Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 16 (1): 93–101
https://doi.org/10.15560/16.1.93

PENSOFT

First record of *Astyanax bifasciatus* Garavello & Sampaio, 2010 (Teleostei, Ostariophysi, Characidae) in the Piquiri river basin, upper Paraná river basin

Mayara P. Neves[1], Priscilla C. Silva[2], Rosilene L. Delariva[3], Clarice B. Fialho[1], Andre L. Netto-Ferreira[1]

1 Pós-graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Departamento de Zoologia, Avenida Bento Gonçalves 9500, bloco IV, prédio 4343-5, CEP 91501-970, Porto Alegre, RS, Brazil. **2** Universidade Federal de Viçosa, Departamento de Biologia Animal, Avenida Ph. Rolfs s/n, Anexo II CCB, CEP 36570-000, Viçosa, MG, Brazil. **3** Universidade Estadual do Oeste do Paraná, Centro de Ciências Biológicas e da Saúde, Programa de Pós-Graduação em Conservação e Manejo de Recursos Naturais, Rua Universitária 2069, 85819-110, Cascavel, PR, Brazil.

Corresponding author: Mayara P. Neves, mayara-nevesbio@hotmail.com

Abstract

Astyanax bifasciatus Garavelo & Sampaio, 2010 was originally described as endemic to the Iguaçu river basin. Between March 2017 and January 2018, specimens of *A. bifasciatus* were sampled during expeditions to headwater streams of the Piquiri river basin, upper Paraná river basin. The identification was confirmed both by morphological and molecular analyses, representing, therefore, the first record of the species outside of the basin of the Iguaçu River. In addition, the lack of structuring in the haplotype network confirms that the representatives of *A. bifasciatus* from both basins appear to comprise a single population.

Keywords

Distribution range, DNA barcode, endemicity, headwater streams, species identification.

Academic editor: Cristiano Moreira | Received 28 October 2019 | Accepted 14 January 2020 | Published 31 January 2020

Citation: Neves MP, Silva PC, Delariva RL, Fialho CB, Netto-Ferreira AL (2020) First record of *Astyanax bifasciatus* Garavello & Sampaio, 2010 (Teleostei, Ostariophysi, Characidae) in the Piquiri river basin, upper Paraná river basin. Check List 16 (1): 93–101. https://doi.org/10.15560/16.1.93

Introduction

Astyanax Baird & Girard, 1854 is the richest genus of the Characidae with about 180 valid species (Fricke et al. 2019). Most species are highly morphologically similar and lack unique diagnostic characters making the process of accurately identifying species difficult (Rossini et al. 2016; Silva 2017). In the Iguaçu river basin, *Astyanax* is the most diverse and abundant group of fishes (Pavanelli and Oliveira 2009; Baumgartner et al. 2012; Pini et al. 2019). Among its representatives, *Astyanax bifasciatus* Garavello & Sampaio, 2010 is widely distributed throughout the basin (Garavello and Sampaio 2010; Baumgartner et al. 2012), where it is highly abundant both in the main channel (Delariva et al. 2013) and in smaller streams (Larentis et al. 2016; Delariva et al. 2018). According to Garavello and Sampaio (2010: 856), *A. bifasciatus* is diagnosed from other congeners by the following combination of characters: “infraorbital 3 deep, but not in contact with the preopercle, leaving a narrow, naked area between its border and the preopercle; the premaxillary outer series with 4 (rarely 5) pentacuspid teeth; the non-symphysial teeth in premaxillary inner series heptacuspid; the vertically elongate humeral blotch with the dorsal portion much wider than the ventral; the presence of a faint and diffuse 'post-humeral spot'; the presence of 36–40 perforated scales on the lateral line; the presence of 7–9, usually 8, gill-rakers on the first epibranchial and 10–13, usually 12, on the ceratobranchial".

The Iguaçu river basin is bordered by the Ribeira, Tibagi, Ivaí, Piquiri, and Paraná III basins (Parolin 2010). Among those, the Piquiri river basin has the third largest drainage area, also with extensive stretches free of damming in the upper Paraná river basin (Affonso et al. 2015; Cavalli et al. 2018). Recent ichthyofaunistic inventories in Piquiri river basin recorded a high diversity of fishes (152 species), mainly represented by small species belonging to the Loricaridae and Characidae (Gubiani et al. 2010; Delariva and Silva 2013; Dei Tos et al. 2014; Cavelli et al. 2018). However, those studies did not record *A. bifasciatus*. We report herein a geographic range extension for *A. bifasciatus* in the Piquiri river basin, representing the first record outside from the Iguaçu river basin. Additionally, we provide a brief description of these individuals, based on morphological characters and Cytochrome c oxidase subunit 1 (COI) gene sequences.

Methods

The Piquiri and Iguaçu rivers are direct tributaries of the Paraná River (Parolin et al. 2010). The Iguaçu basin is characterized by have a low diversity of species (Baumgartner et al. 2012). Piquiri basin, besides the high diversity, is one of the only rivers free of dams in Paraná state. Specifically, in western Paraná, both basins are mainly affected by agricultural activities, with monocultures of soybean, corn, and sugar cane (Parolin et al. 2010). At the border between these two basins, in the municipality of Cascavel, the fish fauna was sampled in nine headwater streams (Fig. 1).

The samplings were performed using electrofishing technique between March 2017 and January 2018. A collecting license was provided by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (Process 30182) and approved by the Ethics Committee on Animal Use of the Universidade Federal do Rio Grande do Sul (CEUA; Permit number 32734) in accordance with protocols in their ethical and methodological aspects for the use of fish. Specimens were deposited at the ichthyological collection of the Universidade Federal do Rio Grande do Sul (UFRGS).

The identification of the specimens was based on Garavelo and Sampaio (2010). Measurements and counts followed Fink and Weitzman (1974), with the exception of the number of scale rows below the lateral line, which were counted from the scale row ventral to the lateral line

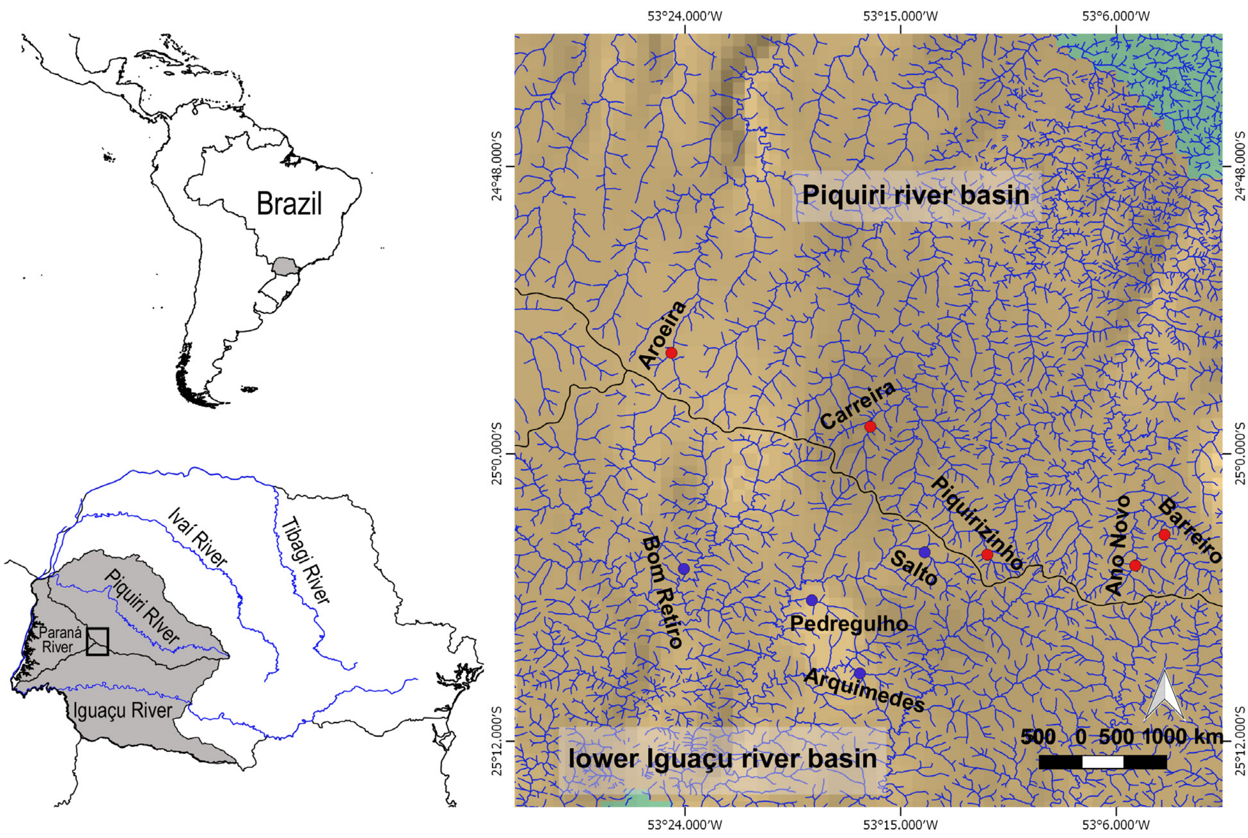

Figure 1. Distribution of *Astyanax bifasciatus*. Red circles are new records from the Piquiri river basin. Blue circles are records from the lower Iguaçu river basin. Black lines indicate the limits among Piquiri, lower Iguaçu, and Paraná III basins.

to the scale row nearest to the origin of the first pelvic-fin ray. The measurements were taken point to point with electronic calipers to the nearest 0.1 mm on the left side of specimens. Counts were taken under a stereomicroscope from the left side of specimens whenever possible.

Morphological variations were evaluated using a Principal Component Analysis (PCA) applied to meristic and morphometric data (based on percentage of standard length and percentage of head length). This analysis was run in R environment version 3.5.2 (R Core Team 2018) with 'vegan' package version 2.4-6 (Oksanen et al. 2019) and 'ggplot2' package version 3.1.0 (Wickham 2016).

Partial sequences of the COI gene were amplified for seven specimens from headwater streams of the lower Iguaçu River (4 specimens) and Piquiri River (3 specimens) basins. In addition, the COI gene of the four sintopic characidae species (*Astyanax* aff. *fasciatus* Cuvier, 1819; *Astyanax* aff. *paranae* Eigenmann, 1914; *Astyanax lacustris* (Lütken, 1875) and *Bryconamericus ikaa* Casciotta, Almirón & Azpelicueta, 2004) was also amplified (Appendix Table A1). DNA was extracted from muscles fragments using CTAB methodology (Doyle and Doyle 1987). COI was amplified with primers cocktail FishF1t1 and FishR1t1 (Ivanova et al. 2007). PCR reactions were conducted in a reaction volume of 20 μL [10.3 μL of H2O, 2 μL of 10× reaction buffer (Platinum®Taq), 0.6 μL of MgCl2 (50 mM), 2 μL of dNTPs (2 mM), 2 μL of each primer (2 μM), 0.1 μL (5U) of Platinum® Taq (Invitrogen), and 100 ng of template DNA], under the following conditions: an initial DNA denaturation at 94 °C for 3 min followed by 35 cycles at 94°C for 30 s, 50 °C for 40 s, and 72°C for 1 min and a final extension at 72 °C for 10 min. The PCR products were purified using the Exosap enzymatic method (25% exonuclease, 25% Shrimp Alkaline Phosphatase and 50% deionized water), and the sequencing was performed by the company ACTGen (Porto Alegre, Rio Grande do Sul). The sequences were aligned using Clustal W in MEGA 6.0 software (Tamura et al. 2013) and the alignments were visually inspected for any obvious base miscall (e.g. base incorporated at the sequence different from the color pic in chromatograms). A Neighbor Joining dendogram and pairwise genetic distances between species were performed using Kimura-2-parameters model (K2P) (Kimura 1980) in the MEGA 6 software (Tamura et al. 2013). Additionally, polymorphic sites were identified using DnaSP software (Librado and Rosas 2009), and haplotype networks were created from Median-Joining (Bandelt et al. 1999) with the use of PopArt 1.7 (Leigh and Bryant 2015) just for *A. bifasciatus* sequences. All generated sequences have been submitted to the GenBank database (Appendix Table A1).

Results

Astyanax bifasciatus Garavelo & Sampaio, 2010

Material examined (morphological data). BRAZIL • Piquiri river basin, upper Paraná river basin: Ano Novo stream (25°04′39.9″S, 053°5′11.9″W), collected by Neves M.P., Larentis C. and Delariva R.L., 12 April 2017 (UFRGS 26224, 5, 52.5–60.4 mm SL), 14 December 2017 (UFRGS 26225, 1, 58.2 mm SL) • Barreiro stream (25°03′22.7″S, 53°03′58.8″W) collected by Neves M.P., Larentis C. and Delariva R.L., 12 April 2017 (UFRGS 26227, 5, 69.6–79.4 mm SL), 14 December 2017 (UFRGS 26229, 4, 51.4–72.3 mm SL; UFRGS 26249, 1, 75.0 mm SL) • Carreira stream (24°58′52.1″S, 053°16′15.8″W), collected by Neves M.P., Larentis C. and Delariva R.L., 29 March 2017 (UFRGS 26231, 5, 74.4–91.3 mm SL), 4 August 2017 (UFRGS 26231, 2, 56.4–65.5 mm SL), 3 January 2018 (UFRGS 26253, 2, 74.0–80.2 mm SL) • Piquirizinho stream (25°04′12.7″S, 053°11′22.6″W), collected by Neves M.P., Larentis C. and Delariva R.L., 29 March 2017 (UFRGS 26237, 8, 59.9–70.2 mm SL), 19 December 2017 (UFRGS 26239, 4, 55.0–60.3 mm SL) • Aroeira stream (24°55′47.4″S, 053°24′33.9″W), collected by Neves M.P., Larentis C. and Delariva R.L., 20 March 2017 (UFRGS 26240, 5, 77.9–63.7 mm SL) • Iguaçu river basin: Arquimedes stream (25°09′10.2″S, 053°16′ 41.9″W), collected by Neves M.P., Larentis C. and Delariva R.L., 27 July 2017 (UFRGS 26235, 10, 69.4–93.1 mm SL), 18 December 2017 (UFRGS 26258, 1 73.5 mm SL) • Salto stream (25°04′06.9″S, 053°13′59.6″W), collected by Neves M.P., Larentis C. and Delariva R.L., 14 March 2017 (UFRGS 26242, 5, 50.1–77.9 mm SL), 25 July 2017 (UFRGS 26243, 5, 45.1–51.4 mm SL) • Bom Retiro stream (25°04′48.4″S, 053°24′02.9″W), collected by Neves M.P., Larentis C. and Delariva R.L., 28 July 2017 (UFRGS 26244, 10, 77.9–100.1 mm SL) • Pedregulho stream (25°6′7.2″S, 53°18′42.2″W), collected by Neves M.P., Larentis C. and Delariva R.L., 27 July 2017 (UFRGS 26247, 5, 56.1–82.0 mm SL), 18 December 2017 (UFRGS 26248, 5, 62.1–74.4 mm SL; UFRGS 26251, 3, 46.5–80.5 mm SL).

Comparative material examined. BRAZIL • Piquiri river basin, upper Paraná river basin: *Astyanax* aff. *fasciatus*: Aroeira stream (24°55′47.4″S, 053°24′33.9″W), collected by Neves M.P., Larentis C. and Delariva R.L., 11 January 2018 (UFRGS 26256, 1, 62.0 mm SL) • *Astyanax* aff. *paranae*: Carreira stream (24°58′52.1″S, 053°16′15.8″W), collected by Neves M.P., Larentis C. and Delariva R.L., 3 January 2018 (UFRGS 26252, 2, 50.1–54.5 mm SL) • *Astyanax lacustris*: Aroeira stream (24°55′47.4″S, 053°24′33.9″W), collected by Neves M.P., Larentis C. and Delariva R.L., 11 January 2018 (UFRGS 26255, 1, 84.2 mm SL) • *Astyanax bifasciatus*: rio Iguaçu basin: stream in Palmas PPBIO (26° 30′33″S, 51°36′50″W), collected by Dala-Corte R., Camana M., Dalmolin M. and Jacob C., 20 January 2016 (UFRGS 22090, 2 of 3, 70.0–75.5 mm SL) • Municipality of Foz do Iguaçu: Tamanduazinho river (25.55°S, 054.52°W), collected by Oliveira, C. and Roxo F. F., 13 September 2012 (LBP 16180, 4, 68.3–78.1 mm SL) • *Astyanax gymnodontus*: rio Iguaçu basin: rio Iguaçu, municipality of Laranjeiras, collected by

Garavello J. C., Soares A. S., Rodrigues R. J., Veber M. J. and Paschoa M. M., 10 November 1990 (UFRGS 11923, 3, 84.7–118.9 mm SL). • *Astyanax gymnogenys*: rio Timbó basin: rio dos Pardos (26.43°S, 050.96°W), collected by Geuster C. J. and Spier E. F., 27 November 2010 (UFRGS 14070, 1, 150.6 mm SL). • *Astyanax minor:* rio Iguaçu basin: Reservatório Vossoro, tributary of rio São João (25.82°S, 049.07°W), collected by GERPEL team, June 2007 (UFRGS 14780, 10, 72.1–82.0 mm SL). • *Astyanax serratus*: rio Iguaçu basin: rio Lageado, municipality of Porto Amazonas (25.54°S, 049.89°W), collected by Bertaco V. A., Artioli L, Wingert J. M., 15 October 2009 (UFRGS 11435, 3,47.4–72.1 mm SL) • *Astyanax troya*: arroyo Cuña Pirú basin: Misiones, collected by Azpelicueta M., Aichino D. and Méndez D., 29 October 2004 (UFRGS 10079, 5, 44.1–61.1 mm SL). • *Astyanax ribeirae*: rio Iguape basin: stream on the road between Iguape and Icapara (24.08°S, 047.53°W), collected by Bertaco V. A., Carvalho F. R., Jerep, F. C. and Thomaz A., 27 November 2010 (UFRGS 13689, 3, 36.0–42.6 mm SL).

Identification. Two hundred and twenty-two representatives of *Astyanax bifasciatus* were sampled from five headwater streams of the Piquiri river basin. Most diagnostic characters proposed for those species were confirmed in representatives of both basins, with the only exception being the number of teeth cusps on the inner premaxillary series, which were pentacuspid in all sampled specimens. Garavello and Sampaio (2010) stated that *A. bifasciatus* have heptacuspid teeth; however, in their description contradicts that information by stating that the inner premaxillary teeth have five "pentacuspid teeth, rarely 4" (p. 856), similar to the conditions observed in all the specimens examined herein (39 from the Piquiri River and 40 from the Iguaçu River). In addition, the specimens share other meristic (Table 1) and coloration characters (i.e. fins red in life; the presence of two humeral blotches; Fig. 2) consistent with the original description of *A. bifasciatus*.

The comparisons between specimens from the Iguaçu and Piquiri rivers employing morphological (PCA; Fig. 3) and molecular tools (COI gene; Fig. 4, Appendix Table A2) showed homogeneity between representatives of both basins, confirming those samples represented a single species. Molecular results indicate a low genetic divergence between the specimens sampled from streams in the Iguaçu and the Piquiri basins (≤0.008). In addition, no structure for each basin (Piquiri and Iguaçu) was observed in haplotype distributions in the network (Fig. 4). The BLAST comparison of the sequences obtained here with available sequences in GenBank resulted in a 100% match with *A. bifasciatus* from the Iguaçu river basin. However, similar matches were also observed in samples attributed to *Astyanax minor* Garavello & Sampaio, 2010 (99.7%), also from the Iguaçu River, and *Astyanax* aff. *intermedius* Eigenmann, 1908 (99.8%) from the Jordão River, a tributary from the Iguaçu River.

Ecological notes. The specimens of *A. bifasciatus* were captured synoptically with other species of the genus reported for the Piquiri river basin, such as *A.* aff. *fasciatus*, *A. lacustris*, and *A.* aff. *paranae* in third-order streams 4.5 m (mean) wide, 50 cm deep, and moderate with marginal vegetation (Fig. 5). Several microhabitats were observed including with backwaters, large pools, and rapids. The substrates were composed of rocks,

Table 1. Morphometric data of *Astyanax bifasciatus* specimens originating from the lower Iguaçu and Piquiri river basins.

Basin	Lower Iguaçu River				Piquiri River			
	N	Range	Mean	SD	*N*	Range	Mean	SD
Standard length (mm)	40	45.1—100.2	72.2	—	39	51.4—91.3	67.4	—
Percentage of standard length								
Predorsal distance	40	48.5—52.8	51.3	1.0	39	49.1—53.9	51.7	1.2
Prepelvic distance	40	43.7—49.4	46.6	1.3	39	41.6—49.3	45.8	1.6
Prepectoral distance	40	22.3—26.4	24.2	1.0	39	20.7—26.0	23.3	1.1
Preanal distance	40	61.6—68.2	65.2	1.9	39	59.3—68.9	64.0	2.0
Depth at dorsal-fin origin	40	30.1—36.9	33.5	1.6	39	30.3—35.4	32.8	1.5
Caudal peduncle depth	40	10.1—12.2	11.0	0.5	39	10.2—12.1	11.2	0.4
Caudal peduncle length	40	11.5—14.3	12.6	0.6	39	10.9—13.9	12.6	0.7
Anal-fin base	40	20.9—28.3	24.2	1.5	39	21.8—27.0	23.8	1.5
Dorsal fin length	40	20.1—26.8	24.0	1.4	39	21.0—26.2	23.5	1.1
Pelvic fin length	40	13.8—18.6	16.2	1.2	39	13.7—17.1	15.6	0.8
Pectoral fin length	40	18.1—23.5	21.4	1.3	39	18.1—23.8	20.9	1.0
Head length	40	22.8—27.0	24.5	1.1	39	23.2—26.2	24.6	0.6
Percentage of head length								
Snout length	40	23.6—31.8	29.0	1.3	39	26.8—31.8	29.4	1.4
Upper jaw length	40	33.3—44.0	37.1	2.1	39	32.6—42.1	36.4	1.9
Orbital diameter	40	31.0 — 39.3	34.7	2.0	39	30.0 — 37.7	34.4	1.7
Interorbital width	40	29.2 — 35.6	31.6	1.4	39	26.4 — 34.8	31.8	1.5

Figure 2. *Astyanax bifasciatus*. **A.** UFRGS 26235, 85.0 mm SL, Arquimedes stream, lower Iguaçu river basin, Paraná, Brazil. **B.** UFRGS 26231, 91.3 mm SL, Carreira stream, Piquiri river basin, upper Paraná river basin, Paraná, Brazil.

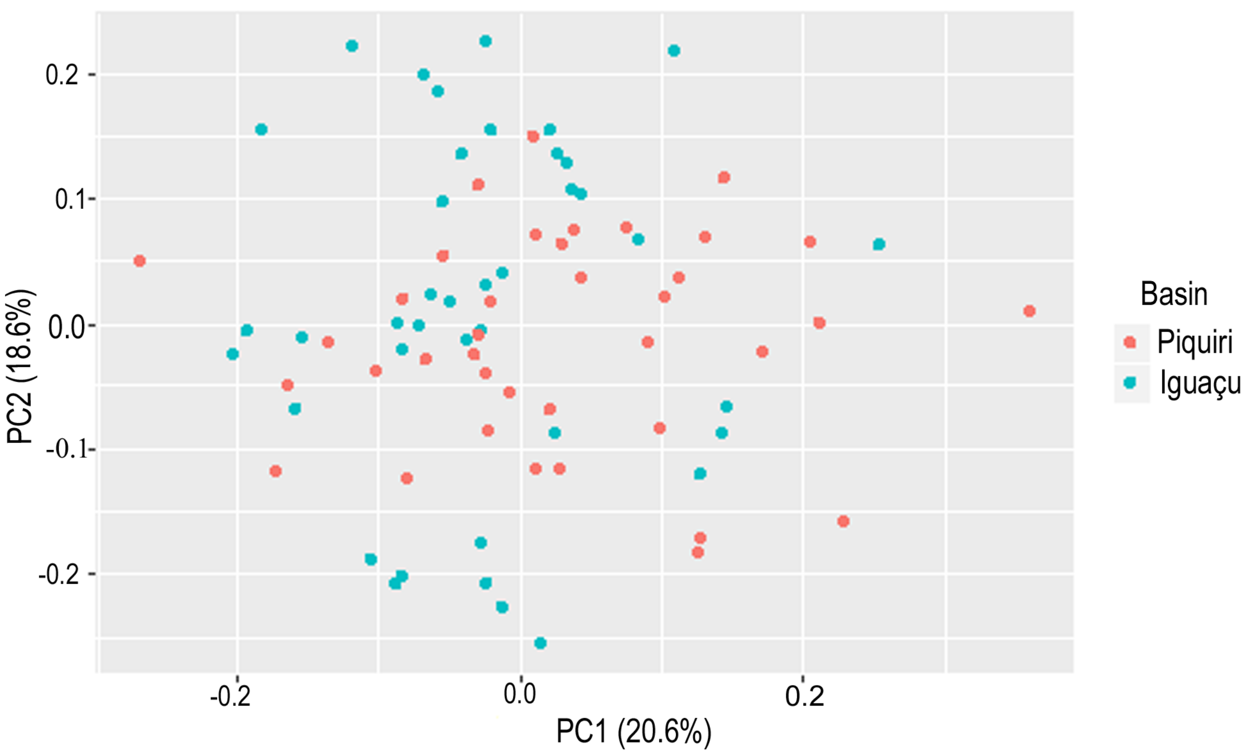

Figure 3. Ordination of populations of *Astyanax bifasciatus* in lower Iguaçu and Piquiri river basins, Paraná, Brazil, produced by the first two axes of the principal component's analysis (PCA 1 and PCA 2) applied to the correlation of 16 morphometric and meristic data of the specimens (based on percentage of standard length and percentage of head length).

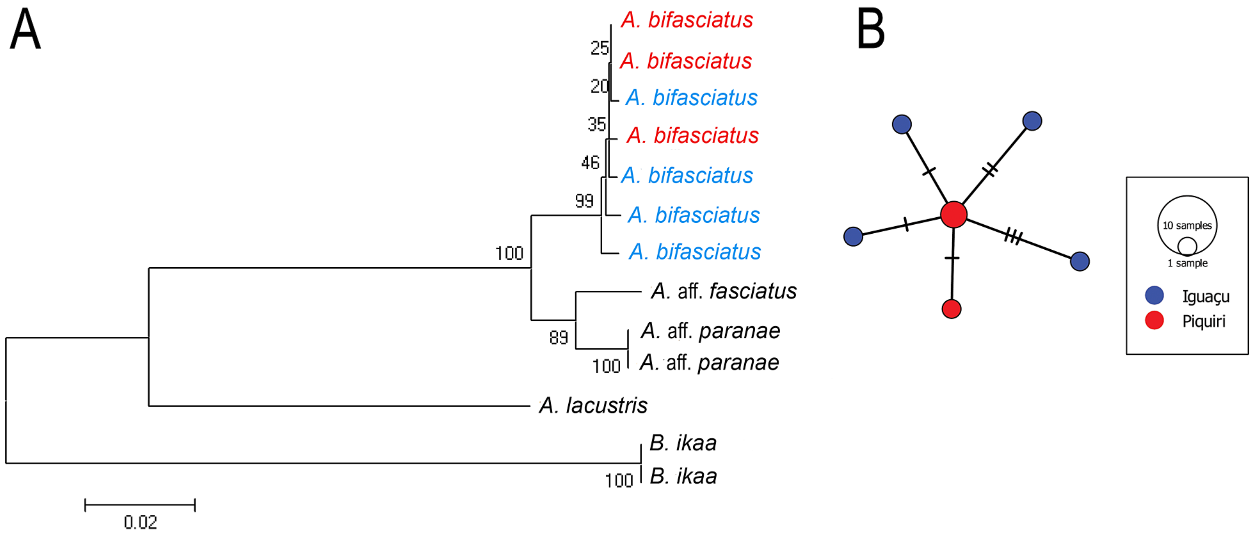

Figure 4. Genetic analyses of *Astyanax bifasciatus* in streams of lower Iguaçu and Piquiri river basins based on COI gene. **A.** Neighbor-joining tree of 13 sequences (Table A1) using genetic distances, with the branch lengths equivalent to the number of base substitutions per site. Bootstrap values (1000 replications) are indicated next to the branches. Blue: specimens from Iguaçu River basin. Red: specimens from Piquiri river basin, upper Paraná River basin. **B.** Haplotype network of *A. bifasciatus*. Traces in each branch refer to number of mutational steps between haplotypes.

gravel, and pebbles, and the water flow was moderate. The areas around the Ano Novo, Barreiro, and Piquirizinho streams are deforested, whereas the Carreira stream has its headwaters within a conservation unit (Centro de Educação Ambiental Suely Marcondes de Moura Festugatto, Cascavel city). The Aroeira stream is within the city of Cascavel and is impacted by pollution and urbanization.

Preliminary results from stomach content analysis of 130 specimens corroborate the omnivorous habit of *A. bifasciatus*, as suggested by Neves et al. (2015); the diet consists mainly of aquatic (Ephemeroptera) and terrestrial insects (Coleoptera and Hymnoptera), seeds, and detritus.

Discussion

Astyanax bifasciatus is the most abundant species in the Iguaçu river basin (Baumgartner et al. 2012). It is omnivorous and easily found in several streams (Neves et al. 2015; Delariva et al. 2018). The species is recognized as highly resilient to many stressors caused by human activities, such urbanization and agriculture (Nimet et al. 2020). Herein, the morphological, meristic, and morphometric characters obtained from the specimens collected in the streams of the Piquiri River are largely congruent with those provided by Garavello and Sampaio (2010) and observed for the Iguaçu River specimens examined herein, despite the imprecise diagnosis of that species. In addition, the molecular tools also confirm that both samples represent a single species, justifying their identification as *A. bifasciatus.*

The absence of structure between the two basins in the haplotype network points to a single population, although the present data is unable to determine the presence of gene flow between representatives of both basins neither other biogeographic processes behind each possible scenario. On the other hand, considering the small genetic distance between the samples of *A. bifasciatus* examined herein, the isolation of the Iguaçu river basin (~22 mya, Oligo-Miocene period) with the uplift of the Iguaçu Falls (Severi and Cordeiro 1994), does not explain the current distribution pattern of the species. A similar scenario was observed by Morais-Silva et al. (2018) with *Trichomycterus davisi* (Haseman 1911) and *T. stawiarski* (Miranda Ribeiro 1968), species that were initially considered endemic to the Iguaçu river basin, but are also present in the Piquiri and the Ivaí river basins, and consist of a single population.

Considering yet the efficiency of the COI marker to elucidate taxonomic problems (Kwong et al. 2013; Rossini et al. 2016), the similarity between the sequences and those identified as "*A. minor*" (99.7%), from the Iguaçu River, and "*A.* aff. *intermedius*" (99.8%), from the Jordão River (Iguaçu river basin), seem indicative of a single species being mistakenly identified under at least three different names. Although the present results indicate a broader distribution for *A. bifasciatus* and refutes the endemism of the species for the Iguaçu river basin, other studies did not detect the species in river basins adjacent to the Iguaçu River, such as the survey on the main channel and tributaries of the Piquiri river basin (Cavalli et al. 2018). The absence of *A. bifasciatus* in Cavalli et al.'s checklist may be due to both the difficulty in the identification of *Astyanax* species, but also in the assumption that *A. bifasciatus* of Garavello and Sampaio (2010) was endemic to the Iguaçu river basin. Similarly, it is possible that *A. bifasciatus* might be present in adjacent drainages such as the Ivaí and the Tibagi river basins (Viana et al. 2013; Claro-García et

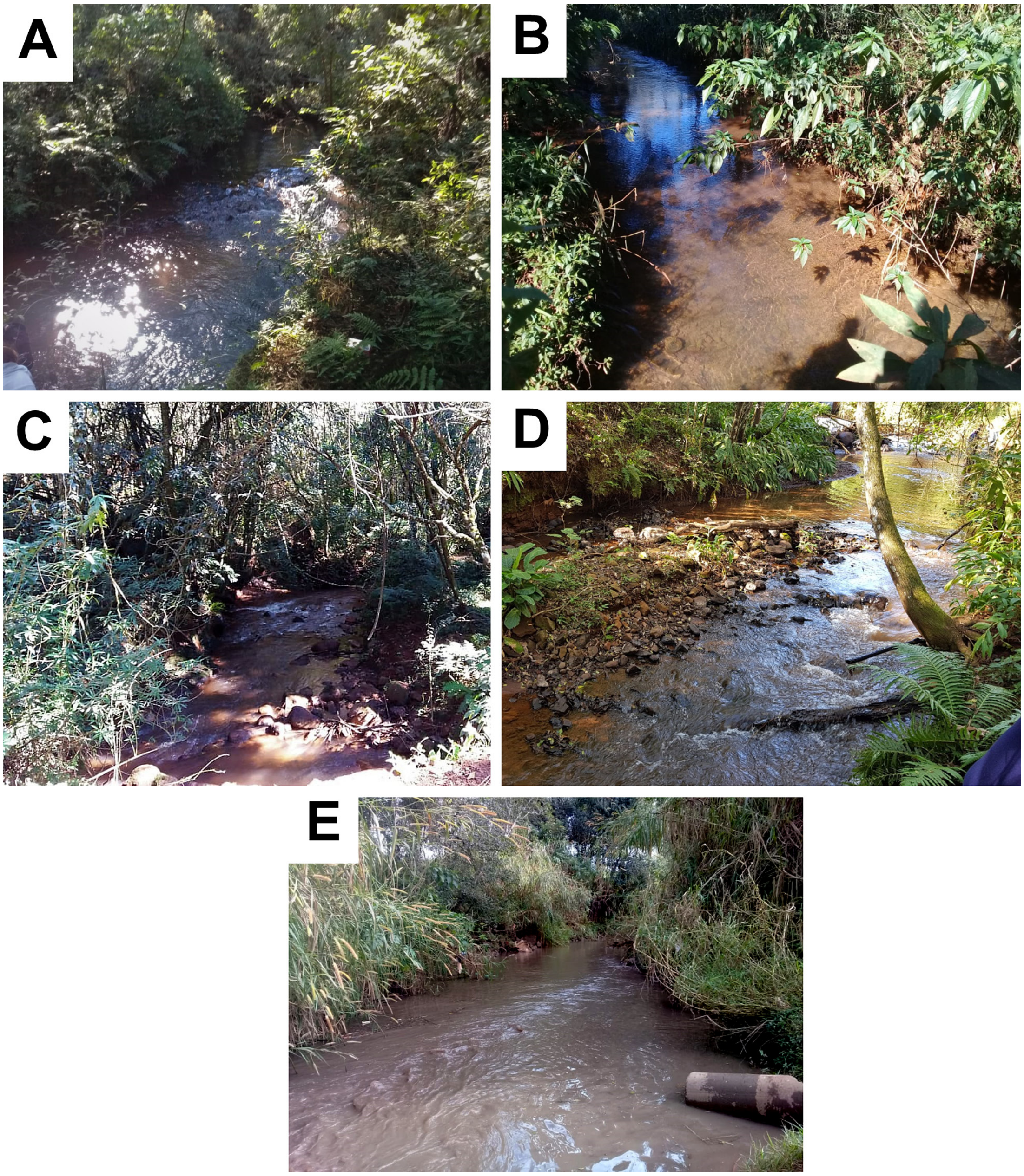

Figure 5. Localities where *Astyanax bifasciatus* specimens were recorded in Piquiri river basin, upper Paraná river basin. **A.** Ano Novo. **B.** Barreiro, both from sub-basin of the Tourinho River. **C.** Piquirizinho. **D.** Carreira, both from sub-basin of the Piquirizinho River. **E.** Aroeira, from sub-basin of the Melissa River.

al. 2018). In addition, it is necessary to investigate the border regions between those basins to better understand the processes that lead to the sharing of this species and possibly other species currently considered endemic of the Iguaçu River. Finally, a robust and integrative taxonomic review of *Astyanax* in the Iguaçu and adjacent river basins would elucidate the distribution pattern of the species and provide accurate tools for the identification of *Astyanax* samples in those basins.

Acknowledgements

We are thankful to the Universidade Estadual do Oeste do Paraná for all support during field expeditions. We thank the LIEB members, Suelen Pini, and Jonatas F. Neves for help during field work; the Laboratório de Ictiologia da Universidade Federal do Rio Grande do Sul, especially to Luiz R. Malabarba for his helpful insights during the identification process of the specimens; the Conselho Nacional de Desenvolvimento Científico e Tecnológico for grantings and PhD scholarship (CNPq Proc. 152847/2016-2, to MPN); and the Programa de Pós-Graduação em Biologia Animal/UFRGS.

Authors' Contributions

MPN and RLD collected the specimens; MPN, PCS, and ALN-F identified the specimens, MPN conducted morphological, molecular procedures, and statistical analysis; MPN, PCS, and ALN-F interpreted the data; MPN and ALN-F wrote the text; and PSC, RLD and CBF improved and reviewed the manuscript.

References

Affonso IP, Azevedo RF, Santos NLC, Dias RM, Agostinho AA, Gomes LC (2015) Pulling the plug: strategies to preclude expansion of dams in Brazilian rivers with high-priority for conservation. Natureza & Conservação 13 (2): 199–203. https://doi.org/10.1016/j.ncon.2015.11.008

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16 (1): 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Baumgartner G, Pavanelli CS, Baumgartner D, Bifi AG, Debona T, Frana VA (2012) Peixes do baixo rio Iguaçu. Editora da Universidade Estadual de Maringá, Maringá, 203 pp.

Cavalli D, Frota A, Lira AD, Gubiani ÉA, Margarido VP, Graça WJD (2018) Update on the ichthyofauna of the Piquiri river basin, Paraná, Brazil: a conservation priority area. Biota Neotropica 18 (2): e20170350. https://doi.org/10.1590/1676-0611-BN-2017-0350

Claro-García A, Assega FM, Shibatta OA (2018) Diversity and distribution of ichthyofauna in streams of the middle and lower Tibagi river basin, Paraná, Brazil. Check List 14: 43–53. https://doi.org/10.15560/114.1.43

Dei Tos C, Gomes LC, Rodrigues MA (2014). Variation of the ichthyofauna along the Goioerê River: an important tributary of the Piquiri-Paraná basin. Iheringia, Série Zoologia 104 (1): 104–112. https://doi.org/10.1590/1678-476620141041104112

Delariva RL, Hahn NS, Kashiwaqui EAL (2013) Diet and trophic structure of the fish fauna in a subtropical ecosystem: impoundment effects. Neotropical Ichthyology 11 (4): 891–904.

Delariva RL, Neves MP, Larentis C, Kliemann BCK, Baldasso MC, Wolff LL (2018) Fish fauna in forested and rural streams from an ecoregion of high endemism, lower Iguaçu river basin, Brazil. Biota Neotropica 18 (3): e20170459. https://doi.org/10.1590/ 1676-0611-bn-2017-0459

Delariva RL, Silva JC (2013). Fish fauna of headwater streams of Perobas Biological Reserve, a conservation unit in the Atlantic Forest of the northwestern Paraná state, Brazil. Check List 9 (3): 549–554. https://doi.org/10.15560/9.3.549

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Fink WL, Weitzman SH (1974) The so-called *Cheirodontin* fishes of Central America with descriptions of two new species (Pisces: Characidae). Smithsonian Contributions to Zoology 172: 1–56.

Fricke R, Eschmeyer WN, van der Laan R (2019) Eschmeyer's catalog of fishes: genera, species references. https://www.calacademy.org/scientists/projects/eschmeyers-catalog-of-fishes. Accessed on: 2019-12-29.

Garavello JC, Sampaio FAA (2010) Five new species of genus *Astyanax* Baird & Girard, 1854 from Rio Iguaçu, Paraná, Brazil (Ostariophysi, Characiformes, Characidae). Brazilian Journal of Biology 70 (3): 847–865.

Gubiani ÉA, Gomes LC, Agostinho AA, Baumgartner G (2010). Variations in fish assemblages in a tributary of the upper Paraná River, Brazil: a comparison between pre and post-closure phases of dams. River Research and Applications 26 (7): 848–865. https://doi.org/10.1002/rra.1298

Ivanova NV, Zemlak TS, Hanner RH, Hebert PDN (2007) Universal primer cocktails for fish DNA barcoding. Molecular Ecology Resources 7 (4): 544–48.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16 (2): 111–120. https://doi.org/10.1007/BF01731581

Kwong S, Srivathsan A, Meier R (2012) An update on DNA barcoding: low species coverage and numerous unidentified sequences. Cladistics 28 (6): 639–644. https://doi.org/10.1111/j.1096-0031.2012.00408.x

Larentis C, Delariva RL, Gomes LC, Baumgartner D, Ramos IP, Sereia DADO (2016) Ichthyofauna of streams from the lower Iguaçu river basin, Paraná, Brazil. Biota Neotropica 16 (3): e20150117. https://doi.org/10.1590/1676-0611-bn-2015-0117

Leigh JW, Bryant D (2015) PopART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution 6 (9): 1110–1116. https://doi.org/10.1111/2041-210x.12410

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25 (11): 1451–52. https://doi.org/10.1093/bioinformatics/btp187

Morais-Silva JP, Oliveira AVD, Fabrin TM, Diamante NA, Prioli SM, Frota A, da Graça WJ, Prioli AJ (2018) Geomorphology influencing the diversification of fish in small-order rivers of neighboring basins. Zebrafish 15 (4): 389–397. https://doi.org/10.1089/zeb.2017.155

Neves MP, Delariva RL, Wolff LL (2015) Diet and ecomorphological relationships of an endemic, species-poor fish assemblage in a stream in the Iguaçu National Park. Neotropical Ichthyology 13(1): 245–254. https://doi.org/10.1590/1982-0224-20140124

Nimet J, Neves MP, Viana NP, Amorim, JPA, Delariva RL (2020). Histopathological alterations in gills of a fish (*Astyanax bifasciatus*) in Neotropical streams: negative effects of riparian forest reduction and presence of pesticides. Environmental Monitoring and Assessment 192 (1): 1–13. https://doi.org/10.1007/s10661-019-8030-y

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2019) Vegan: Community Ecology Package. R package version 2.5-3. https://CRAN.R-project.org/package=vegan. Accessed on: 2018-11-25.

Parolin M, Volkmer-Ribeiro C, Leandrini JA (2010) Abordagem interdisciplinar em bacias hidrográficas no Estado do Paraná. Editora Fecilcam, Campo Mourão, 158 pp.

Pavanelli CS, Oliveira CAM (2009) A redescription of *Astyanax gymnodontus* (Eigenmann, 1911), new combination, a polymorphic characid fish from the rio Iguaçu basin, Brazil. Neotropical Ichthyology 7 (4): 569–578.

Pini SF, Abelha MC, Kashiwaqui EA, Delariva RL, Makrakis S, Makrakis MC (2019) Food resource partitioning among species of *Astyanax* (Characiformes: Characidae) in the Lower Iguaçu River and tributaries, Brazil. Neotropical Ichthyology 17 (4): e190028. https://doi.org/10.1590/1982-0224-20190028

R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed on: 2018-11-25.

Rossini BC, Oliveira CAM, Melo FAG, Bertaco VA, Astarloa JMD, Rosso JJ, Foresti F, Oliveira C (2016) Highlighting *Astyanax* species diversity through DNA barcoding. PLoS ONE 11 (12): e0167203. https://doi.org/10.1371/journal.pone.0167203

Severi W, Cordeiro AAM (1994) Catálogo de peixes da bacia do rio Iguaçu. IAP/GTZ, Curitiba.

Silva PC (2017) Sistemática integrativa: diversidade e relações de *Deuterodon* Eigenmann 1907 (Teleostei: Characidae) e gêneros afins. PhD thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, 188 pp. https://www.lume.ufrgs.br/handle/10183/2/browse?value=Silva%2C+Priscilla+Caroline&type=author. Accessed on: 2018-11-27.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30 (12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Viana D, Zawadzki CH, Oliveira EF, Vogel HF, Graça WJ (2013) Estrutura da ictiofauna do rio Bonito, bacia hidrográfica do rio Ivaí, sistema alto rio Paraná, Brasil. Biota Neotropica 13 (2): 218–226.

Wickham H (2016) Ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York, 258 pp.

Appendix

Table A1. Voucher number and genetic sequences accession numbers (GenBank) of *Astyanax bifasciatus* specimens and other syntopic Characidae species from the basins of the lower Iguaçu River and Piquiri River (upper Paraná river basin) generated in this study.

Specimen voucher	Accession number	Species	Basin
UFRGS 26251(TEC 8953A)	MK557882	*A. bifasciatus*	Iguaçu
UFRGS 26251(TEC 8953B)	MK557883	*A. bifasciatus*	Iguaçu
UFRGS 26251 (TEC 8953C)	MK557884	*A. bifasciatus*	Iguaçu
UFRGS 26258 (TEC 8960)	MK557881	*A. bifasciatus*	Iguaçu
UFRGS 26253 (TEC 8955A)	MK557885	*A. bifasciatus*	Piquiri
UFRGS 26253 (TEC 8955B)	MK557886	*A. bifasciatus*	Piquiri
UFRGS 26249 (TEC 8951)	MK557880	*A. bifasciatus*	Piquiri
UFRGS 26256 (TEC 8958)	MN053307	*A.* aff. *fasciatus*	Piquiri
UFRGS 26252 (TEC 8954A)	MN053305	*A.* aff. *paranae*	Piquiri
UFRGS 26252 (TEC 8954B)	MN053306	*A.* aff. *paranae*	Piquiri
UFRGS 26255 (TEC 8957)	MN053308	*A. lacustris*	Piquiri
UFRGS 26250 (TEC 8952A)	MN053303	*B. ikaa* (I)	Iguaçu
UFRGS 26250 (TEC 8952B)	MN053304	*B. ikaa* (I)	Iguaçu

Table A2. Pairwise distance between of the *Astyanax bifasciatus* specimens and other syntopic Characidae species from the basins of the lower Iguaçu River and Piquiri River (upper Paraná river basin) generated in this study. I = Iguaçu river basin, P = Piquiri river basin.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1. TEC 8960	*A. bifasciatus* (I)	—												
2. TEC 8953A	*A. bifasciatus* (I)	0.006	—											
3. TEC 8953B	*A. bifasciatus* (I)	0.005	0.008	—										
4. TEC 8953C	*A. bifasciatus* (I)	0.003	0.006	0.005	—									
5. TEC 8955A	*A. bifasciatus* (P)	0.002	0.005	0.003	0.002	—								
6. TEC 8955B	*A. bifasciatus* (P)	0.002	0.005	0.003	0.002	0.000	—							
7. TEC 8951	*A. bifasciatus* (P)	0.003	0.006	0.005	0.003	0.002	0.002	—						
8. TEC 8954A	*A.* aff. *paranae* (P)	0.034	0.034	0.032	0.034	0.032	0.032	0.034	—					
9. TEC 8954B	*A.* aff. *paranae* (P)	0.034	0.034	0.032	0.034	0.032	0.032	0.034	0.000	—				
10. TEC 8958	*A.* aff. *fasciatus* (P)	0.036	0.039	0.034	0.036	0.034	0.034	0.036	0.022	0.022	—			
11. TEC 8957	*A. lacustris* (P)	0.153	0.157	0.155	0.155	0.155	0.155	0.153	0.156	0.156	0.162	—		
12. TEC 8952A	*B. ikaa* (I)	0.227	0.223	0.229	0.229	0.229	0.229	0.227	0.227	0.227	0.233	0.212	—	
13. TEC 8952B	*B. ikaa* (I)	0.227	0.223	0.229	0.229	0.229	0.229	0.227	0.227	0.227	0.233	0.212	0.000	—