

First record the rare parasitic *Pseudostegophilus paulensis* in Paranapanema River and geographic distribution in Brazil

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Received: December 16, 2022 / Accepted: November 26, 2023 / Published online: December 29, 2023

Abstract

Pseudostegophilus paulensis Miranda Ribeiro 1918 is an endemic parasitic species from the state of São Paulo, southeastern Brazil, with few registered occurrences in databases (15 records). Here, we present the first record of *P. paulensis* in the middle portion of the Anhumas River, a tributary of the Paranapanema River right bank. Specimens were collected in two environments with different ecological characteristics (lotic water running over gravel sediment and semi-lotic water with silty sediment on the bottom). We also present an updated occurrence map of the species in Brazil. This new record of *P. paulensis* in the upper Paraná River basin extends the geographic distribution of the species to the South of the state of São Paulo. Furthermore, important ecological information remains unknown due to the lack of data about this group, such as habitat niches, vertical migration, growth patterns, reproduction, and feeding behavior.

Keywords: Candirú; Neotropical region; South America; Upper Paraná River basin.

Primeiro registro de um raro peixe parasito *Pseudostegophilus paulensis* no rio Paranapanema e sua distribuição atual no Brasil

Resumo

Pseudostegophilus paulensis Miranda Ribeiro 1918 é uma espécie de peixe parasita endêmica do estado de São Paulo, sudeste do Brasil, com poucas ocorrências registradas em bancos de dados (15 registros). Aqui, apresentamos o primeiro registro de *P. paulensis* na porção média do rio Anhumas, afluente da margem direita do rio Paranapanema. Os espécimes foram coletados em dois ambientes com características ecológicas distintas (água lótica correndo sobre sedimento pedregulho e água semilótica com sedimento arenoso no fundo). Apresentamos também um mapa de ocorrência atualizado da espécie no Brasil. Este novo registro de *P. paulensis* na bacia do alto rio Paraná estende a distribuição geográfica da espécie para o sul do estado de São Paulo. Além disso, informações ecológicas importantes permanecem desconhecidas devido à falta de dados sobre esse grupo, como nichos de habitat, migração vertical, padrões de crescimento, reprodução e comportamento alimentar.

Palavras-chave: Candirú; Região Neotropical; América do Sul; Bacia do Alto Rio Paraná.

Introduction

The Trichomycteridae family has 326 species (Fricke, Eschmeyer, & Van der Laan, 2022), one of the richest groups of freshwater catfish (Ochoa *et al.*, 2020). It is currently divided into eight subfamilies (Baskin 1973), with a wide distribution in the Neotropical river basins of South and Central America (De Pina & Wosiacki 2003), including both sides of the Andes Mountains (De Pinna & Wosiacki 2003). Most trichomycterids are small (≤ 100 mm standard length) and are generalist predators of small invertebrates, only the Stegophilinae and Vandelliinae subfamilies are exclusively semi-parasites or parasites: Stegophilinae feeds on mucus,

scales, and some species on skin and pieces of flesh; and Vandelliinae are hematophagous and parasitize gills of larger fish, being known as "candirus" (De Pinna & Wosiacki 2003; Fricke *et al.*, 2022). Stegophilinae is the second richest subfamily with 28 valid species, with nine of them belonging to the genus *Pseudostegophilus* Eigenmann & Eigenmann 1889 (Fricke *et al.*, 2022). *Pseudostegophilus paulensis* Miranda Ribeiro 1918 is a species of rare occurrence, with only 13 records in online biodiversity databases and five specimens identified in zoological collections. The species is native to the Upper Paraná River basin, being endemic to the state of São Paulo, Southeastern Brazil (De Pinna & Wosiacki 2003). *Paravandellia oxyptera* e *P. paulensis* are

the only known parasitic fish species in the state of São Paulo (Britski, 1972).

Traditional taxonomy is still the most used method for identifying fish specimens. However, molecular markers have corroborated in taxonomic studies integrated with morphological data to provide more consistent identification and species delimitation. The 5' end of the gene encoding the cytochrome c oxidase subunit I (COI) is considered the universal marker for animal genetic barcode (Hebert, Cywinska, Dewaard, 2003; Ward, Hanner, Hebert, 2009). These markers are being used successfully to identify fish species, including cryptic species with taxonomic problems or in early stages of development such as eggs, larvae, and juveniles (Hubert *et al.* 2015).

As important as the correct identification of species is the knowledge about their exact geographic distribution. In this context, scientific collections and databases gather and make available information to solve our lack of knowledge of species distribution. However, those databases need to be constantly reviewed and updated with correctly identified species to be precise in biodiversity conservation and management (Watanabe, 2019).

In this context, understanding the spatial patterns of fish distribution provides essential information to mitigate the effects of climate and anthropogenic changes and knowledge of new areas of occurrence of the species. Detailed knowledge of the distribution at different temporal and spatial scales is essential for conserving its environment, as management and conservation plans are far from being completed due to a lack of information (Riaño, 2020). Information on the distribution, structure, and spatial patterns of *P. paulensis* in the Upper Paraná River basin is scarce. This study aimed to report the first record of *P. paulensis* in the Paranapanema River basin, gathering all the occurrence information in Brazil.

The Paranapanema River is located on the left bank of the Paraná River, and it is one of the main tributaries of the upper Paraná basin. Its headwaters lie on the Serra de Paranapiacaba, and it flows to the West for approximately 930 km forming most of the border between the states of Paraná and São Paulo until it flows out in the Paraná River (Sampaio, 1944). In the low portion of the Paranapanema River basin, the Anhumas River is one of its main tributaries and possibly one of the most important maintainers of its ichthyofauna richness (Leme *et al.*

2015; Geller *et al.* in prep.). A fish inventory was carried out in the Anhumas River by the Laboratório de Ecologia de Peixes e Invasões Biológicas (LEPIB) from the Universidade Estadual de Londrina (UEL), in the municipality of Narandiba, State of São Paulo, Brazil. The expedition was registered in the Biodiversity Information and Authorization System (SISBIO) as number 72912. The specimens were captured using a 6.36 m² trawl net (2 mm mesh) with a sampling effort of 60 minutes by four people. The specimens were anesthetized and euthanized by overexposure in a clove oil solution, fixed in 4% formalin for 48 hours, and then preserved in 70% ethanol. For molecular analysis, specimens were deposited in 99% ethanol after euthanized. Testimony individuals are deposited at the Museu de Zoologia da Universidade Estadual de Londrina (MZUEL). The following specimens were used in this study (alc = alcohol; c&s = cleared and stained; mol = molecular).

The quality of the obtained sequence was analyzed using the Electropherogram Quality Analysis online application (available in: <http://asparagin.cenargen.embrapa.br/phph/>). Then, sequence alignment was performed using the Mega X software (Kumar *et al.*, 2018) using the Clustal W tool (Thompson *et al.*, 1994). The four sequences obtained referring to the four specimens analyzed were submitted to the Boldsystems database (<http://www.boldsystems.org/>) to check the correspondence and similarity with the sequences deposited in the database. The deposited sequences with the best matches (>99%), together with the sequences of the analyzed specimens, were incorporated into the intraspecific and interspecific genetic distance analyses based on the evolution model Kimura-2-Parameters (K2P) (Kimura 1980) using Mega X software. Interspecific genetic divergence values (<2%) were defined at the species level (Ratnasingham & Hebert 2007).

The new record of the species *Pseudostegophilus paulensis* occurred in Brazil in the Paranapanema river basin, Anhumas river, municipality of Narandiba. The species *P. paulensis* (Figure 1) were collected in two distinct environments (Figure 2). In the semi-lotic environment (22°29'34.09"S; 51°27'50.99"W) (Figure 2A) with a sandy-muddy substrate containing deposited leaf, average depth of 50 cm, visibility of 20 cm, average width of 30 meters (Figure 2A).



Figure 1. Lateral view of *Pseudostegophilus paulensis* MZUEL 21207, 22.0mm, middle Anhumas River, Lower Paranapanema River, Upper Paraná basin, Paraná, Brazil.



Figure 2. New records sites of *Pseudostegophilus paulensis* in the middle Anhumas River, right bank tributary of the Paranapanema River. A) Semi-lotic environment. B) lotic environment.

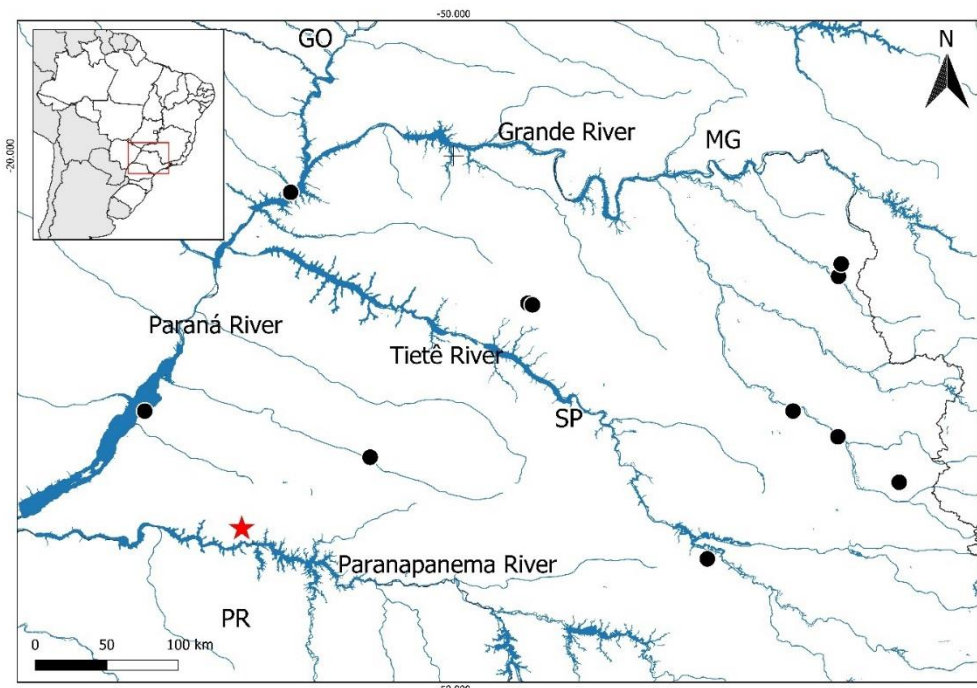


Figure 3. Geographic distribution of *Pseudostegophilus paulensis* in the state of São Paulo, southeastern Brazil. Database records are represented by black dots and the new record by the red star in the Paranapanema River basin.

On the following water conditions: average temperature 25.7°C; pH 8.8; conductivity 29 (µs); turbidity 7.5 (utn); dissolved nitrate 140 (NO₃) and average saturation of O₂ 72.1%. In the lotic environment (22°33'15.6"S; 51°27'05.7"W) (Figure 2B). With a sandy and rocky substrate, average depth of 1 m, visibility of 60 cm on the following water conditions: average temperature 18.1°C; pH 8.4; conductivity 28,2; turbidity 7.3; dissolved and average saturation of O₂ 77.2%. Our findings expand the species' occupation area to the South of the State of São Paulo, being the first record in the Paranapanema River basin (Figure 3).

Specimens' identification was obtained through external morphology analysis according to Miranda Ribeiro (1918), and osteological characters according to Do Nascimento (2015). Data were obtained from two cleared and stained specimens from each sampled locality. The genus *Pseudostegophilus* can be identified by combining the following characters: a horizontally oriented coronoid dental process (present in all

Stegophilinae); the dorsal edge of the quadrate bone convex or straight (concave in other members of the Trichomicteridae family); branchiostegal membrane with a short posterior margin laterally free from the isthmus; occipital region of the head covered by thick opaque skin; posterior nostril completely surrounded by a continuous fleshy margin, forming a tubular structure; lateral line channel reaching distal end of pectoral fin inner ray; the distal edge of the pelvic fin not reaching the anus.

P. paulensis has an elongated body; depressed head; lower mouth; hook present in the interoperculum and operculum; hyaline fins, seven anal-fin rays; origin of anal fin posterior to dorsal fin; forked caudal fin with rounded lobes; yellow body with small, rounded spots. The congener *Pseudostegophilus maculatus* found in the Lower Paraná and Uruguay basins (De Pinna & Wosiacki 2003) has larger rounded black spots than *P. paulensis* arranged longitudinally. In addition, it has a caudal fin with a rounded

black spot at the base of the upper lobe and black spots at the ends of the upper and lower lobes (vs. two small black patches that are not parallel, with hyaline ends and small black spots arranged in a irregular shape in *P. paulensis*).

Here, the interspecific distances of the studied specimens with six *P. paulensis* sequences available in the database were all < 1%, with a minimum distance of 0.50% and maximum of 0.69%. As all distances were < 2%, and all belong to the same BIN (Barcode Index Number) BOLD: AAD7057, we conclude that all sequences belong to specimens correctly identified as *P. paulensis*.

Pseudostegophilus paulensis is known from the Upper Paraná River basin, with records in some of its main tributaries: Tiête River basin, Grande River basin, and now to Paranapanema River basin. According to the two largest online databases on fish biodiversity, SpeciesLink and Gbif, there are 15 records of *P. paulensis* in Brazil, belonging to five zoological collections, evidencing the species rarity of capture. The most recent records were in the Peixe River basin, in 2016 (Bifi, Dias & Frota, 2017), and in the Sapucaí-Mirim River, in 2014 (Brambilla, Uieda & Nogueira, 2018). Extinction is often unavoidable for many rare species when local environments become unsuitable due to habitat loss or modification. The last survey of ichthyofauna for the Paranapanema River basin carried out by Jarduli *et al.*, (2020) did not present any record of *P. paulensis* for that basin.

The presence of *P. paulensis* inhabiting lotic environments (Fig. 2B) is not mentioned in the literature as characteristic for the species (De Pina & Wosiacki, 2003; Zanata & Primitivo, 2013). Our results highlight the occurrence of *P. paulensis* in areas of rapids, crystalline water with rocks, and little muddy sediment at the bottom of rivers and streams. Previous records found on databases are placed in semi-lotic or lotic habitats (Fig. 2A). This discovery brings more information about the species' life habits and their plasticity for survival.

Along with *P. paulensis*, two specimens of *Geophagus brasiliensis* (Quoy and Gaimard 1824) were captured (71.52 g e 18.27 g) in the semi-lotic environment. They were lacerated and bleeding in the caudal fin and operculum region (personal observation), while the individuals of *P. paulensis* had their digestive system filled with blood. This species commonly attacks *Salminus brasiliensis* (Cuvier 1816) and *Brycon* sp. (Ihering 1930) (De Pina & Wosiacki 2003) and herein we register *P. paulensis* parasitizing *G. brasiliensis*. Trichomycteridae exhibit one of the widest trophic diversity found in Siluriformes and among Actinopterygii in general, including predators of small aquatic invertebrates, algivores (Copionodontinae), scavengers (*Pareiodon microps*), scale and mucus eaters (Stegophilinae), and the highly specialized hematophagous Vandellinae (Zanata & Primitivo, 2013). Parasitic fish have high ecological importance in aquatic communities and biological diversity. They can provide valuable information about their hosts and the environment in which they live, in addition to possible biological indicators of environmental conditions (Takemoto *et al.*, 2009).

The accuracy of species identification using molecular markers depends on high-quality reference sequences in databases such as Boldsystems (Becker, Sales, Santos, Santos & Carvalho, 2015). Herein, the interspecific distance of the

studied specimens with six sequences of *P. paulensis* available in the database were all < 1%, with a minimum distance of 0.50% and a maximum of 0.69%. Since all distances were < 2%, we concluded that all sequences belong to specimens correctly identified as *P. paulensis*.

Pseudostegophilus paulensis is not on the red list of endangered species from Brazil (ICMBio, 2018), most likely due to a lack of information about the species. In the Book of Endangered Fauna in the State of São Paulo (2009), this species was cataloged as Data Deficient freshwater fish (DD). However, in the last decree of the state of São Paulo (decree n° 63.853, of November 27, 2018), updating the list of enamed species, *P. paulensis* is no longer present, is removed from the list without justification.

Conclusion

This new record of *P. paulensis* in the upper Paraná River basin extends the geographic distribution of the species to the South of the state of São Paulo. The knowledge about the diversity of parasitic fish in freshwater in Brazil is based on scientific expeditions and on the scarcity of specimens deposited in zoological collections. *Pseudostegophilus paulensis* may be more abundant and widely distributed in Brazilian waters than is currently known, and more efforts should be applied to survey small species with parasitic characteristics. Furthermore, important ecological information remains unknown due to the lack of data about this group, such as habitat niches, vertical migration, growth patterns, reproduction, and feeding behavior.

Acknowledgements

This study was carried out with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001. We want to thank China Three Gorges Corporation (CTG) Brazil for the financial support to the project Pesquisa e Desenvolvimento – ANEEL (project n° 11218/UEL/FAUEL) and the postdoctoral scholarships to DAZG and LRJ. We are thankful to the technician Aparecido Souza and students Augusto Jatobá and Matheus Chueire for their assistance in field works. We are thankful Ana Carolina Vizintim for contributing to the creation of the distribution map. We are thankful to Vitor P. Abrahão for identifying and discussing the data.

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