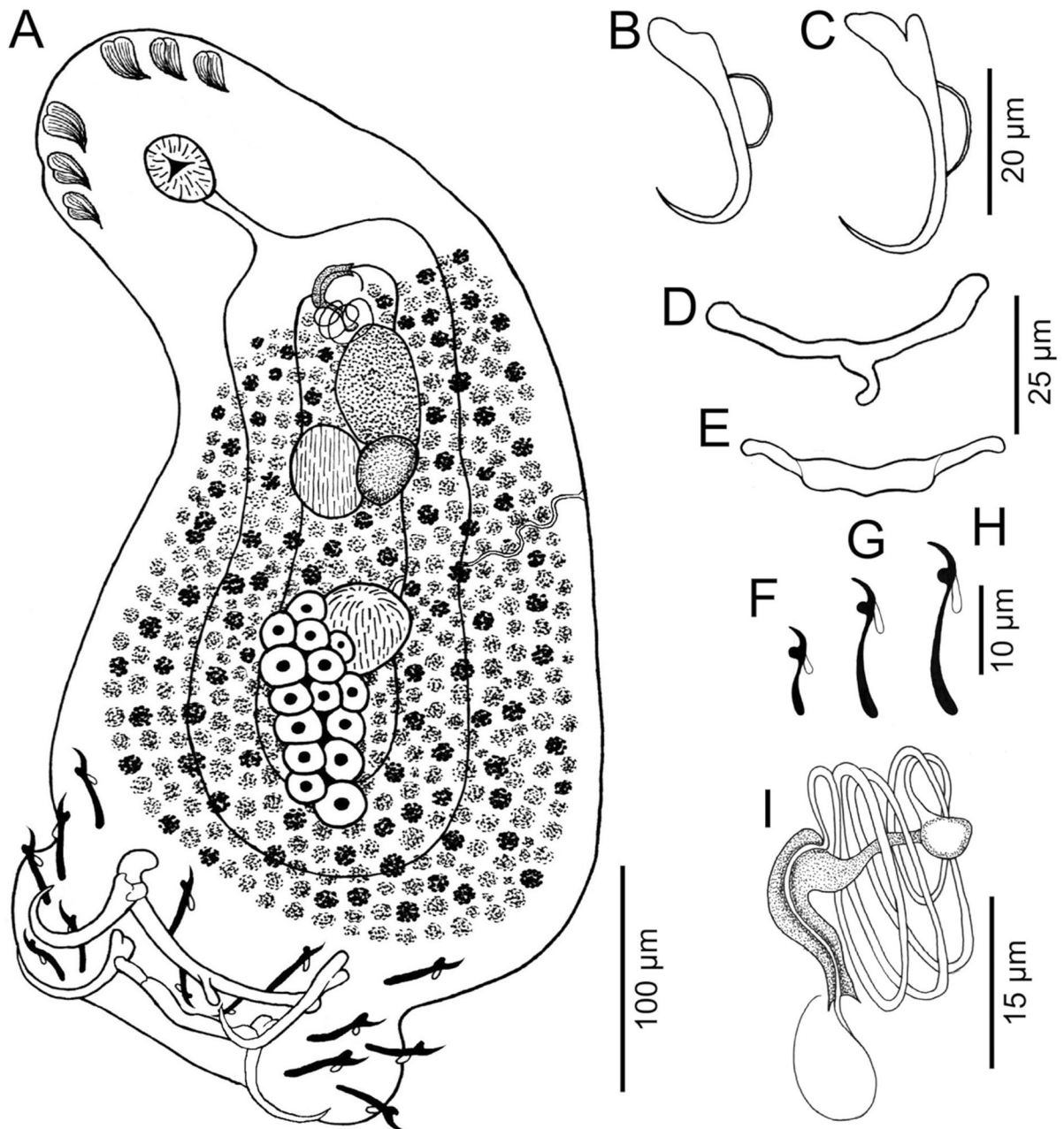


Fig. 1 *Ameloblastella amazonica* n. sp. ex *Pimelodus blochii*, collected in River Iaco, State of Acre, Brazil. Holotype. A, Whole mount (ventral view); B, Ventral anchor; C, Dorsal anchor; D, Ventral bar (ventral view); E, Dorsal bar (ventral view); F, Hook of pair 5; G, Hook of pair 1; H, Hook of pairs 2–4, 6 and 7; I, Copulatory complex (ventral view)

fishes from the Rivers Paraná and São Francisco, Brazil (França et al., 2003; Monteiro et al., 2010). However, *A. amazonica* n. sp. differs from *A. paranaensis* based on the shape of the dorsal bar (slightly U-shaped vs pronounced U-shaped), the origin of its flap (in the anterior edge vs in the

posterior edge) and by having hooks of three different sizes (vs all hooks of similar size) (see Monteiro et al., 2010). All these features were confirmed in *A. paranaensis* after observation of the type-specimens (CHIOC 34588a, b; 34598b). Moreover, hooks with similar size were also described in *A. chavarriai*

(Price, 1938), *A. edentensis* Mendoza-Franco, Mendoza-Palmero & Scholz, 2016, *A. mamaevi* (Kritsky & Thatcher, 1976), *A. satoi* Monteiro, Kritsky & Brasil-Sato, 2010 and *A. unapi* Mendoza-Franco & Scholz, 2009, and hooks with two different sizes were described in *A. unapioides* Mendoza-Franco, Mendoza-Palmero & Scholz, 2016 and *A. peruensis* Mendoza-Franco, Mendoza-Palmero & Scholz, 2016 (see Kritsky et al., 2000; Mendoza-Franco & Scholz, 2009; Mendoza-Franco et al., 2016). However, all the above species differ from *A. amazonica* n. sp. based on the size of the hooks in addition to the dorsal bar structure. Although *A. formatrium* Mendoza-Franco, Mendoza-Palmero & Scholz, 2016 and *A. platensis* (Suriano & Incrovaia, 1995) are the only two congeners having hooks of three different sizes as in the new species, their dorsal bars lack the median flap and are V-shaped (Suriano & Incrovaia, 1995; Kritsky et al., 2000; Mendoza-Franco et al., 2016) (vs slightly U-shaped bearing a median flap).

The MCO and the accessory piece also distinguish *A. amazonica* n. sp. from the remaining congeners as follows. *Ameloblastella chavarriai*, *A. formatrium*, *A. peruensis* and *A. unapi* have 2.5, 2, 5.5 and 13–14 counterclockwise rings in the MCO, respectively (vs 3–4 in the new species) (Kritsky et al., 2000; Mendoza-Franco & Scholz, 2009; Mendoza-Franco et al., 2016). The structure articulating the base of MCO with the accessory piece in *A. paranaensis*, *A. peruensis*, *A. platensis*, *A. satoi* and *A. unapioides* is thin and filamentous, in *A. edentensis* is a zig-zag tube, in *A. formatrium* is a very short thin tube and in *A. unapi* is a long tube bearing several small sclerotised spines, contrasting to *A. amazonica* n. sp. that has a thick, medium-sized, smooth and tubular articulation between the MCO base and the accessory piece (Suriano & Incrovaia, 1995; Kritsky et al., 2000; Mendoza-Franco & Scholz, 2009; Monteiro et al., 2010; Mendoza-Franco et al., 2016). The accessory piece in *A. chavarriai* has two subunits vs single in the new species (Kritsky et al., 2000).

The anchor points in *A. amazonica* n. sp. are at the same level or slightly passing the superficial root tip, differentiating it from *A. formatrium*, *A. mamaevi*, *A. peruensis*, *A. satoi* and *A. unapioides* that have at least one pair of anchors with points largely passing the level of the superficial root tip (Kritsky & Thatcher, 1976; Monteiro et al., 2010; Mendoza-Franco et al., 2016).

In addition to *A. amazonica* n. sp., other two monogenean species were found infecting *P. blochii* in the present study, *D. leptosynophallus* (accession number ZUFMS-PLA00031) on the gills and *S. yuncensi* Jara & Cone, 1989 (accession number ZUFMS-PLA00029) on the body surface. The identity of *D. leptosynophallus* was confirmed after comparison with the specimens deposited in the CHIOC (CHIOC 34586b, 34587c, 34589, 34590, 34591, 34592, 34593a–b). Unfortunately, we had no access to the types of *S. yuncensi*, but its original description has sufficient and clear morphological details (see Jara & Cone, 1989) making it possible to confirm the specific status of the present specimens. The photomicrographs showing the diagnostic features of *D. leptosynophallus* and *S. yuncensi*, collected in the present study are provided in the Supplementary Figures S1 and S2.

Discussion

Although the present specimens of *S. yuncensi* showed a larger body and longer anchors and bars, the morphology of all sclerotised structures along with the morphometry of hooks and MCO were highly similar when compared with the type-material (see Table 1 and Jara & Cone, 1989). These differences may be accounted as intraspecific variation and are possibly a consequence of dissimilarities on host and parasite microhabitat attributes.

Since *D. leptosynophallus* and *S. yuncensi* have their diagnostic features well-represented in the literature (see Jara & Cone, 1989; Kritsky & Gutiérrez, 1998; França et al., 2003; Cohen & Kohn, 2008), we refrain from describing and representing these species in detail, but a comparative table with their measurements and information regarding hosts and localities is given (see Table 1), as well as their diagnostic features are provided in photomicrographs in the Supplementary Figures S1 and S2.

Considering the high biodiversity potential of both fish and monogeneans in the Neotropical region, it is acceptable that our current knowledge on their species richness and host-parasite relationships is poor. An example is that the Pimelodidae, one of the endemic families of Siluriformes in the Neotropics, allocates 134 species with only 25 examined for monogeneans (Aguar et al., 2017). The present results also illustrate this situation, in which *D. leptosynophallus* and

Table 1 Host, geographical distribution (river, country) and the most important comparative measurements (as ranges in μm) of *Scleroductus yuncensi* and *Demidospermus leptosynophallus*. The measurements are based on the newly collected specimens and those from the type-series. The type-host and type-locality are in bold

	<i>Scleroductus yuncensi</i> (n = 6)	<i>Scleroductus yuncensi</i> (n = 6)	<i>Demidospermus leptosynophallus</i> (n = 10)	<i>Demidospermus leptosynophallus</i> (n = 16)
Host: locality (reference)	<i>Pimelodus blochii</i> ^a : Acre, Brazil (present study)	<i>Pimelodella yuncensis</i>^b: Chicama, Perú (Jara & Cone, 1989); <i>Pimelodus albicans</i> ^a and <i>P. maculatus</i> ^a : La Plata, Argentina (Gutiérrez & Martorelli, 1999a, b); <i>Leporinus copelandii</i> ^c : Guandu, Brazil (Azevedo et al., 2010)	<i>Pimelodus blochii</i> : Acre, Brazil (present study)	<i>Bergiaria westermanni</i>^a: La Plata, Argentina (Kritsky & Gutiérrez, 1998); <i>Pimelodus maculatus</i> ^a : Guandú, Brazil (Azevedo et al., 2010); <i>Iheringichthys labrosus</i> ^a , <i>Pimelodus</i> sp. ^a and <i>Pimelodella</i> sp. ^b : Paraná, Brazil (França et al., 2003; Cohen & Kohn, 2008; Takemoto et al., 2009)
Body length	440–667	400–470 ^d	320–549	405–648
Body maximum width	93–163	90 ^d	79–96	122–163
Pharyngeal bulb width	35–42	35–45 ^d	21–31	34–40
Posterior pharyngeal bulb width ^c	42–57	–	–	–
Testicle length × width	26–30	–	40–65 × 26–33	71–103 × 26–33
Germarium length × width	12–12	–	37–56 × 20–27	38–57 × 31–42
MCO length ^f	12–16	15–16 ^d	65–79	75–95
Acessory piece	–	–	35–45	62–65
Dorsal anchors length	49–142	75–78 ^d	42–50	35–41
Ventral anchors length	–	–	58–63	34–44
Dorsal bar length	–	–	57–76	63–78
Ventral bar length ^g	14–40	12–18 ^d	73–100	80–90
Hook length	19–22 (all similar)	20–21 ^d (all similar)	pair 1: 38–41; pair 2: 26–31; pair 3: 16–19; pair 4: 15–19; pair 5: 23–25; pair 6: 24–34; pair 7: 40–43	pair 1: 39–46; pair 2: 31–33; pair 3: 16–18; pair 4: 16–18; pair 5: 22–25; pair 6: 22–25; pair 7: 39–46

^aPimelodidae; ^bHeptapteridae; ^cAnostomidae; ^dMeasurements based on the type material; ^eExclusive of *Scleroductus*; ^fMeasured as diameter in *Scleroductus*; ^gCorresponds to the superficial bar of *Scleroductus*

S. yuncensi represent new host and locality records, being reported for the first time in the Brazilian Amazon.

The genus *Ameloblastella* includes host-specific species mostly infecting pimelodid fishes, and its geographical distribution ranges from South Mexico, Central and South America, extending to southern Brazil (Boeger & Vianna, 2006; Aguiar et al., 2017). The new species represents the eleventh species in the genus, the third from Brazil and the first parasitising a pimelodid catfish from the Brazilian Amazon, thus improving our knowledge of the biodiversity aspects of this genus of ectoparasites.

It seems that, at least in some cases, the close relatedness between host taxa overshadows the influence of environment in the geographical distribution of some monogenean species (Braga et al., 2015). It can be noted for *D. leptosynophallus* and *S. yuncensi*, which infect mostly fishes belonging to the family Pimelodidae, also occur in the closely related Hep-tapteridae (Vergara et al., 2008) from different and unconnected river basins (see Table 1). It should be mentioned that the report of *S. yuncensi* infecting *Leporinus coperlandii* Steindachner (Characiformes: Anostoidae) (Azevedo et al., 2010), may represent a misidentification because the parasite seems to be specific to catfishes (Siluriformes). However, we had no response from the collection where the vouchers have been deposited (Coleção Helminológica do Instituto de Biociências de Botucatu, accession number CHIBB 036L) and thus could not confirm the identification of this material.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures involving animals were permitted and were strictly according to the rules of the Sistema de Autorização e Informação em Biodiversidade (acronym SISBIO, No. 60899–1) and the Committee on Ethics of Animal Experiments of the Embrapa Amapá (Protocol No. 002- CEUA-CPAFAP).

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