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ECOSYSTEMS

Updated distribution and morphological variation of the genus *Pseudanos* in the Río de la Plata basin (Characiformes: Anostomidae)

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Abstract: *Pseudanos* is a fish genus with cis-Andean distribution in South America. *Pseudanos trimaculatus* is originally known from the Amazon and Orinoco basins. Three decades ago, a few specimens collected in the Río de la Plata basin were identified as *P. trimaculatus*, what remained to be confirmed and understood. The aim of this contribution is to analyze these specimens. Consequently, the morphological variation of *P. trimaculatus* is discussed and updated. Morphometric and meristic data were taken from the specimens and compared with those of the type and non-type specimens of the species. Multivariate analyses of the size-corrected measurements were used to explore the morphological variation. Size-corrected PCA revealed that the specimens collected in the Río de la Plata basin nested with the remaining specimens, being slightly closer to those from the Guaporé, Napo and Uatumã rivers. Measurements such as caudal peduncle depth, body depth, and body width affected more heavily the first components. Cluster analysis did not show well-defined groups based on the hydrogeographic basins. The studied specimens from the Río de la Plata basin are herein confirmed as conspecific with *P. trimaculatus*. The species is added to the list of fish species shared between the Amazon and Río de la Plata basins.

Key words: Neotropical fish, Paraná basin, size-corrected PCA, widespread species.

INTRODUCTION

The genus *Pseudanos* Winterbottom is a small group of Neotropical fishes of Anostominae (Winterbottom 1980), a subfamily of Anostomidae (Dillman et al. 2016, Sidlauskas & Vari 2008). The genus is recognized by the combination of the following characteristics: 16 scales around caudal peduncle, mouth opening dorsally with lower lip plicate, and four teeth in each lower jaw (bony portion of the lower jaw as long as its wide), three branchiostegal rays, 41 or more lateral-line scales, and 39 or more vertebrae (Winterbottom 1980). To date, *Pseudanos* is composed of four cis-Andean species as follow: *P. gracilis* (Kner), *P. trimaculatus* (Kner), *P. varii* Birindelli, Lima & Britski, and *P. winterbottomi* Sidlauskas & Santos, which are distributed in the Negro, Branco, Uatumã, Madeira, Tapajos rivers in the Amazon basin, and the Casiquiare, Cinaruco, Síapa and Atabapo rivers in the Orinoco drainage, and also in the Essequibo river basin (Winterbottom 1980, Sidlauskas & dos Santos 2005, Birindelli et al. 2012, Fricke et al. 2023).

Pseudanos trimaculatus was originally described in *Schizodon* Agassiz, placed in *Anostomus* Scopoli in Garman (1890), and treated as *Pseudanos* by Winterbottom (1980) during his review of the Anostominae. In that contribution, he diagnosed the species based mainly by its color pattern composed of narrow dark transverse bars across the dorsum (vs. absent in *P. gracilis*), light spots in the center of body scales (vs. dark spot on center of body scales in *P. irinae*), and by the angle of 110-180° between the three posterior scale *radii* (vs. 40-90° in *P. gracilis*). Winterbottom (1980) additionally listed a combination of diagnostic characteristics including a few overlapping morphometric (e.g. body depth, body width) and meristic features (number of lateral-line scales and vertebrae). Birindelli et al. (2012) described *P. varii* based on specimens previously identified as *P. gracilis* by Winterbottom (1980) and Sidlauskas & dos Santos (2005), and discussed the validity of *P. irinae*. In that paper, Birindelli et al. (2012) examined a large series of specimens of *P. trimaculatus* and noticed that there is a great degree of variation in the conspicuousness of the dark spots on body scales in specimens from the Amazon basin. The authors suggested that *P. irinae* should be considered a junior synonym of *P. trimaculatus*, since this feature was the only diagnostic characteristic to distinguish both species.

The first mention of *Pseudanos* in the Río de la Plata basin was presented by Ringuelet & Arámburu (1961) for Argentina, who cited *Anostomus trimaculatus* within "Anostomatinae" without reference to any voucher specimen. One year later, Alonso de Arámburu et al. (1962) reported a short description of the single specimen (cited as an uncatalogued MLP, 84 mm of size, apparently SL) that was identified as *A. trimaculatus*. Alonso de Arámburu et al. (1962) also indicated that the recently specimen deposited in MLP had three blotches along the body, on the opercular area, at vertical through the fifth dorsal-fin ray, and at the base on the middle caudal-fin rays, and a red pigmentation on the caudal fin (features that fit into the known color pattern of *P. trimaculatus*). In their book of freshwater fishes from Argentina, Ringuelet et al. (1967), revised the same uncatalogued MLP specimen and added some morphological data as the silvery pigmentation of the body and the presence of five dark vertical bands on the predorsal region. In the description of *Pseudanos* proposed by Winterbottom (1980), *P. trimaculatus* was designated as its type species and the record by Ringuelet et al. (1967) for the Río de la Plata basin was included in the distributional range of the species, but the MLP specimen was not examined. In her taxonomic contribution of the anostomid from Argentina, Braga (1993) described a second specimen of *P. trimaculatus* from the Río de la Plata, at Buenos Aires city (MACN-ict 6207), and also examined the specimen cited by Alonso de Arámburu et al. (1962) and Ringuelet et al. (1967), referred as MLP 12-X-61-1. These records from Argentina remained unstudied in the further systematic literature on the genus or the species (Birindelli et al. 2012, Sidlauskas & dos Santos 2005). Additionally, López et al. (2005) registered *P. trimaculatus* for the Delta of the Río Paraná in Entre Ríos.

The aim of this contribution is to evaluate these records in view of our current knowledge on the taxonomy of *Pseudanos*.

MATERIALS AND METHODS

Material studied are deposited in the following institutions: BMNH, NMW, FMNH, INPA, UFRO, MACN-ict, MLP, MZUSP, and UFRO-I (abbreviations according to Sabaj 2020). Measurements and meristic data, which follow Winterbottom (1980) and Birindelli et al. (2012), were used to corroborate the identification of the specimens and to conduct the statistical analyses. Morphometric variables were taken as point-to-point distances with digital caliper and are expressed as percents of standard length (SL) or head length (HL) for units of the head. Additionally, measurements were taken from images using tpsDig2 v.2.31 (Rohlf 2013) for one specimen that is indicated by an "*" in the respective section. Counts were taken on the left side of the specimens when possible. Additional collection data for the specimens under study were estimated from the metadata obtained in the collection catalogs and historical records informed by the collection staff of each institution. These are presented between square brackets in "Material examined" section. Total number of vertebrae were counted by means of radiographs, which were obtained from Kodak 2100 (60kv, 7 mA, 0.250s) and Toshiba DC-12M x-ray machines (50 kv, 50 mAs). Total vertebral count includes the four vertebrae of the Weberian apparatus as separate elements. The first preural centrum plus first ural centrum (PU1+U1) were counted as single vertebra.

Statistical procedures

For comparative purposes, the specimens were pooled into geographic groups according to major river basins. In order to compare the size-free shape differences between the specimens from the Río de la Plata basin and the type and non-type specimens of *P. trimaculatus* from its main range of distribution, the measurements were processed under Burnaby's allometric correction (Burnaby 1966, Humphries et al. 1981, Rohlf & Bookstein 1987). In that procedure, the log-transformed variables are projected onto a space orthogonal to the first principal component. The size-corrected measurements were analyzed using a principal component analysis (PCA). The number of significant components was chosen by two criteria: the broken-stick model (Frontier 1976) and the scree plot method (Cattel 1966). For the morphometric data of *P. trimaculatus* from the Amazon basin, cases of single missing values were imputed from predictor values obtained under maximum likelihood from the EM algorithm (Dempster et al. 1977, Pigott 2001) using 500 iterations. A hierarchical cluster analysis was performed in the size-corrected morphometric data to compare the morphological dissimilarity between specimens by means of Ward's method (Ward 1963) and Euclidean squared distances (Sneath & Sokal 1973). When needed, uni and multivariate normality was tested using a Shapiro–Wilk statistic (W) and Mardia test ($\alpha < 0.05$), respectively. Statistical procedures were carried out in PAST 4.10 (Hammer et al. 2001) and IBM SPSS Statistics 26.0 (IBM 2019). Additional data reported as "Supplementary Files" from the resulting analysis, as well as a digitalized radiograph from one of the studied specimens from Argentina, are available through Morphobank (Project 4490; https://morphobank.org/index.php/LoginReg/login).

RESULTS

Material examined

MLP 7281, 1, 79.9 mm SL, Argentina, Corrientes, Itatí, [Río Paraná basin c. 27°15'S, 58°14'W, 61 m a. s. l.] 1961, E. Arriguti. MACN-ict 6207, 1, 69.1 mm SL, Argentina, "Relevamiento Integral" in area from Río de la Plata [Buenos Aires, water intake of Obras Sanitarias de la Nación, OSN (= AYSA), near Aeroparque, c. 34°32'40.98"S, 58°24'59.39"W 2 m a. s. l.], 1964 to 1965, G. Fonseca & H. Castello.

Description

Mouth small and strongly upturned, opening on the dorsal surface of the head, and with plicate lips (Figures 1-2). Premaxilla with four compressed teeth, each with two to five cusps along a continuous serrated edge (see Braga 1993: Figures 14 and 15). Maxilla edentulous. Dentary with four compressed teeth, each with two to five cusps.

Dorsal fin with ii,10 rays. Anal fin with ii–iii,7–8 rays. Pectoral fin with i,13–14. Pelvic fin with i,8 rays. Caudal fin forked, with 8–9/8 rays. Three branchiostegal rays. Lateral line bearing 42-43

Figure 1. ***Pseudanos trimaculatus*** **from the Río de la Plata basin: a. MLP 7281, 79.9 mm SL (flitted image horizontally from left side because body spots are faded on it); b. MACN-ict 6207, 69.1 mm SL (left side).**

pored scales. Six scales rows between dorsal fin and lateral line. Five scales rows between lateral line and anal-fin origin. Five scales rows between lateral line and pelvic-fin origin. 13 to 14 predorsal scales. 16 scales around caudal peduncle. 41 vertebrae (Supplementary Material File SI - Media, Morphobank P4490). Angle of more than 110° formed between the dorsal and ventralmost *radii* of body scales (Figure 3).

Coloration

Both specimens with faded coloration. Ground pigmentation faded, color yellowish tan. A weakly-dark round midlateral blotch below dorsal-fin base present in both specimens, but slightly more conspicuous in MACN-ict specimen. MLP specimen with a small weakly-dark midlateral blotch posterior to opercle and another at base of the caudal-fin rays. Dorsum bearing five transversal weakly-dark bars along predorsal region in MLP specimen (Figure 1). Dorsum uniform in MLP specimen, bars likely completely faded.

Morphometric comparison

The measurements are summarized in Table I. The scree plot method suggested to extract up to the fifth component, while the Broken-stick method revealed that the breaking point should be between the third and fourth components. As consensus between both procedures, the first four principal components (PCs), which accounted for 75.4 % of the total variation, were used to include as maximum as possible of

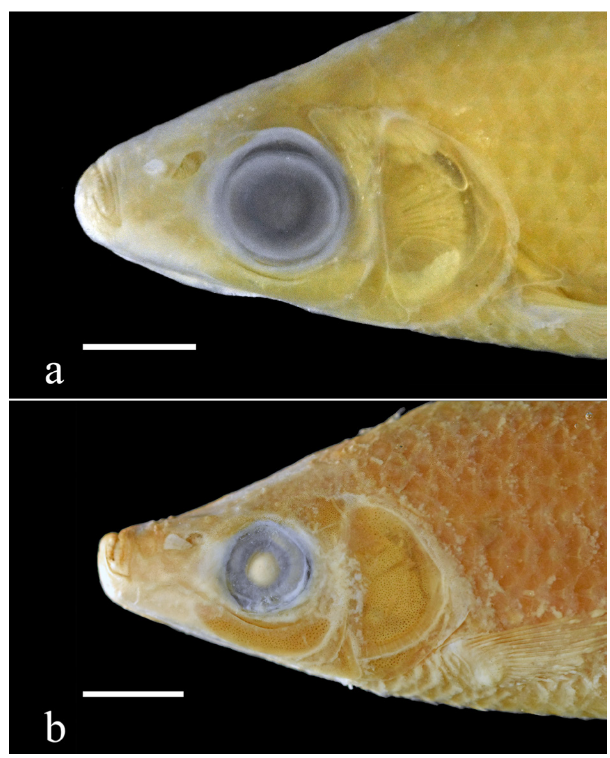

Figure 2. **Head of** ***Pseudanos trimaculatus*** **from the Río de la Plata basin: a. MLP 7281, 79.9 mm SL; b. MACN-ict 6207, 69.1 mm SL. Left side, lateral view. Scale 5 mm.**

the underlying biological variation. The size-corrected PCA plot of the PC1 *vs*. PC2 (Figure 4a: representing 48.3 % of the total variance, File SII) suggested that the specimens from the Río de la Plata are within the range of variation of the rest of the specimens of *P. trimaculatus*. Moreover, these specimens (Figure 4: Río de la Plata group) grouped closer to the specimens from the Guaporé, Napo and Uatumã groups than any other along the PCs. In particular, through PC1, the specimens from the Branco, Jari, Madeira (in part), Xingu groups were distributed quite distant from the specimens of the Río de la Plata. PC1 was mostly influenced positively by the caudal peduncle length (0.7), and negatively by the body depth (-0.2), and body width (-0.2) (Table II). PC2 was most heavily affected by measurements such as the head depth (-0.5) and body width (-0.3) (Table II). The remaining components selected, PC3 and PC4, explained 27.1 % of the variance in the dataset (Figure 4b). Comparatively, these PCs were most strongly influenced by the body width (-0.4: PC3 and PC4) and eye diameter (0.2: PC3; -0.1: PC4), and bony interorbital length (0.2: PC3; -0.4: PC4) (Table II). Along PC3, the specimens from the Río de la Plata basin were placed slightly separated between each other but located closer to other specimens. Full morphometric loadings are presented in Table II, other PCA results are informed as File SII.

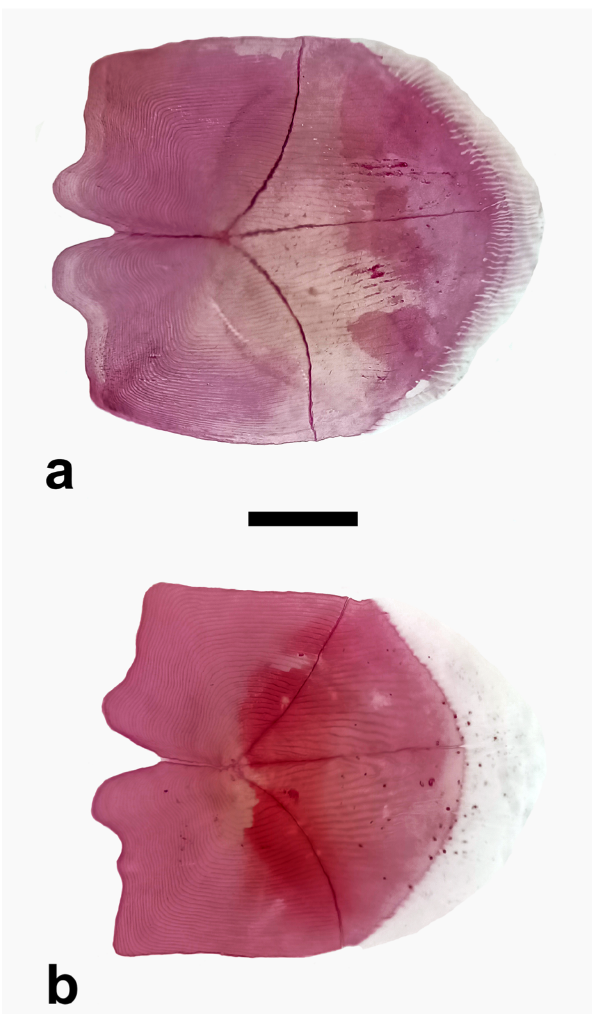

Figure 3. Lateral body scale of *Pseudanos trimaculatus* from the Río de la Plata basin: a. MLP 7281; b. MACN-ict 6207. Left side, lateral view. Scale 1 mm.

The cluster analysis did not reveal completely exclusive sets of specimens based on our geographic origins (Figure 5), but showed results that were somewhat similar in part to those obtained by the PCA. The specimens from the Río de la Plata were grouped together in a medium-size cluster that is divided into two smaller ones, in which each specimen is distributed. The medium-size cluster is composed of specimens from the Orinoco and Uatumã. The Argentinean specimens, as it was observed in the PCA results, are more closely associated with another specimen than with each other.

Comparative material examined

Pseudanos trimaculatus: Brazil: INPA 2664, 1, 158.8 mm SL, Roraima, Maracá, Río Uraricoera, at Ilha de Maracá, 11–17 Mar 1988, M. Jégu. INPA 3204, 1, 150.3 mm SL, Amazonas, Río Pitinga, at Bom Futuro, c. 1°52’S 59°37’W, 17 Oct 1989, F. Marinho. INPA 15180, 2, 71.4–74.6 mm SL, Rondônia, Pimenteiras do Oeste, Río Guaporé, 13°29’S 61°3’W, 1 Dec 1984, G. M. Santos. INPA

Table I. Comparative morphometric data of the specimens analyzed of *Pseudanos trimaculatus*. SD: standard deviation.

Measurements	Río de la Plata			Amazon			Orinoco
	n	Range	Mean±SD	n	Range	Mean±SD	
Standard length (mm)	2	69.1–79.7	74.4±7.5	30	59.8–162.0	107.0±28.3	69.4
Percents of SL:							
Lower jaw to anal-fin origin	2	79.3–81.2	80.3±1.3	30	73.8–82.4	80.1±1.9	78.6
Lower jaw to adipose-fin origin	2	83.7–85	84.4±0.9	30	83.4–86.6	85.0±1.0	84.8
Lower jaw to dorsal-fin origin	2	48.6–50.6	49.6±1.5	30	45.7–50.7	48.1±1.3	50.4
Lower jaw to pelvic-fin origin	2	49.9–50.1	50.0±0.2	30	38.6–52.0	49.8±2.4	50.8
Dorsal-fin origin to caudal-fin origin	2	53.1–54.5	53.8±1	30	54.0–58.2	55.7±1.2	54.1
Dorsal-fin origin to adipose-fin origin	2	38.1–38.2	38.1±0	30	36.8–41.8	39.4±1.3	37.3
Caudal-peduncle length	2	12.6–13.9	13.3±0.9	30	9.4–13.1	11.2±0.8	12.8
Body depth	2	24.8–25.2	25±0.2	30	24.7–32.7	27.1±1.9	27.1
Body width	2	13.0–13.3	13.2±0.2	30	11.3–17.2	13.9±1.5	12.8
Caudal-peduncle depth	2	10.0–10.4	10.2±0.2	30	9.7–11.6	10.7±0.5	10.0
Head length	2	24.9–27.1	26±1.6	30	22.4–26.9	24.2±1.3	26.3
Percents of HL:							
Preopercle length	2	72.7–76.5	74.6±2.7	30	66.8–77.3	72.9±2.2	70.5
Snout length	2	36.7–39.8	38.2±2.2	30	35.8–41.2	38.9±1.4	30.5
Head depth	2	78.2–86.9	82.6±6.2	30	67.5–89.3	75.2±5.8	85.4
Preopercle depth	2	64.8–69.0	66.9±3	30	58.4–67.3	62.1±2.5	64.9
Snout depth	2	36.6–41.1	38.8±3.1	30	38.4–46.9	42.0±2.3	36.7
Eye diameter	2	27.2–29.1	28.1±1.3	30	20.9–29.5	24.5±2.5	28.5
Bony interorbital	2	40.3–42.1	41.2±1.3	30	38.9–45.6	41.8±1.9	32.3

26862, 1, 114.3 mm SL, Amazonas, Presidente Figueiredo, Río Uatumã, at Cachoeira Morena, 2°7'23"S 59°19'49"W, 12 Feb 2007, E. Ferreira. INPA 35266, 1, 82.9 mm SL, Amazonas, Apuí, Igarapé Pajurá, tributary of Río Guariba, at Resex Guariba, Río Aripuanã basin, 8°46'18"S 60°23'37"W, 6 Nov 2008, W. Pedroza et al. MZUSP 31255, 1, 68.5 mm SL), Amazonas, Río Negro, at, Anavilhanas, c. 2°42'S 60°45'W, 21 Nov 1979, M. Goulding. MZUSP 31441, 1, 119.8 mm SL, Roraima, Maracá, Río Uraricoera, c. 3°21'N 61°25'W, 13 Jan 1984, M. Goulding. MZUSP 31476, 1, 118.2 mm SL, Rondônia, Calama, Río Madeira, c. 8°3'S 62°53'W, 1980, M. Goulding. MZUSP 35581, 1, 121.0 mm SL, Amazonas, Humaitá, Igarapé Banheiro, c. 7°31'S 63°2'W, Jul 1975, U. Caramaschi. MZUSP 36927, 1, 151.3 mm SL, Mato Grosso, Vila Bela da Santíssima Trindade, Río Alegre, tributary of Río Guaporé, c. 15°2'S 59°58'W, 29 Sep 1984, J. C. Garavello et al. MZUSP 101569, 3, 85.2–91.3 mm SL, Amapá, Laranjal do Jari, Rio Iratapuru at Cachoeira de São Raimundo, 0°33'59"S 52°34'40"W, 13 Sep 2008, J. L. Birindelli and P. Hollanda-Carvalho. MZUSP 101593, 2, 94.9–100.8 mm SL, Pará, Monte Dourado, Rio Jari, downstream of Cachoeira Santo Antônio, 0°41'27"S 52°30'44"W, 18 Sep 2008, C. R. Moreira and A. Akama. MZUSP 103386, 1, 96.7 mm SL, Pará, Monte Dourado, Rio

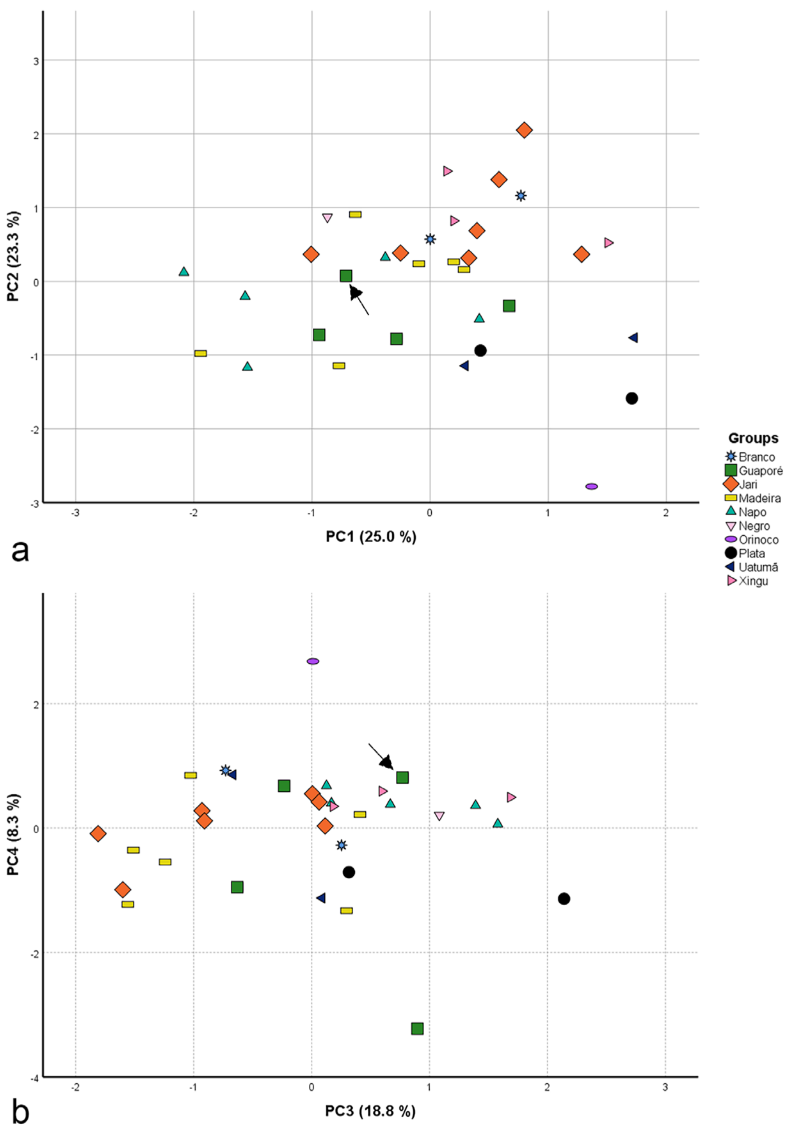

Figure 4. Principal component analysis of the size-corrected morphometric data of *Pseudanos trimaculatus* including the specimens analyzed. a. PC1 vs PC2; b. PC3 Vs PC4. Plata = Río de la Plata. Black arrow = NMW 62692.

Jari, downstream of Cachoeira Santo Antônio, 0°41’10”S 52°36’10”W, 19 Feb 2009, J. L. Birindelli et al. MZUSP 103423, 1, 103.8 mm SL, Pará, Monte Dourado, Rio Jari, upstream of Cachoeira Santo Antônio, 0°36’9”S 52°31’35”W, 20 Feb 2009, J. L. Birindelli et al. MZUSP 103540, 2, 101.0–113.4 mm SL, Amapá, Laranjal do Jari, Rio Jari, upstream of Cachoeira Santo Antônio, 0°34’18”S 52°34’40”W, 22 Feb 2009, J. L. Birindelli et al. MZUSP 105848, 3, 96.1–103.8 mm SL, Pará, Altamira, Rio Xingu, at Boa Esperança, 3°33’45”S 52°20’59”W, 12 Jan 2001, Equipe UFPA. UFRO-I 107, 3, 76.6–127.8 mm SL, Rondônia, Porto Velho, Igarapé Jaciparanã, tributary of Rio Madeira, c. 8°45’S 63°54’W, 6 Dec 2004, Equipe UNIR. NMW 62692, holotype, 156.9 mm SL, (“Matto Grosso” = Rio Guaporé at Vila Bela da Santíssima Trindade), c. 15°0’S 59°57’W, 1828–1829, J. Natterer. Ecuador: FMNH 102122 ,13, 66–162 mm SL, Napo, Río Yasuni, tributary of Río Jatuncocha, 1°0’18”S 75°31’24”W, 24 Oct 1981, D. J. Stewart et al. FMNH 102125, 5, 58–129 mm SL, Napo, Río Yasuni or Río Salado, 0°58’36”S 75°26’6”W, 27 Oct 1981, D. J. Stewart et al. FMNH 102127, 2, 97.4–134.4 mm SL, Napo, Quebrada Zancudococha, tributary of Río Aguarico, 0°33’30”S 75°30’0”W, 2 Nov 1983, D. J. Stewart

Table II. Loading matrix produced by the size-corrected PCA comparing the specimens studied of *Pseudanos trimaculatus*.

Variables	Components			
	1	2	3	4
Standard length (mm)	0.0	0.0	0.0	0.0
Lower jaw to anal-fin origin	0.0	0.0	0.0	0.0
Lower jaw to adipose-fin origin	0.0	0.0	0.0	0.0
Lower jaw to dorsal-fin origin	0.0	0.0	0.0	0.0
Lower jaw to pelvic-fin origin	0.0	0.1	-0.1	0.6
Dorsal-fin origin to caudal-fin origin	0.0	0.1	-0.1	0.0
Dorsal-fin origin to adipose-fin origin	0.0	0.1	-0.1	-0.1
Caudal-peduncle length	0.7	-0.1	0.0	-0.3
Body depth	-0.2	-0.1	0.0	0.2
Body width	-0.2	-0.3	-0.4	-0.4
Caudal-peduncle depth	0.0	0.0	-0.1	0.1
Head length	0.0	0.0	0.1	0.0
Preopercle length	0.0	0.0	0.1	0.0
Snout length	-0.1	0.2	0.1	-0.2
Head depth	0.0	-0.5	0.2	0.1
Preopercle depth	0.0	-0.1	0.1	0.0
Snout depth	-0.1	0.0	0.1	0.1
Eye diameter	0.0	0.0	0.2	-0.1
Bony interorbital	-0.1	0.1	0.2	-0.4

et al. MZUSP 38680, 2, 58.0–59.8 mm SL, Napo, Laguna Jatuncocha, 0°59’7”S 75°27’2”W, 23 Oct 1981, D. J. Stewart and M. Ibarra. Guyana: BMNH 1911.10.31.463, paratype of *Pseudanos irinae*, 53.7 mm SL, Río Essequibo, at Crab Falls, c. 5°23’N 58°53’W, Nov 1908, C. H. Eigenmann. Venezuela: CAS 58809*, 1, holotype of *Pseudanos irinae*, 69.4 mm SL (plus radiograph), Amazonas state, Orinoco basin, Orinoco bifurcation, Tamatama rock, c. 3°8’26.03”N, 65°52’32.90”W, 4 Mar 1925, C. Ternetz.

DISCUSSION

We could not observe discrete differences between the meristic data of the specimens from Argentina and the Amazon basin (see, Birindelli et al. 2012: Table I). Based on the PCA results, variables such as the snout length, body width, head length, and caudal peduncle length are among the most informative to characterize the intraspecific variation observed in *P. trimaculatus*. In fact, these variations are subtle and could only be detected by multivariate methods (i.e. the descriptive statistics of the morphometric data between the MLP and MACN-ict specimens is fully overlapped with the specimens from the Amazon basin). It is not possible based on our results to point out the specific factors that would explain these differences. Usually, the intraspecific variation can be interpretated as part of a divergence

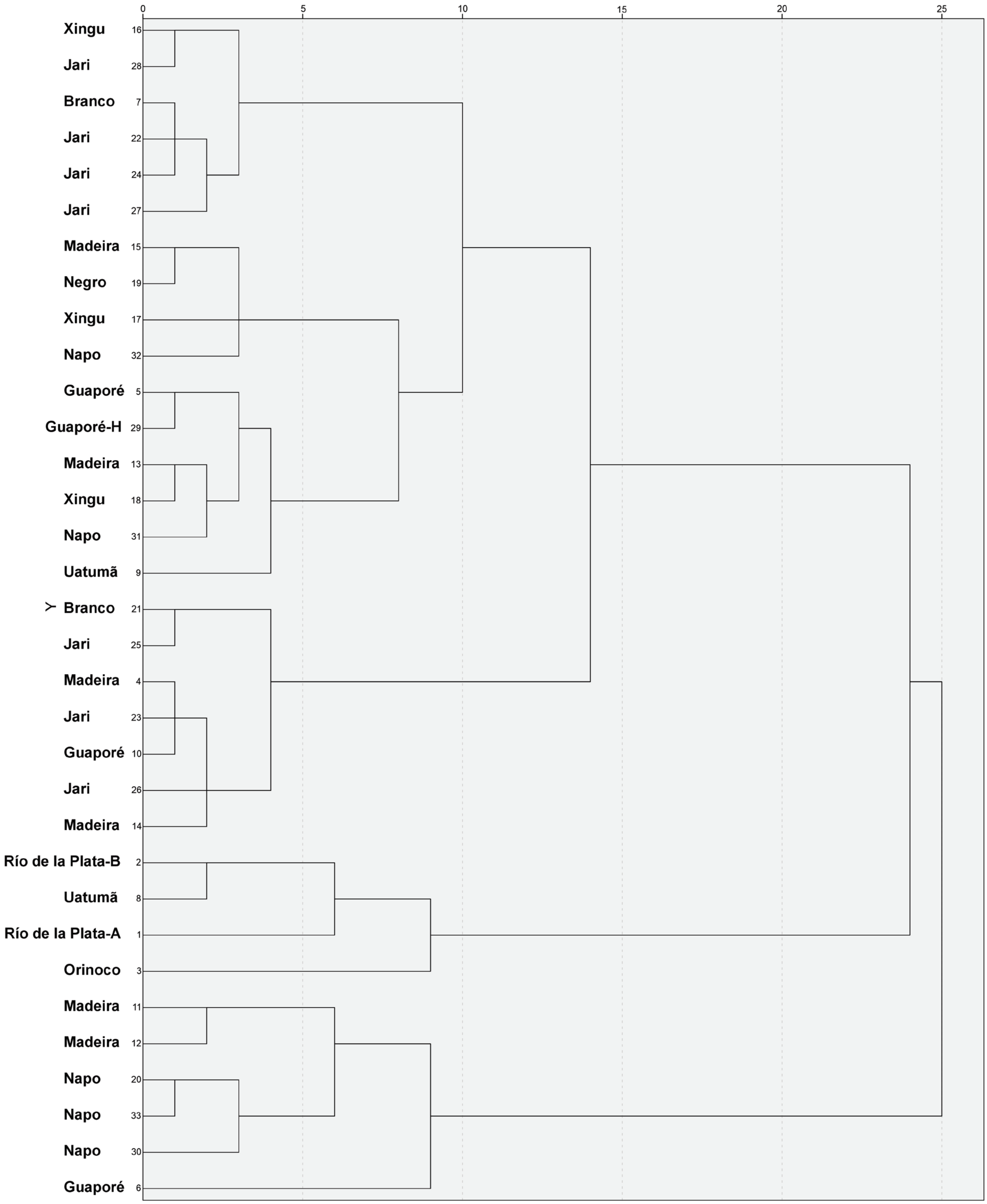

Figure 5. Dendrogram obtained by the Ward's method of the specimens compared of *Pseudanos trimaculatus*. Río de la Plata-A = MLP 7281; Río de la Plata-B = MACN-ict 6207; Guaporé-H = NMW 62692.

resulting from a geographical discontinuity (Aguirre et al. 2016, Lazzarotto et al. 2017, Marinho & Langeani 2016, Vanegas-Ríos 2016, Vanegas-Rios et al. 2019). Therefore, the subtle variations observed here in *P. trimaculatus* may likely respond to a gradual divergence accumulated by the disjunct distribution or spatial disaggregation between the groups, specially whether the specimens from the Río de la Plata are only compared with all the specimens from the Amazon basin as a single group. Usually, the integration of morphology, DNA and cytogenetic data reveals supportive explanations to validate and understand the population structure in fish species with disjunct distribution (Aguilera et al. 2022, Guimarães et al. 2021). The morphology data compared between the specimens under study gave us supportive arguments to accept the presence of the species in two disjunct basins and, in part, to elucidate the intraspecific patterns within the species.

Our results revealed a relatively moderate population variation among examined specimens, which can be explained in part by the limited number of specimens available from the Río de la Plata basin, and the somewhat steady phenotypic variation within *P. trimaculatus* in terms of body shape. However, it is expected that such a widespread species exhibits intraspecific variation despite the similarity among its specimens (e.g. head measurements, see Table I). Such variation is usually expected as part of processes associated with differential adaptations to diverse habitats (Garavello et al. 1992, Vanegas-Rios et al. 2019). In fact, some minor differences were detected between the MLP and MACN-ict specimens in some characteristics as the head depth, snout depth, eye diameter and dorsal profile of the head (see Figure 2 and Table I), in this way (respectively): 78.2 % HL vs. 86.9 % HL, 41.1 % HL vs. 36.6 % HL, 27.2 % HL vs. 29.1 % HL, and straight profile vs. slightly concave. Although these values are within the range of variation for the species (Table I), the specimens from the Amazon and Orinoco basins have somewhat different tendencies in the data. For example, the head depth on average was 75.2 % HL (showing a high internal deviation ± 5.8) in the specimens from the Amazon basin (85.4 % HL in the Orinoco specimen), a value below the variation mentioned for the MLP and MACN-ict specimens. For the snout depth, the mean value in the specimens from the Amazon basin was 42.0 % (± 2.3 standard deviation), which is more similar to the variation of the MLP specimen, but the Orinoco specimen (36.7 % HL) had a value closer to the MACN-ict specimen. In the eye diameter, the specimens from the Amazon basin tended to have a smaller eye (24.5 % HL, ± 2.5), whereas it is slightly greater in the Orinoco specimen (28.5 % HL, closer to the variation of the MLP and MACN-ict specimens). In general, the specimens examined in the Amazon and Orinoco basins are characterized by the slightly concave dorsal profile of the head (Birindelli et al. 2012: Figure 6), which is more similar to the MACN-ict specimen. These patterns influenced the cluster analysis (Figure 5), in which the MLP and MACN-ict specimens were grouped slightly distant to each other in the dendrogram. Comparing *P. trimaculatus* and congeners (based on Birindelli et al. 2012: Table I), the head depth tended to be smaller in *P. gracilis*, and *P. varii*, and *P. winterbottomi* (mean = 62.4 % HL ± 3.7; 70.2 % HL ± 4.9; 67.0 % HL ± 5.5). In the snout depth, *P. varii* presented a greater average tendency (41.5 % HL ± 2.7) with respect to the other species (mean values below 39.0 %). *Pseudanos winterbottomi* tended to has a larger eye (27.3 ± 3.3), in comparison with *P. gracilis* and *P. varii* (24.0 % HL ± 2.2; 25.5 % HL ± 2.8), and is more similar to the MLP and MACN-ict specimens (and some specimens of *P. trimaculatus* in the Amazon basin). The dorsal

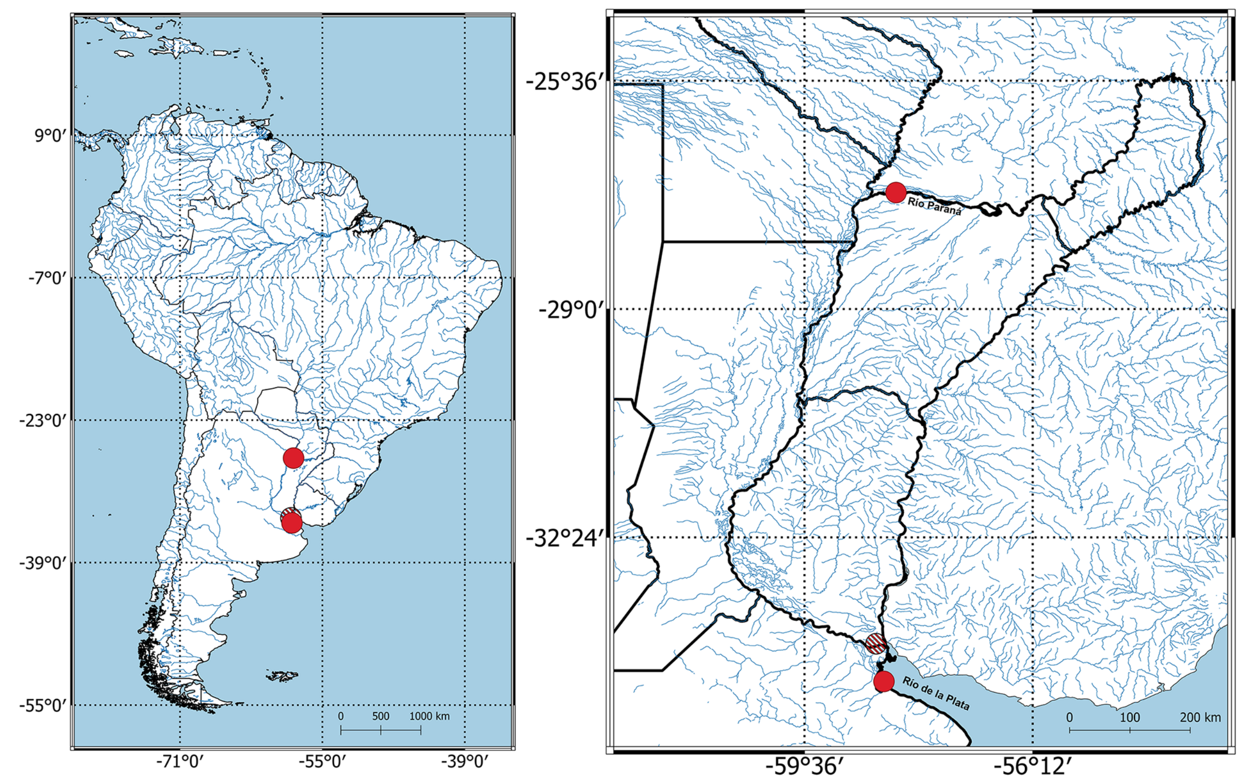

Figure 6. Distribution map of *Pseudanos trimaculatus* (circles) in the Río de la Plata basin, Argentina. Coordinates (33°59S; 58°32'W) corresponding to the uncorroborated record (striped circle) in the Delta of the Río Paraná (López et al. 2005; more details in the text) were estimated using the closest intermediate point between the provincial limit and the Delta.

profile of the head is straight or slightly concave in *P. gracilis*, *P. varii*, and *P. winterbottomi*, but is slightly concave or concave in the specimens from the Amazon and Orinoco specimens of *P. trimaculatus*, reason for which the dorsal profile in the MLP specimen is more aligned with the variation reported in other *Pseudanos* species.

Currently the state of preservation of the two specimens from Argentina herein examined is slightly different to what was shortly described by Alonso de Arámburu et al. (1962), Ringuelet et al. (1967) and Braga (1993). The MLP specimen is faded, its predorsal pigmentation is completely lost and the midlateral blotches are almost inconspicuous, except for the one below the dorsal fin on the right side of the body. The MACN-ict specimen is more similar in pigmentation to what observed by Braga (1993). In fact, in this specimen, it is possible to observe the body spots and the predorsal bars. Nevertheless, dentition and fin rays are in rather good conditions. In addition to the meristic and morphometric conclusions aforementioned, the two specimens have three branchiostegal rays, coloration composed of four dark midlateral blotches plus narrow dark transversal bars on the dorsum, body scales with theirs ventralmost and dorsalmost *radii* forming an angle of more than 110° (Figure 3), as well as four multicuspid compressed teeth on both premaxillary and dentary bones, and fringed lips. These data confirm their identification as co-specific with *P. trimaculatus*.

We considered that our results are enough to corroborate the presence of *P. trimaculatus* in the Río de la Plata basin. We could not find any specimen cited (or deposited in collections) or photo supporting the record of the species in the Delta of the Río Paraná (Entre Ríos province), as was presented by López et al. (2005) and then compiled in Arias et al. (2013) and Rosso & Liotta (2021). Thus, although we are unable to validate that record at the moment, the locality is plotted in our distributional map (Figure 6). It is rather possible that the record may be a misidentification or that never can be corroborated, but further attempts will be made by us to try to find any potential clue to the origin of this matter.

The records of these MLP and MACN-ict specimens in the Río de la Plata basin are clearly odd and unlikely. All other species of Anostominae are currently known to occur in the Amazon and Orinoco basins and in the coastal rivers of the Guyanas and Suriname. We analyzed the validity of the records from Argentina trying to uncover any possible human mistake that may have caused a confusion of specimens or collection data. However, we could not find consistent evidence to support such an assumption at this time, based on the data provided by the curatorial staff of each collection. In any case, this possibility will remain latent until new specimens of *P. trimaculatus* are collected from the Río de la Plata basin and/or until a stronger argument can be tested (beyond speculations). For these reasons, the present contribution is key to provide a greater dissemination of these records between the scientific and fishing communities.

Based on our results, *P. trimaculatus* is added to the list of fish species shared between the Amazon and Río de la Plata regions. Carvalho & Albert (2011) listed 111 fish species shared between the Paraguay and Amazon drainages. Considering that list and the recent nomenclatural changes updated in the Eschemeyer's Catalog of Fishes (Fricke et al. 2023), we counted 109 species (including the recently described *Saxatilia ploegi* (Varella, Loeb, Lima & Kullander) and *Hoplias misionera* Rosso, Mabragaña, González-Castro, Delpiani, Avigliano, Schenone & Díaz de Astarloa *Moenkhausia mutum* Dagosta & Marinho) (Guimarães et al. 2021, Varella et al. 2018) shared between the Amazon and Río de la Plata basins. In addition to *P. trimaculatus*, other anostomids, such as *Abramites hypselonotus* (Günther), *Leporellus vittatus* (Valenciennes), *Leporinus friderici* (Bloch), *L. striatus* Kner, and *L. octomaculatus* Britski and Garavello, have been previously reported to be conjointly present in those basins (Carvalho & Albert 2011, Garavello et al. 1992).

It is remarkable to notice that the southernmost record in the Amazon basin of *P. trimaculatus* is located in the Rio Guaporé at Vila Bela da Santíssima Trindade (Matto Grosso, Brazil) (Birindelli et al. 2012) and the northernmost record in the Río de la Plata basin is located in Itatí (Corrientes, Argentina), which represents a linear distance of about 1368 km between both localities (according to Google Earth Pro 7.3). The species has not been recorded along the Paraná-Paraguay basin in Brazil or Paraguay (Birindelli et al. 2012, da Silva et al. 2020, Koerber et al. 2017, Ota et al. 2018), which bring out a large gap in the geographic distribution of *P. trimaculatus*. What is clear to us is that it breaks the usual trends found in the biogeographic patterns of the freshwater fishes shared between the Amazon and Río de la Plata basin, which may be characterized as follow: a) species widely distributed with a continuous occurrence along the Paraná-Paraguay basin and their populations occurring more closely between each other along the Amazon and Rio de la Plata basins (e.g. Belonidae: *Potamorhaphis eigenmanni*; Anostomidae: *A. hypselonotus*) (Carvalho & Albert 2011, Collete 1982, Garavello et al. 1992, Ota et al. 2018, Polaz et al. 2014); b) species in which their populations are less widely distributed, with a more restricted occurrence along the Paraguay-Paraná basin, usually located in the upper portions of its tributaries (e.g. Cetopsidae: *Cetopsis starnesi* Vari, Ferraris & de Pinna; Cichlidae: *S. ploegi*) (Varella et al. 2018, Vari et al. 2005). The distributional gap is really a mystery that we cannot explain at the present based on the data available, but it may be associated with an example of a threatened population, whose distribution is more restricted to the middle Paraná basin in the Río de la Plata basin, as consequence of its inherent rareness.

The conservation status of the species currently is considered as “least concern” by the global IUCN Redlist (https://www.iucnredlist.org/es/species/49829553/160294967). In Argentina, only two specimens of *P. trimaculatus* are currently known. This scenario could lead one to consider the species as “data deficient” in Argentina (IUCN 2012). However, this would imply assuming that there are potential threats that might lead to the extinction of the species in the country in the next decades or so, which seems implausible given its putative wide range. In fact, the lack of collection efforts directed towards sampling the species does not allow us to understand its distribution and abundance in Argentina. Nevertheless, it is likely that the species may be best classified as “least concern” in Argentina. Additional sampling efforts should be made focusing on collecting additional individuals of *P. trimaculatus* in Argentina.

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JAVR and JB designed the study, examined the fishes, took the morphological data. JAV made the data analysis, edited the figures, and wrote the first draft of the manuscript. JB contributed with writing and revision of the final manuscript.