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TWO NEW SPECIES OF *PAVANELLIELLA* (MONOGENEA, DACTYLOGYRIDAE) PARASITIC ON PIMELODID FISHES FROM MOGI GUAÇU RIVER, SOUTHEASTERN BRAZIL, AND NOTES ON THE MORPHOLOGY OF *P. PAVANELLII*

DOS NUEVAS ESPECIES DE *PAVANELLIELLA* (MONOGENEA, DACTYLOGYRIDAE) PARÁSITOS DE PECES PIMELÓDIDOS DEL RIO MOGI GUAÇU, SUDESTE DE BRASIL Y NOTAS SOBRE LA MORFOLOGÍA DE *P. PAVANELLII*

Julio C. Aguiar[1], Paulo S. Ceccarelli[2] & José L. Luque[3]
[1] Curso de Pós-Graduação em Ciências Veterinárias, Universidade Federal Rural do Rio de Janeiro, BR-465, Km 7, CEP.: 23.890–000, Caixa Postal 74.508, Seropédica, Rio de Janeiro, Brazil.
[2] Centro Nacional de Pesquisa da Conservação de Peixes Continentais, Instituto Chico Mendes para Conservação da Biodiversidade, Rodovia Pref. Euberto Nemésio Pereira de Godoy, Km 6,5, CEP 13.630-970, Caixa Postal: 64, Pirassununga–SP, Brazil
[3] Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro. E-mail: jlluque@ufrrj.br

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Abstract

Two new species of *Pavanelliella* (Dactylogyridae) were described and some details about the morphology of *Pavanelliella pavanellii* Kritsky & Boeger, 1998 were added based on specimens collected from pimelodid fishes from Mogi Guaçu River, Brazil. *Pavanelliella takemotoi* sp. n., collected from nasal cavities of *Pimelodus maculatus* Lacepède, 1803 differs from its congeners by having a male copulatory organ (MCO) with 2–5 rings, vaginal canal sinuous without loops in distal portion of vaginal canal and anteriorly directed vagina containing five irregular rings around the vestibule. *Pavanelliella laertei* sp. n. collected from *P. heraldoi* Azpelicueta, 2001 is separated from the other species of the genus by lacking a vaginal vestibule and by distal end shape of accessory piece, which resemble flames or is bifurcated with one flank hooked in *P. laertei* sp. n. The specimens of *P. pavanellii* examined in this study showed morphological variations especially on MCO, vaginal canal and vagina morphology with respect to the holotype and paratype and are noted in this paper. Significant differences among the described species were confirmed on the light of multivariate morphometric statistical analysis. Initially a Principal Component Analysis (PCA) showed the multivariate distinction between the three species and after a Linear Discriminant Analysis (LDA) revealed that the Maximum number of MCO ring and the MCO length fairly contributed to the discrimination between all *Pavanelliella* species (Wilk´s lambda = 0.006; p<0.000). This is the first record of *Pavanelliella* species in Mogi Guaçu River.

Key words: Ancyrocephalinae – Biodiversity - nasal parasites - Neotropical Region - *Pavanelliella laertei* sp. n. - *Pavanelliella takemotoi* sp. n. - South America.

Resumen

Dos nuevas especies de *Pavanelliella* (Dactylogyridae) fueron descritas y algunos detalles sobre la morfología de *Pavanelliella pavanellii* Kritsky & Boeger, 1998 se han añadido sobre la base de especímenes colectados en los peces pimelódidos del rio Mogi Guaçu, Brasil. *Pavanelliella takemotoi* sp. n., colectado de las cavidades nasales de *Pimelodus maculatus* Lacepède, 1803 se diferencia de sus congéneres por tener un órgano copulador masculino (OCM) con 2-5 anillos, canal vaginal sinuoso,

pero nunca con lazos en la porción distal del canal vaginal y la vagina es dirigida anteriormente y tiene cinco anillos irregulares alrededor del vestíbulo. *Pavanelliella laertei* sp. n. colectados de *P. heraldoi* Azpelicueta, 2001 se diferencia de las otras especies del género por La falta de un vestíbulo vaginal y por la forma de extremo distal de la pieza accesoria, que se asemeja a las llamas o se bifurca con un flanco enganchado en *P. laertei* sp. n. las muestras de *P. pavanellii* examinadas en este estudio mostraron variaciones morfológicas, especialmente en la morfología de OCM, canal vaginal y de la vagina con respecto al holótipo y parátipo, y se discuten en esta investigación. Todas las diferencias entre las especies estudiadas fueron confirmadas com el uso del análisis estadístico morfométrico multivariada. Inicialmente, un Análisis de Componentes Principales (ACP) mostró la distinción multivariado entre las tres especies y después un Análisis Discriminante Lineal (ADL) reveló que el número máximo de anillos del OCM y la longitud del OCM contribuyeron bastante a la discriminación de todas las especies de *Pavanelliella* (Wilk´s lambda = 0,006; p<0,000). Este es el primer registro de especies de *Pavanelliella* en el río Mogi Guaçu.

Palabras clave: América del Sur – Ancyrocephalinae – Biodiversidad - parásitos nasales - *Pavanelliella laertei* sp. n. - *Pavanelliella takemotoi* sp. n. - Región Neotropical.

INTRODUCTION

Papers about monogeneans parasitic on Brazilian pimelodids focused on descriptions of dactylogyrid species infecting gills of the hosts (Boeger & Vianna, 2006; Cohen & Kohn, 2008; Monteiro *et al*., 2010). Kritsky & Boeger (1998) proposed *Pavanelliella* Kritsky & Boeger 1998 to include dactylogyrid species parasitic on nasal cavities of pimelodid fishes, *although* later Kritsky & Mendoza-Franco (2003) described another *Pavanelliella* species parasitic on a heptaterid host. *Pavanelliella* includes specimens with few features compared to other ancyrocephalin species, and this makes still more difficult the distinction between the species. After a carefully morphological examination, we separated three putative species which shared based in their morphological characteristics and performed to multivariate analysis, to confirm statistically, the differences between the species observed by us.

Here, two new species of *Pavanelliella* are described and new morphological data are added to *P. pavanellii* Kritsky & Boeger 1998 from specimens collected from the nasal cavities of *P. maculatus* Lacepède, 1803 and *Pimelodus heraldoi* Azpelicueta, 2001 in São Paulo, Pirassununga, southeastern Brazil.

MATERIAL AND METHODS

Host fish and parasites collection and identification

One hundred-one fishes were collected between February 2008 and March 2010 from Cachoeira de Emas (21º 58'S, 47º 26' W) on Mogi Guaçu River, Pirassununga, São Paulo. Samplings were carried out with nets and cast nets. Were collected 57 individuals of *P. maculatus* (Mean of total length = 26.24 ± 6.14; mean of weight = 258.42 ± 191.85) and 44 of *P. heraldoi* (Mean of total length = 12.03 ± 2.21; mean of weight = 18.46 ± 12.61). The fishes were identified according to Britski *et al*. (1999) and Azpelicueta (2001). The fish nasal cavities were disrupted with a probe and washed with 1:4000 formalin solutions. The parasites were posteriorly fixed in 4% formalin. The parasites were stained with Gomori's trichrome and mounted in Canada balsam for visualization of soft tissues, or mounted in Gray and Wess medium to study of sclerotized structures (Kritsky *et al*., 1986), or even stained with Gomori's trichrome and mounted in Gray and Wess medium. The identification of the parasites was carried out use of an optical microscope with differential interference contrast (DIC) Olympus BX 51. The illustrations were made with a drawing tube attached to a phase contrast microscope Opton™. The measurements (in micrometers) are represented by the average, with ranges in parentheses and followed by the number (n) of specimens. All measurements were taken as straight-line distance extending between the two most distant parts of such structures, using a calibrated eyepiece micrometer according to Mizelle & Klucka (1953). The diagnosis of the genus was based on Kritsky & Mendoza-Franco (2003). The quantitative population descriptors (prevalence, abundance and mean intensity of infestation) are those suggested by Bush *et al*. (1997). Type specimens were deposited in the Helminthological Collection of the Institute Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil.

Statistical analysis

Morphological features measured were those in accordance with Kritsky & Mendoza-Franco (2003). However, the choice of variables for morphometric analysis follows the parameters suggested by Du-Preez & Maritz (2006) that are those easily measurable, repeatable, not be geometrically redundant, representative and non-negative. So, an univariated analysis was performed to verify the variance (Bartlett's test *P* levels < 0,05) of the variables values and the Shapiro-Wilk test (*P* levels < 0,05) were run for evaluating normality of data (McGarigal *et al.*, 2000) using the MYSTAT 12 (2007) (SYSTAT 12 ©) also utilized for Principal Component Analysis (PCA) and a Linear Discriminant Analysis (LDA). The variables who had significant variance and non-normal distribution were $\log_{10}(x)$ transformed. The measurement of the body (total length and greatest wide), haptor, germarium and testis (length and wide) weren't used because their values can to suffer drastic variations as consequence of deformation of the soft parts during the fixation and mounting of the parasites (Strona *et al.*, 2005; Vignon & Sasal, 2010; Vignon, 2011). Only the hard structures (male copulatory organ length, proximal ring diameter and hooks length), the pharynx diameter and accessory piece length values were also used for morphometric analysis, so, only five out 14 features measured were selected. A Pearson's correlation coefficient with 95 percent confidence interval was performed from five selected variables according to exposed above, since that the redundancy of variables chosen is indicate by high Pearson's values (Vignon & Sasal, 2010; Vignon, 2011) indicating a multicollinearity. Additionally, nine categorical variables alone and in combination with continuous variables were used in multivariate analysis (Table 1). An ordination analysis (PCA) was conducted in order to evidence the presence of multivariate distinction between the three species (Strona *et al.*, 2005). A backward stepwise LDA was run to describe how the species grouping differ between their (McGarigal *et al.*, 2000) and to select the best set of morphometric variables in order to contribute for groupings distinction.

RESULTS

Descriptions

Family Dactylogyridae Bychowsky, 1933

Pavanelliella takemotoi sp. n. (Figs. 1–8)

Diagnosis (based on 48 specimens: 6 stained with Gomori's trichrome and mounted in Canada balsam, 5 stained with Gomori's trichrome and mounted in Grey and Wess's medium, 37 mounted in Grey and Wess's medium): Body 426 (246–580; n = 19), long, fusiform, tapering posteriorly, peduncle absent; greatest width of trunk 140 (86–240; n = 19) at level of copulatory complex. Cephalic region with 2 pairs of poorly developed cephalic lobes, each lobe with 3 head organs; cephalic glands present. Eyes 4 subequal, equidistant; accessory granules few in cephalic region. Pharynx subspherical 23 (14–36; n = 22) in diameter; esophagus moderately long; intestinal caeca confluent in posterior trunk after the germarium. Haptor 82 (51–122; n = 15) wide, 59 (46–69; n = 15) long, comprising delicate extension of posterolateral trunk velum-like, with slightly rounded tip. Hooks 16 (13–21; n = 96), with terminally expanded protruding, slightly depressed thumb, a delicate point; filamentous hook loop reaching union of shank subunits. Testis 67 (42–104; n = 5) long, 20 (19–27; n = 5) wide, elongate ovate, tapering posteriorly, posterodorsal to germarium; seminal vesicle sigmoid, lying to left of midline in anterior trunk, vas deferens observed until looping left intestinal. Prostatic reservoir saccate. Male copulatory organ (MCO) 35 (23–52; n = 29) long, 19 (16–27; n = 29) proximal ring diameter, delicate, tubular, coiled with 2–5 rings; base with 4 flanges, 2 proximal and 2 distal, 3 variations of flanges are observed. Accessory piece 27 (21–40; n = 25) long, comprising sheath enclosing medial or distal portion of MCO, distally partite. Germarium elongate, posteriorly tapering, 64 (45–104; n = 7) long, 18 (6–30; n = 7) wide; oviduct, ootype, not observed. Vagina sinistroventral, distal sclerotized vestibule, anteriorly directed, with 4–5 irregular rings around it; vaginal canal slightly sclerotized, seminal receptacle subspherical, overlapped germarium partially. Vitellaria dense, absent in the region of

Taxonomic summary

Type host: *Pimelodus maculatus* Lacepède, 1803, mandi amarelo (Siluriformes, Pimelodidae).

Site of infection: Nasal cavity.

Type locality: Cachoeira de Emas, Mogi Guaçu

River, Pirassununga, State of São Paulo, Brazil (21º 58'S; 47º 26' W).
Prevalence of infection: 35.1%.
Mean intensity of infection: 2.3.
Mean abundance of infection: 0.8.

Specimens deposited: Holotype (CHIOC 37567a), paratypes (CHIOC 37568a-f, 37569, 37570a-b, 37571, 37572, 37573, 37574, 37575a-b, 37576a-b, 37577a-b, 37578, 37579a-b, 37567b, 3758037582a-C).

Etymology: The specific name is in honor to Ricardo Massato Takemoto in recognition to his contribution to fish parasitology in Brazil.
Remarks
Pavanelliella takemotoi sp. n. is close to *P. pavanellii* and *P. scaphiocotylus* because these species have a vaginal vestibule. However, *P. takemotoi* sp. n. differs from the latter two species by the vagina shape, which in *P. pavanellii* is represented by a simple vaginal vestibule without ornamentations and a vaginal canal with 1–3 loops in the distal portion. *Pavanelliella scaphiocotylus* have a simple vestibule and a vaginal canal with 4–5 loops in the distal portion around the vestibule. In *P. takemotoi* sp. n., the vagina is anteriorly directed and apparently longest, besides having 4–5 irregular rings around of the vaginal vestibule and lack the loops of the distal portion of vaginal canal. *Pavanelliella takemotoi* sp. n. also differs of *P. pavanellii* and *P. scaphiocotylus* by presenting 4 flanges (1–3 flanges in *P. pavanellii*; 2 flanges in *P. scaphiocotylus*) on the bases of MCO; The number of MCO rings of *P. takemotoi* sp. n. is usually 4, but was found two specimens with 2 and 5 MCO rings, differentiating it from *P. pavanellii* (1–2 MCO rings) and *P. scaphiocotylus* (5–6 MCO rings). *Pavanelliella takemotoi* sp. n. also differs from *P. scaphiocotylus* because this last species haves a prominent tissue ridge along anterodorsal haptoral surface. Additionally *P. takemotoi* sp. n. has a more larger MCO (35) than *P. pavanellii* (23) and a more larger hook length (16) than *P. pavanellii* (12) and *P. scaphiocotylus* (14).

Pavanelliella laertei sp. n.
(Figs. 9–15)

Diagnosis (based on 2 specimens stained with Gomori's trichrome and mounted in Grey and Wess's medium and 5 specimens mounted in Grey and Wess's medium): Body 334 (274–374; n = 7) long, ellipsoid, peduncle absent and differentiated haptor; greatest width of trunk 135 (92–159; n = 4) in posterior third of body. Cephalic margin rounded, with 3 pairs of head organs; cephalic glands present. Eyes 4 subequal with two anterior more closely than posterior eyes, sometimes not observed. Pharynx subspherical 25 (20–30; n = 5) in diameter; esophagus short; intestinal caeca confluent posterior to germarium. Haptor 70 (60–79; n = 2) wide, 51 (42 –66; n = 5) long, comprising delicate extension of postero lateral trunk velum-like, with rounded tip, differentiated from trunk by a constriction anterior to seventh pair of hooks. Hooks 14 (11–17; n = 23), with terminally expanded protruding erected thumb, a delicate point, proximal subunit of shank slightly expanded; filamentous hook loop reaching union of shank subunits. Testis 30 (20–43; n =3) long, 17 (8–29; n = 3) wide, mediodorsal to germarium; vas deferens observed until looping left intestinal; seminal vesicle not observed. Prostatic reservoir saccate. MCO 43 (21–70; n = 5) long, 18 (17–20; n = 6) first ring diameter, tubular, forming coils with 2–3 rings or sometimes with 2 anterior rings and 2 posterior rings in relation to accessory piece; base with 2–4 flanges. Accessory piece 20 (18–21; n = 3) long, comprising sheath enclosing distal or medial portion of MCO, with irregular distal end flame-like or bifurcated with one of them hooked. Germarium elongate 57 (38–86; n = 3) long, 18 (10–29; n =3) wide, oval; oviduct, ootype, not observed. Vagina sinistroventral, vaginal vestibule not observed; vaginal canal sclerotized, elongate, sinuous with 2–3 loops in distal portion with a duct inside; seminal receptacle partially overlapped to germarium. Vitellaria dense, absent in the region of the reproductive organs.

Taxonomic summary
Type host: *Pimelodus heraldoi* Azpelicueta, 2001, mandi branco (Siluriformes, Pimelodidae).
Site of infection: Nasal cavity.

Type locality: Cachoeira de Emas, Mogi Guaçu River, Pirassununga, State of São Paulo, Brazil (21º 58'S; 47º 26' W).
Prevalence of infection: 14%.
Mean intensity of infection: 1.5.
Mean abundance of infection: 0.2.
Specimens deposited: Holotype (CHIOC 37561), paratypes (CHIOC 37562, 37563, 37564, 37565, 37566).

Etymology: The specific name is in honor to Laerte Batista de Oliveira Alves by his contribution to fish conservation studies in Brazilian waters.
Remarks
Pavanelliella laertei sp. n. is more close to *P. pavanellii* and *P. scaphiocotylus* because these two species share the presence of loops in distal portion of vaginal canal (2–3 loops in *P. laertei*; 1–3 in *P. pavanellii*; and 4–5 in *P. scaphiocotylus*). However, the new species is different from all congeneric species because *Pavanelliella laertei* sp. n. lacking a vaginal vestibule. Moreover, *Pavanelliella laertei* sp. n. has a larger hook (14) and MCO length (43) than *P. pavanellii* (12 and 23, respectively). But, the accessory piece length of *P. laertei* sp. n. (20) is lower than *P. pavanellii* (27). Although *P. scaphiocotylus* has the same hook length (14) of *P. laertei* sp. n., the former species has a prominent tissue ridge along anterodorsal haptoral surface (absent in *P. laertei* sp. n.). The shape of distal end of accessory piece can be single, flame-like or bifurcated with one end hooked on *P. laertei* sp. n. The single distal end of accessory piece is present too on *P. pavanellii*, *P. scaphiocotylus* and *P. takemotoi* sp. n., but the flame-like and hooked distal ends never are present on these last three species. Additionally, *P. laertei* sp. n. was found in another host species, *Pimelodus heraldoi*, which to date hadn't a parasitological record.

Pavanelliella pavanellii Kritsky & Boeger, 1998 (Figs. 16–18)

Diagnosis (based on 9 specimens: 1 stained with Gomori's trichrome and mounted in Grey and Wess's medium, 8 mounted in Grey and Wess's medium): Body 340 (250–451; n = 7) long; greatest width of trunk 102 (69–126; n = 7). Pharynx diameter 19 (13–26; n = 4). Haptor 88 (87–120; n = 7) wide, 60 (48–77; n = 7) long. Hooks 12 (8–14; n = 7). Testis (35; 39; n = 2) long, (11; 14; n = 2) wide. MCO 23 (11–34; n = 7) long, 19 (13–24; n = 7) first ring diameter. Accessory piece 27 (18–48; n = 4) long. Germarium 55 (40–77; n = 4) long, 16 (9–23; n = 4) wide.

Taxonomic summary
Host: *Pimelodus maculatus* Lacepède, 1803, mandi amarelo (Siluriformes, Pimelodidae).
Site of infection: Nasal cavity.
Locality: Cachoeira de Emas, Mogi Guaçu River, Pirassununga, Brasil (21º 58'S e 47º 26' W) (Marcho 2008; September 2010).

Prevalence of infection: 14%.
Mean intensity of infection: 1.25.
Mean abundance of infection: 0.2.
Specimens deposited: Voucher specimens (CHIOC 37554a, 37555, 37560, 37559, 37558, 37557, 37556, 37554b, 37553a, 37553b).

Other specimens studied: Holotype (INPA PLH 365), paratype (INPA PLH 366a).
Remarks
The specimens of *P. pavanellii* collected from Mogi Guaçu River and examined on this study showed morphological differences in MCO, vaginal canal and vagina compared to the descriptions and deposited material of Kritsky & Boeger (1998). The accessory piece described by Kritsky & Boeger (1998) has a proximal lobe. In the specimens observed by us this proximal lobule was less developed or even absent as the accessory piece was more or less contracted, respectively. In the holotype of *P. pavanellii* the accessory piece cannot be observed, but in the paratype was quite evident as it has been observed by the authors. Kritsky & Boeger (1998) observed that the proximal and distal margins of base of the MCO were sclerotized, but they gave not any information about the MCO base flanges existence. We observed the presence of 3 sclerotized flanges in our specimens, one proximal and 2 distal, and the exam of the holotype and paratype described above, revealed the presence of 2 miniature flanges at distal margin of MCO of paratype and one in each margin of holotype. The vagina of the specimens examined by us shows a large vaginal vestibule, only observed in the paratype. The vaginal vestibule of the holotype is equal to the Kritsky & Boeger (1998) illustration, ie, longer than wide. The vaginal canal of *P. pavanellii* according to Kritsky & Boeger (1998) is coiled and has 1 loop in its distal portion (Kritsky & Mendoza-Franco, 2003); however in our specimens we observed of 1–3 loops and in the holotype we observed 2 loops. It was also observed in our specimens that the vaginal canal extends into the vaginal vestibule reaching near the lateral end of the vestibule. Kritsky & Boeger (1998) did not observe this because in the holotype and paratype the vaginal canal ends closest to the proximal portion of vaginal vestibule. We had access to only

the holotype and one paratype, and, although morphological variations was observed in this parasites examined, especially with respect to vaginal morphology, we don't redescribe this species, since, possibility has that the total type series of *P. pavanellii* can show more morphological diversity.

Statistical analysis

On the first PCA, with all variables, the three first factors (Eingenvalues > 1) accounts for 79% of the variance, with the first factor mainly represented by the minimum number of vaginal canal; the second, by the minimum number of MCO ring and the third, by the presence of the accessory piece with flame-like distal end. In this analysis the categorical variables were those that contributed for the longest variability of the total sample. Only continuous variables were used on the second PCA in which the first factor accounts for 35.8% of the variance and the second, 23.6%. In this analysis the hook length, and the accessory piece length were that captured the almost of variance between species groups (59%). The backward stepwise LDA with the set of total variables revealed that six out nineteen variables were the best set of characters to explain the variability within groups composed by three species. The predictor variables selected by stepwise were pharynx diameter, hook length, MCO length, proximal ring diameter, maximum number of MCO ring, and accessory piece length. The model was successfully discriminating among the three species (Wilk´s lambda = 0.006; $p<0.000$). The first canonical variable captures most of the difference among the groups, accounting for 99% (eigenvalue = 87.69) of the total dispersion of the groups, whereas the second canonical variables accounted for 0.84% (0.75) (Table 2). The cross validated classification showed that overall 100% of monogeneans were correctly classified.

The scatter plot (Fig. 19) considering two canonical variables showed that the first (Maximum number of MCO ring) and the second variable (MCO length) fairly contributed to discriminated all *Pavanelliella* species.

DISCUSSION

The morphometrical analysis has been used to distinguish monogeneans species (Vignon, 2011). In many researches the data collinearity of variables choice has been a condition to run the multivariate analysis (Shinn *et al.*, 2004; Du-Preez & Maritz, 2006). Vignon & Sasal (2010) and Vignon (2011) having stated that the data collinearity of variables can not be useful for morphometric multivariate analysis because it contribute to the same direction in the species discrimination. In present paper we used the non-collinearity of data and this resulted on well species distinctiveness.

Although the present research don't had as objective to evaluate the phylogenetic relationships between the *Pavanelliella* species, make some general considerations could be important. Specimens belong to *Pavanelliella* Kritsky and Boeger, 1998 have a few morphological characteristics and so, is necessary to look for others characters, beyond of the MCO and accessory piece morphology to support the description of new species. The vaginal vestibule when present or absent; the number of loops in distal portion of vaginal canal or it absence, seems reasonable morphological characters to contribute on distinguishment of the *Pavanelliella* species. *Pavanelliella laertei* sp. n. lacking the vaginal vestibule, it was collected on *Pimelodus heraldoi*, whereas *Pavanelliella pavanellii* and *P. takemotoi* sp. n. have a vaginal vestibule and were both collected on *P. maculatus*. But unlike of *P. pavanellii*, *Pavanelliella takemotoi* sp. n. hasn't loops on the distal portion of the vaginal canal. This can be results of an inter-host speciation (Poisot & Desdevises, 2010). However, we don't know if this evolutive process was result of a co-speciation, or host switch and speciation or speciation followed by host switch. But one of these events seems have occurred on the three *Pavanelliella* species found on pimelodid fishes from Mogi Guaçu river basin. The largest distance between centroids of *P. takemotoi* sp. n. and *P. laertei* sp. n. (266.730), between P. *takemotoi* sp. n. and *P. pavanellii* (411.468) and the shorter distance between centroids (10.418) of *P. pavanellii* and *P. laertei* sp. n. reinforces this possibility. However, these questions about these speciation events can't be answered fully with morphometric analysis alone (Poisot & Desdevises, 2010).

Two set of morphological variations were found for *P. laertei* sp. n. The first set (Figs.9–11) showed by

smaller specimens had a minor MCO, and lacking hook loops while the second morphological set (Figs. 12–15) represented by the largest specimens had the largest MCO and hook loops present. Although these differences have suggested us belong to different species, we consider more careful to keep them as intra-specific morphological variations, especially given the similarity of the morphology of the vagina.

Previous to present paper, *Pavanelliella* Kritsky and Boeger, 1998 was represented by two species. The type species, *P. pavanellii*, was described from specimens collected from the nasal cavity of *Pseudoplatystoma corruscans* (Spix & Agassiz 1829) from the Baía River, Mato Grosso (type locality), from the Paraná River, State of Paraná and from *Callophysus macropterus* (Lichtenstein 1819) collected from Solimões River in the State of Amazonas, Brazil (Kritsky & Boege, 1998). Subsequently, Brasil-Sato & Pavanelli (2000) reported this species in association with *Pimelodus maculatus* Lacepède, 1803 collected from the Paraná River and São Francisco River, Brazil. Later, another species was proposed, *P. scaphicotylus* Kritsky and Mendoza-Franco, 2003, collected from the Yucatán Peninsula in Mexico, parasitizing nasal cavity of *Rhamdia guatemalensis* Kritsky & Mendoza-Franco, 2003 (Kritsky & Mendoza-Franco, 2003).

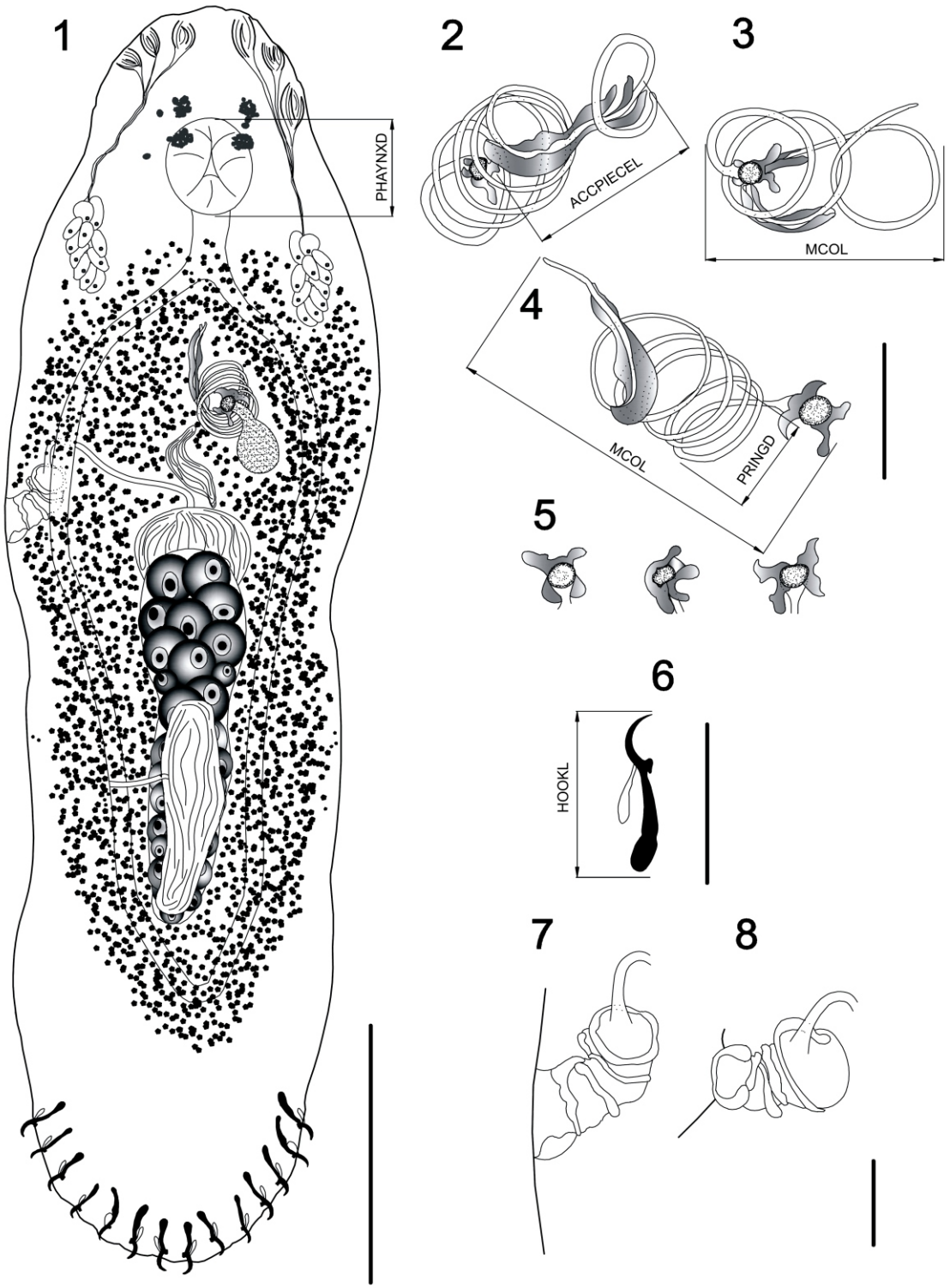
Currently, *Pavanelliella* species are distributed in fishes from 3 siluriform genera (Boeger & Vianna, 2006). As siluriforms are very speciose in the neotropics (Lundberg & Littmann, 2003), new records and new *Pavanelliella* species will certainly be described in other species and localities in this region.

Table 1. Categorical variables of *Pavanelliella* spp. Minimum and Maximum number of MCO ring (Ringmin; Ringmax); Vaginal vestibule (Vagvest; 1 = present; 0 = absent); Minimum and Maximum number of loops of the distal portion of the vaginal canal (Canvagmin; Canvagmax); Distal end of accessory piece (Single = Accsing; Partite = Accpart; Flamed = Accflam; Hooked = Acchook; 1 = present; 0 = absent).

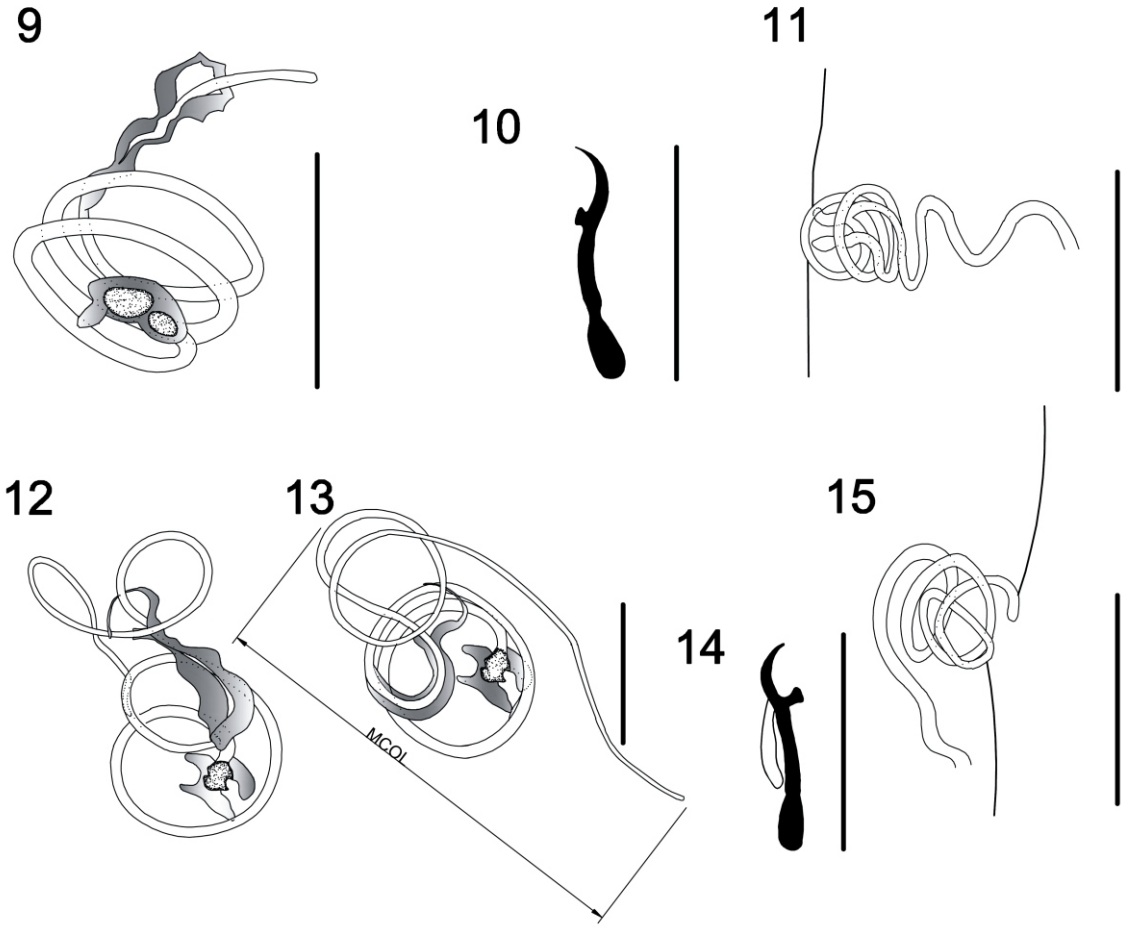
	Ringmin	Ringmax	Vagvest	Canvagmin	Canvagmax	Accsing	Accpart	Accflam	Acchook
P. pavanellii	1	2	1	1	3	1	0	0	0
P. takemotoi sp. n.	2	5	1	0	0	1	1	0	0
P. .laertei sp. n.	2	3	0	2	3	1	0	1	1

Table 2. Canonical discriminant functions of categorical and continuous variables of *Pavanelliella* spp. selected by backward stepwise LDA.

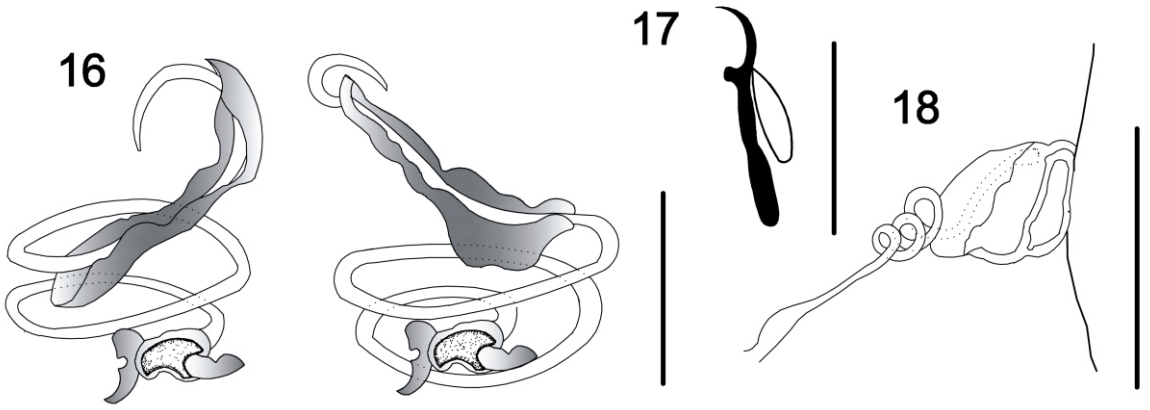
Variables	Factor 1	Factor 2
Pharynx diameter	0.232	0.422
Hook length	-0.286	0.082
Male Copulatory Organ length	-0.542	0.813
Proximal ring diameter	0.247	-0.177
Maximum number of MCO ring	-1.157	-0.032
Accessory piece length	0.077	-0.499



Figures 1–8. *Pavanelliella takemotoi* sp. n. **1.** Whole mount, dorsal view. **2–4.** Compulatory complex: ventral view. **5.** Base of MCO. **6.** Hook. **7–8.** Vagina, dorsal view. Scale bars: 100 µm (Fig. 2) and 20 µm (Figs. 3–9). PHARYNXD = Pharynx diameter; ACCPIECEL = Accessory piece length; PRINGD = Proximal ring diameter; MCOL = Male copulatory organ length; HOOKL = Hook length



Figures 9–15. Sclerotized structures of *Pavanelliella laertei* sp. n. **9.** Copulatory complex, dorsal view. **10.** Hook**. 11.** Vagina, dorsal view. **12–13.** Copulatory complex, left side and ventral view, respectively. **14.** Hook. **15.** Vagina, ventral view**.** Scale bars: 25 µm (Fig. 12, 16), 20 µm (Figs.10, 13–14), and 15 µm (Figs. 11,15).



Figures 16–18. *Pavanelliella pavanellii* Kritsky & Boeger, 1998. **16.** Copulatory complex, ventral view. **17.** Hook. **18.** Vagina and vaginal canal, ventral view. Scale bars: 10 µm (Figs. 17, 19) and 25 µm (Fig. 18).

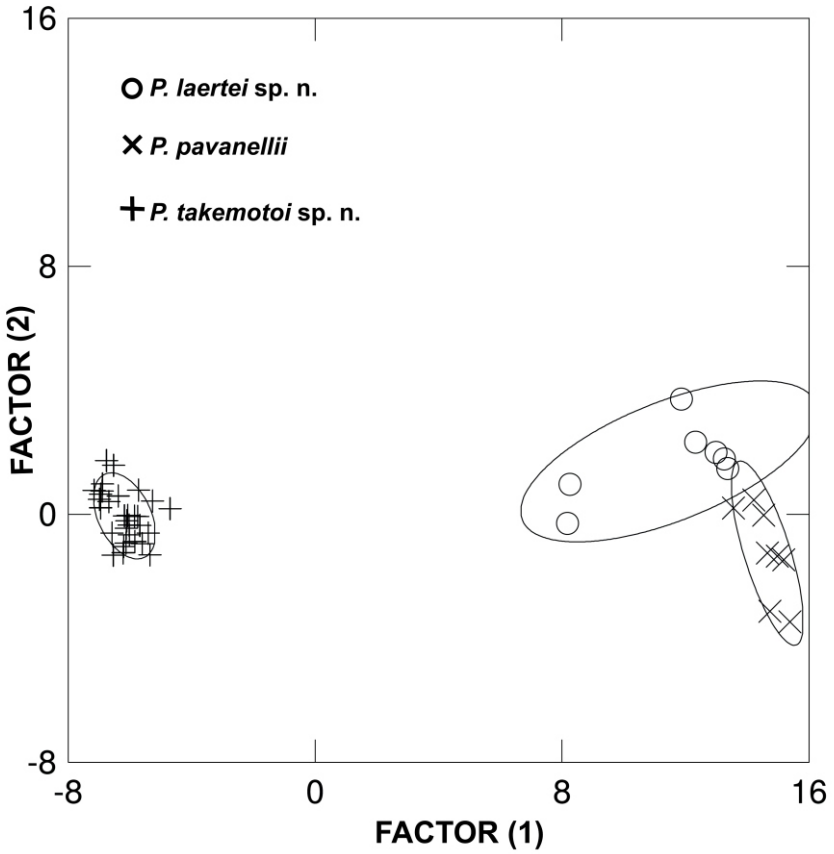


Figure 19 Canonical scores plot from the morphometric variables of *Pavanelliella* spp.

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Correspondense to author /Autor para correspondencia:

José L. Luque
Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro.

E-mail/ correo electrónico:
jlluque@ufrrj.br