

## Rapid Communication

## Occurrence of the matrinxã *Brycon amazonicus* (Agassiz, 1829) in the rivers that form the Paraguay River basin, in Brazil, based on morphological and genetic evidence

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### Abstract

The transfer of species between river basins has been recognized as one of the principal drivers of the loss of biodiversity and changes in aquatic ecosystems. The impacts caused by invasive species include predation, habitat degradation, competition, hybridization, and the transmission of diseases. *Brycon amazonicus*, known in Brazil as the matrinxã or jatuarana, is a fish endemic to the Amazon basin. It is cultivated in fish farms throughout Brazil and is an important fishery resource. Due to its popularity for fish farming, there have been numerous reports of individuals escaping into the natural environment. Specimens of *Brycon amazonicus* were captured during surveys of the Santana River, upstream from a reservoir in the Brazilian state of Mato Grosso, central Brazil. The Santana originates in the Chapada do Parecis and flows into the Paraguay River basin. Morphological analyses and cytochrome *c* oxidase subunit I (COI) gene sequences confirmed the taxonomic identification of the specimens. The present study provides a new record of the species, expanding its known area of occurrence.

**Key words:** allochthonous species, Bryconidae, Characiformes, freshwater fishes, species delimitation

### Introduction

Ecosystem services derived from freshwater biodiversity are essential for human well-being, providing irreplaceable resources for environmental quality (Albert et al. 2020). However, despite their importance, several factors have contributed to the reduction of global biodiversity, with particular emphasis on changes in aquatic ecosystems. These changes have led to a rapid decline, or even the extinction, of freshwater species or populations (Reid et al. 2019; Albert et al. 2020), a phenomenon referred to as the “biodiversity crisis” (Albert et al. 2020; Tickner et al. 2020; Ottoni et al. 2023).

In recent years, the introduction of allochthonous or exotic species into freshwater ecosystems has been one of the principal drivers of the loss of biodiversity and other negative impacts on native species (Agostinho et al.

2005; Clavero and García-Berthou 2005; Casimiro et al. 2018). These introductions are a direct consequence of human activities, such as recreational fishing, the systematic stocking of bodies of water, and the cultivation of both freshwater and marine fish, which is often conducted without adequate planning for the mitigation of potential escapes (Agostinho et al. 2005). The proposed solutions to address the issues related to biodiversity loss include improving the quality of aquatic environments, as well as preventing and controlling the invasions of non-native species (Tickner et al. 2020).

The ecological impacts stemming from the introduction of exotic species may include increased predation pressure and competition for resources, habitat degradation, hybridization, and the transmission of diseases (Latini and Petrere Jr. 2004; Gozlan et al. 2010; Leal et al. 2021). The presence of invasive species may provoke profound shifts in the behavior patterns and trophic relations of native species, which can even lead to extirpation (Blackburn et al. 2011; Franco et al. 2022a, b).

In the specific case of fish farms, fish can escape during the draining of ponds, through the breaching of net tanks or the overflow of ponds during periods of flooding (Orsi and Agostinho 1999). It is nevertheless important to note that not all species may be able to establish a population in the new environment, given the need, not only to survive, but also to reproduce in this novel habitat (Agostinho et al. 2007). Many non-native species are nevertheless able to establish populations in new environments, and even overcome barriers to dispersal, to spread even further into non-native ecosystems (Blackburn et al. 2011).

A number of studies have shown that exotic species may survive at higher rates in bodies of water that have a high degree of endemism, that is, a large proportion of species found only in this environment, as well as in areas regulated by dams (Agostinho et al. 2007). The presence of endemic species may facilitate the adaptation and establishment of introduced species, while impoundment may create favorable conditions for invasive species, especially considering that the species selected for cultivation in fish farms tend to be well adapted to lentic environments.

Species introductions can also have genetic impacts, such as the hybridization of closely-related species or between wild populations and captive populations that have reduced low genetic variability. This may result in shifts in gene frequencies in the resident population, and a decrease in its genetic variability, which may even threaten its persistence, possibly leading to extirpation (Bradbury et al. 2020). Given the progressive advances in genetic methods, the use of molecular markers has become an essential component of research proposals that aim to provide insights for the development of conservation guidelines for native species and the monitoring of aquatic ecosystems.

A number of species of the genus *Brycon* Müller & Troschel, 1844 [(*Brycon amazonicus* (Agassiz, 1829) *Brycon gouldingi* Lima, 2004, *Brycon insignis* Steindachner, 1877, *Brycon orbignyanus* (Valenciennes, 1850), *Brycon opalinus* (Cuvier, 1819)] are widely cultivated in Brazilian fish

farms (Santos-Filho and Batista 2009; Moro et al. 2013). These species are a value subsistence resource, and are target by commercial and sport fisheries, given their abundance in well-preserved riverine ecosystems (Lima 2017). The matrinxã or jatuarana, *Brycon amazonicus* (Agassiz, 1829), is one of the *Brycon* species that is cultivated most widely on Brazilian fish farms, as an alternative to satisfy the commercial demand for this fish, which is one of the most important fishery resources in the Brazilian state of Amazonas (Santos-Filho and Batista 2009). *Brycon amazonicus* is distributed along the Amazon river and its tributaries, in addition to the Orinoco and Essequibo rivers in Venezuela (Lima 2003; Lima 2017). It is a migratory, omnivorous, and opportunistic species, which spawns *en masse* at the onset of the flood period and is found in lowland environments (Arias 2006; Lima 2017).

Given its widespread cultivation on Brazilian fish farms, there exist documented instances of *Brycon amazonicus*, escaping into the drainages of central and southeastern Brazil, as described by Lima (2017). Other *Brycon* species have also been found in hydrographic systems in which they were previously unknown, almost certainly as a consequence of human activities related to unregulated farming practises, and disregard for the legislation governing the introduction of exotic species in certain regions of Brazil.

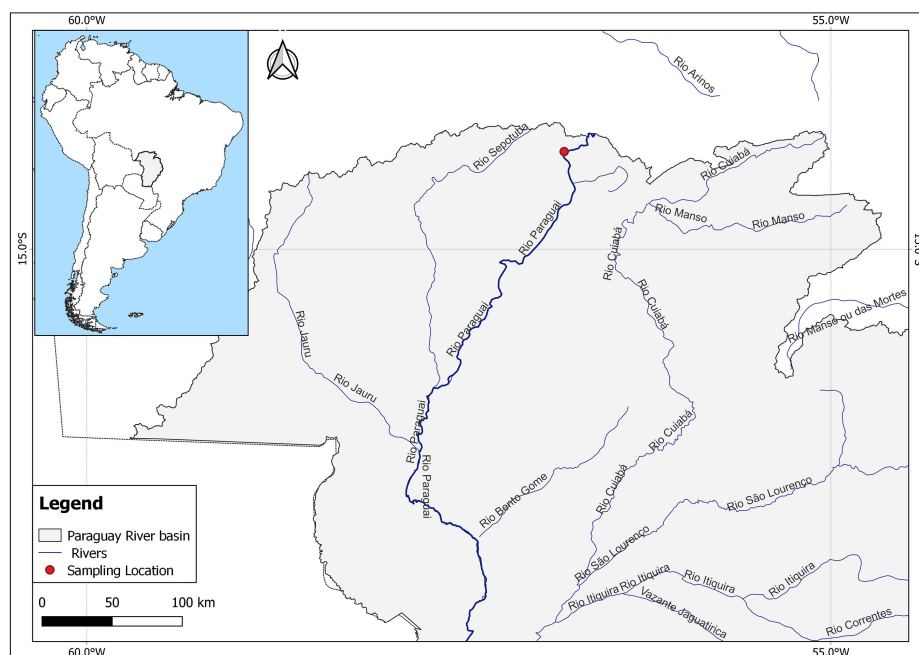
One example of this process is *Brycon opalinus*, which used to be distributed exclusively in the region of the upper Paraíba do Sul River basin and the upper Doce River basin. It is now also found in the Itapemirim River, a distinct coastal basin in the southeastern Brazilian state of Espírito Santo (Rodrigues et al. 2021). Daga et al. (2016) also recorded the introduction of *Brycon hilarii* (Valenciennes, 1850) into the ecoregion of the Iguaçú River, a tributary of the Paraná River. These records of the species occurring in areas outside its natural distribution area may related for a variety of reasons, including aquaculture, ornamental fish, sport fishing, biological control, and by accident (Casal 2006; Vitule et al. 2009). It is essential to monitor and assess the impact of these events, in order to avoid their potential impacts on local biodiversity and the aquatic ecosystems in the invaded regions.

Specimens of *Brycon amazonicus* were collected during a survey of the Santana River, in Mato Grosso state, central Brazil. The species had not been recorded in this region by any previous study. The present study reports the occurrence of the species in this area, updating its known distribution.

## Materials and methods

### *Study area*

The specimens were collected from a locality on the Santana River (latitude  $-14.343200$  and longitude  $-56.791930$ ), in the municipality of Nortelândia, state of Mato Grosso, central Brazil, upstream from a hydroelectric reservoir,



**Figure 1.** Location of the new record of *Brycon amazonicus* from the Santana River, in Paraguay River basin, Brazil.

in a free-flowing stretch of the river (Figure 1). The Santana River originates in the Chapada do Parecis and flows into Paraguay River basin. Samples were collected in July 2019 (collecting license number: 73042). Prior to fixation, small fragments of tissue were extracted from the specimens and preserved in absolute alcohol for molecular analysis. The specimens were subsequently fixed in 10% formalin and then preserved in 70% ethanol.

The specimens collected during the present study were deposited in the Fish Collection of the Federal University of Mato Grosso (CPUFMT7907) and identified using an identification key developed by Lima (2017) for the genus *Brycon*. The diagnosis of *Brycon amazonicus* is consistent with the description provided by Lima (2017). Five adult specimens were collected (LABGEN10687, LABGEN10688, LABGEN10689, LABGEN10690, and LABGEN11054), of which, four were vouchers and one (LABGEN10690) was used only for the collection of tissue samples. Specimen LABGEN11054 was used only for the collection of morphological data.

### *Morphometric and meristic data*

The lateral line scale counts and the morphometric measurements were taken following the methods outlined in Fink and Weitzman (1974), Menezes and Weitzman (1990), and Lima (2017). All the measurements were taken using a precision digital caliper with an accuracy of 0.01 mm and an ichthyometer. The lateral line scales counts they were obtained from all the specimens, were include all pored scales, including those posterior to the hypural joint. We compared our results with the data available in review of the genus (Lima 2017).

### *Molecular identification*

The mitochondrial DNA was sequenced to confirm the identification of the species. The genetic analyses were conducted on four specimens. The total genomic DNA was extracted using the saline extraction protocol of Aljanabi and Martinez (1997). The mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified using the Fish-F1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and Fish-R1 (5'-TAGACTTCTGGGTGGCCAAA GAATCA-3') primers, which were described by Ward et al. (2005).

The Polymerase Chain Reaction (PCR) was geared to a final volume of 25  $\mu$ L, containing 1.5  $\mu$ L of dNTP (1.25 mM), 2.5  $\mu$ L of 10X Buffer, 0.5  $\mu$ L of COI Fish-F1 primer (10 mM), 0.5  $\mu$ L of COI Fish-R1 primer (10 mM), 1.0  $\mu$ L of MgCl<sub>2</sub> (50 mM), 0.2  $\mu$ L of Taq DNA Polymerase (Ludwig, 5U/ $\mu$ L), 1  $\mu$ L of the DNA, and ultrapure H<sub>2</sub>O to complete the final volume. The reactions were run in a thermocycler (Applied Biosystems, Veriti) with initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 55 °C for 60 seconds, extension at 72 °C for 60 seconds, and final extension at 72 °C for 5 minutes. The samples were sequenced by BPI Biotecnologia EPP ([www.bpibiotecnologia.com.br](http://www.bpibiotecnologia.com.br)) in Botucatu, São Paulo (Brazil).

### *Data analysis*

The data matrix was constructed together with sequences from other *Brycon* species (*Brycon amazonicus*, *Brycon orthotaenia*, *Brycon orbignyanus*, *Brycon hilarii*, and *Brycon melanopterus*) extracted from Arruda et al. (2019). *Salminus hilarii*, *Gasteropelecus sternicla*, *Carnegiella striga*, and *Triportheus nematurus* (Supplementary material Table S1) were included as the outgroup, due to their relatively close phylogenetic relationship with the genus *Brycon* (Oliveira et al. 2011).

The editing, alignment, and assembly of the consensus sequences were carried out using the Geneious R9 software (Kearse et al. 2012) with the MUSCLE algorithm (Edgar 2004). These sequences were also subjected to a saturation test implemented in the DAMBE5 program (Xia et al. 2003). We employed the Kimura-2-parameter nucleotide substitution model (K2P) to determine the intra- and interspecific genetic distances in MEGA v7.0 (Tamura et al. 2013). The divergence patterns suggested by the genetic distances were represented graphically in a dendrogram generated by the Neighbor-Joining algorithm (NJ) with 1000 bootstrap replicates (Saitou and Nei 1987). The best-fitting models of nucleotide substitutions were inferred with ModelFinder (Kalyaanamoorthy et al. 2017) using Bayesian information criterion (BIC) as implemented in IQTree (Nguyen et al. 2015) with the data partitioned into three sets by codon position. Models for each partition were determined to be F81+F+I for codon positions 1, TN +F+G4 for codon position 2, and TNe + I for codon position 3. This

algorithm tests best fit models for each partition and the best partitioning scheme. The posterior probability values greater than 0.95 were considered significant (Alfaro and Holder 2006).

The Maximum Likelihood (ML) tree was generated in IQtree (Figure S1) with the node support being estimated using by 1000 bootstrap replicates. We used MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) in the CIPRES portal (Miller et al. 2010) for the Bayesian Inference (BI) analysis, which was based on four chains of 20 million generations, with one tree being sampled every 100 generations, and a burn-in of 25%. The bootstrap values are considered as significant at values greater than 70% (Hillis and Bull 1993).

## Results

### *Morphological identification*

The specimens analyzed in this study were unambiguously identified as *Brycon amazonicus* based in the Lima (2017). The diagnosis conducted by Lima (2017) highlights the differences between *B. amazonicus* and other congeneric species. Some of these differences include the species being distinguished by having a fifth infraorbital bone that is taller than it is wide (versus a fifth infraorbital bone that is nearly as tall as it is wide or wider than it is tall in the other species), a characteristic not analyzed in this study. The species also presents narrow longitudinal stripes along the dorsolateral surface of the body, darkened pelvic and pectoral fins, and the presence of dark pigment on the caudal peduncle and a diffuse caudal fin; some of these characteristics were verified during the identification process using the identification key.

The are presented in Table 1 with the largest examined specimen having a standard lengths (SL) 387.0 mm. All specimens have lateral line complete, from supracleithrum to caudal-fin base, between 68 and 78 scales in lateral line series (LABGEN10688 = 68, LABGEN10689 = 72, LABGEN11054 = 73 and LABGEN10687 = 78). Based on their external morphology, all the specimens were identified as *B. amazonicus* (Figure 2), this result is consistent with the data described by Lima (2017). Morphological identification was agreeing with molecular results using of the COI sequences.

### *Molecular identification*

The alignment of the COI sequences resulted in a final data matrix with a total of 581 base pairs. No evidence of saturation was found in the nucleotide substitutions either by transition ( $R^2 = 0.9642$ ) or transversion ( $R^2 = 0.9188$ ).

The genetic distance analysis using the Neighbor-Joining (NJ) method resulted in two distinct groups of *Brycon amazonicus*. One group comprises specimens from the Amazon River basin and those collected here in the Paraguay River basin, while the other group consists of specimens from the

**Table 1.** Morphometric data on the *Brycon amazonicus* specimens collected during the present study (n = 4) on the Santana River in Mato Grosso, Brazil.

Parameter	Length (mm) in specimen:			
	10687	10688	10689	11054
Standard length (SL)	387	324	378	345
Percentages of standard length				
Snout to dorsal-fin origin	48.08	49.38	49.55	48.57
Dorsal-fin base length	12.41	11.33	12.72	11.73
Posterior terminus of dorsal fin to adipose fin	26.45	23.29	24.07	23.74
Snout to pelvic-fin insertion	45.74	47.60	44.93	47.52
Snout to anal-fin origin	69.51	70.37	69.31	68.12
Anal-fin base length	20.90	18.39	21.30	21.04
Caudal peduncle length	12.47	14.01	14.51	14.40
Pectoral-fin length	18.50	23.15	20.48	19.76
Pelvic-fin length	15.82	17.06	16.38	16.50
Caudal peduncle depth	9.73	9.93	9.84	10.09
Percentages of head length Head length				
Head height	71.86	64.10	70.63	70.83
Snout length	28.97	27.93	29.55	26.75
Upper jaw length	47.01	48.44	49.68	46.20
Horizontal eye diameter	26.26	27.27	27.47	26.80
Post-orbital length	61.09	53.24	62.76	54.58
Least interorbital width	64.90	63.65	65.07	59.79



**Figure 2.** Specimen of *Brycon amazonicus* collected from the Santana River in the state of Mato Grosso, central Brazil. Photo by Hugmar Pains da Silva.

Orinoco River. The genetic distances, calculated using the K2P model, between the different species are shown in Table 2. Overall, the intraspecific genetic divergence values for specimens *Brycon* are low, ranging from 0 to 0.04%. The interspecific distance between *B. amazonicus* lineages 1 (Amazon and Paraguay) and 2 (Orinoco) was 2 percent. In the phylogenetic analysis (Figure 3), the Bayesian Inference and Maximum Likelihood approaches recovered clades with the *B. amazonicus* lineages from the Orinoco and the Amazon/Paraguay river basins with strong branch support.

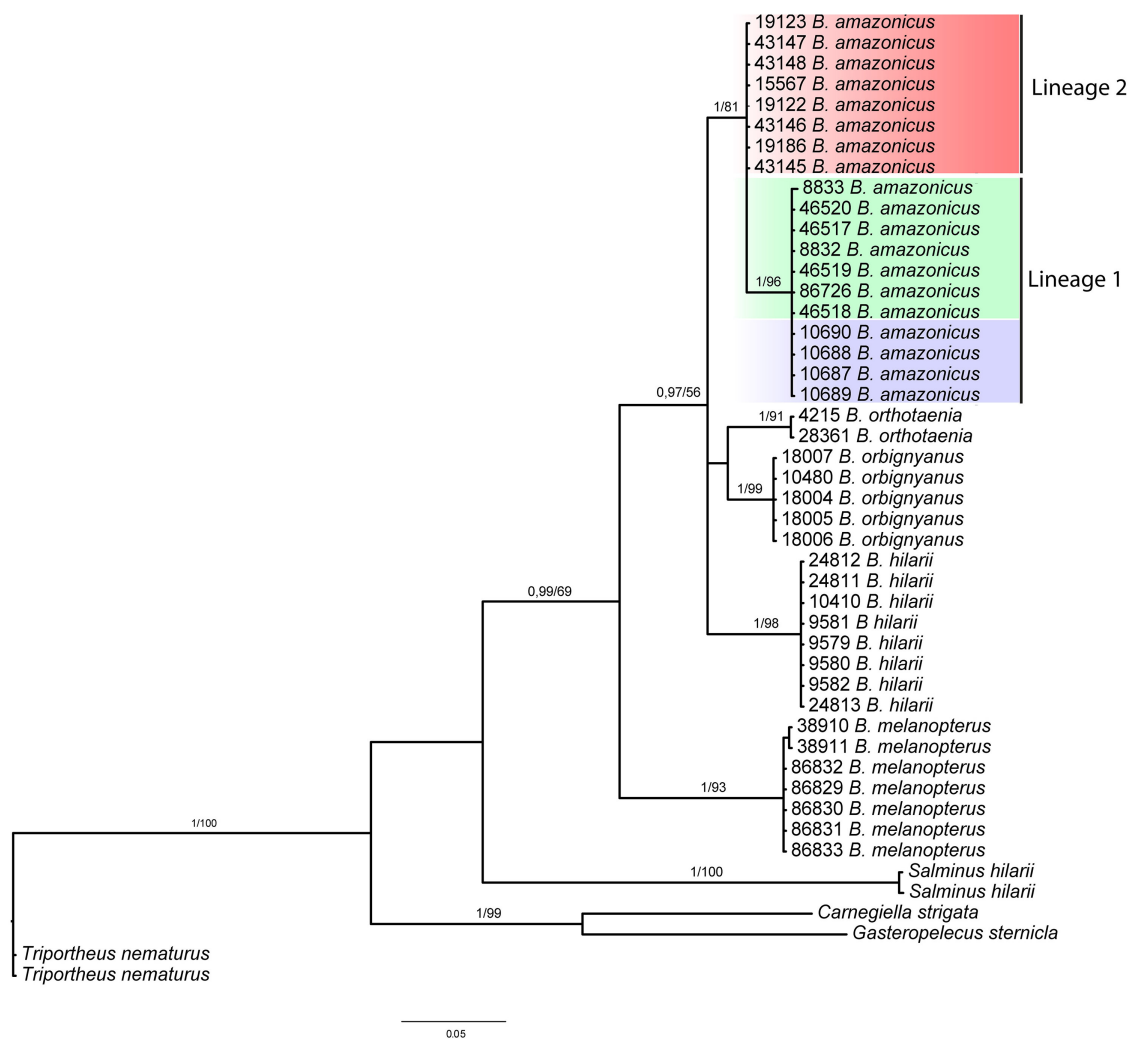
## Discussion

The morphometric data and lateral line scale counts, together with the DNA barcode analysis, confirm the taxonomic identification of the specimens collected in the present study as *Brycon amazonicus*. This confirms a new occurrence of the species in a tributary of the Paraguay River in the Brazilian

**Table 2.** Pairwise genetic distance matrix (calculated using the K2P model) between the two lineages *Brycon amazonicus* and other fish species included in the analyses. The intraspecific distance values are shown in bold script the diagonal. Percentage. n/c not calculated.

Species	1	2	3	4	5	6	7	8	9	10
1. <i>Brycon amazonicus</i> (Lineage 1)	<b>0.04</b>									
2. <i>Brycon amazonicus</i> (Lineage 2)	2.04*	<b>0</b>								
3. <i>Brycon orthotaenia</i>	6.73	5.61	<b>0</b>							
4. <i>Brycon orbignyanus</i>	6.96	5.39	5.17	<b>0</b>						
5. <i>Brycon hilarii</i>	8.58	6.29	7.42	7.16	<b>0</b>					
6. <i>Brycon melanopterus</i>	13.00	12.41	13.02	12.72	12.88	<b>0.09</b>				
7. <i>Salminus hilarii</i>	23.58	24.86	23.51	25.17	25.31	23.64	<b>0</b>			
8. <i>Carnegiella strigata</i>	22.63	21.16	22.29	22.24	22.50	21.29	28.48	<b>n/c</b>		
9. <i>Gasteropelecus sternicla</i>	22.17	21.06	22.22	21.89	21.63	22.53	25.69	17.84	<b>n/c</b>	
10. <i>Triporthesus nematurus</i>	19.65	18.83	21.83	20.98	20.17	21.40	28.25	22.74	23.20	<b>0.19</b>

\* Distance between the two lineages *Brycon amazonicus*.



**Figure 3.** Bayesian Inference topology based on the analysis of the COI gene sequences compiled in the present study. The values near the nodes represent the posterior probability values (left) of the BI and the bootstrap values (right) of the ML analysis (BI/ML). The four specimens collected from the Santana River, in the Paraguay basin, are highlighted in blue.

state of Mato Grosso. In addition to the five specimens examined, other individuals were encountered during fish surveys on the Santana River in subsequent years, although it has not been possible to obtain additional samples for analysis.



The combination of morphological and genetic data was crucial for the accurate identification of the specimens, especially considering that many *Brycon* species are extremely similar to one another, in morphological terms, which hampers their reliable diagnosis. In particular, the coloration pattern of *Brycon amazonicus* is easily confounded with that of both *Brycon gouldingi* and *B. hilarii* from the Amazon basin in Peru (Lima 2017).

The genetic analyses played a pivotal role in the diagnosis of the specimens, especially those of *Brycon amazonicus*. In their species delimitation analyses, Arruda et al. (2019) confirmed the existence of two distinct lineages of *B. amazonicus*, one from the Amazon basin and the other from the Orinoco River. These lineages were differentiated by a mean genetic distance of 2%. In the data matrix, the specimens from the Santana River (Paraguay basin) clustered with those from the Amazonian rivers, which would be expected, given that the aquaculture stocks in Brazil are typically derived from rivers in the Amazon, rather than the Orinoco basin, which is located in Venezuela.

The introduction of *Brycon amazonicus* into non-native environments has been attributed to fish escaping from local fish farming ponds (de Oliveira et al. 2018). In the present study, fish of this species were found in the Santana River, upstream from a reservoir in Mato Grosso, this river is in the headwater region of the Tapajós River. *Brycon amazonicus* is a commercially-important fish in the central Amazon region, and it is farmed extensively, in many regions of Brazil. Its popularity for fish farming derives from its capacity to adapt to intensive cultivation systems, its substantial growth potential, and its popularity as a food (Santos-Filho and Batista 2009).

Fish farming has an enormous potential as an alternative economic activity in the basin of the Paraguay River, although it also entails a high risk of introducing exotic or allochthonous species into the local environment (Rotta 2003). The farming of any non-native species almost always involves some risk of its accidental introduction into the local environment. These invasive species may either compete for resources with the native fauna or act as a predator, as well as modifying habitats and the functioning of the ecosystem, by introducing pathogens and parasites, and even altering gene frequencies (Agostinho et al. 2007).

In Brazil, many fish species have been transferred between river basins. The most notable examples are those of the introductions of the peacock bass (*Cichla kelberi*) and the tambaqui, *Colossoma macropomum* (Cuvier, 1816), which are both endemic to the Amazon region, into the Brazilian Pantanal wetlands (Alho and Reis 2017; Ortega et al. 2015). In other cases, species from the Pantanal have been introduced into other Brazilian drainage systems, as in the case of piavuçu, *Megaleporinus microcephalus* (Garavello and Britski 1988), which is native to the part of the Paraguay basin that lies within this wetland biome (Almeida et al. 2022).

These inter-basin transpositions are relatively common in the Brazilian state of Mato Grosso, which prompted the state environmental agency to publish state legislation 8464 on April 4th 2006 in response. Article 17 of this legislation advocates that the introduction of allochthonous species that affect the survival of native species, or transmit diseases or parasites from other watersheds, constitutes an environmental infraction). This ordinance was subsequently modified through the publication of state law 10669 on January 16<sup>th</sup> 2018 which, in its article 8, states that “fish farming projects aimed at the production of fingerlings and hybrid fish of exotic, native or allochthonous species, in cultivation systems based on excavated ponds, reservoirs, cage tanks, and closed systems, must comply with six criteria. Item VI proposes that “when used, cage tanks must be built with materials resistant to corrosion, traction, and the mechanical action of predators, in order to avoid their rupture, and special care must be taken during their transportation, repair, handling, and harvesting”. For the Paraguay basin, according to State Decree No. 337, published on December 23, 2019, the cultivation of non-native species belonging to the genus *Brycon* is permitted, as well as the species *Arapaima gigas* (pirarucu), *Colossoma macropomum* (tambaqui), and *Leiarius marmoratus* (jundiá).

Despite this legislation, escapes appear to be recurring continuously. During the present study, local residents reported that there had been an escape from a fish farm located upstream from the reservoir on the Santana River. This incident may account for the presence of invasive species in this river system as shown in the present study (Hugmar P. Silva *personal communication*). This reflects the relatively ineffective application and enforcement of the legislation.

Ordinance 8464 also establishes that “the entrepreneur, whether an individual or legal entity, who by action or omission, degrades the environment shall be held responsible administratively, with the obligation of repairing the damage caused”. This passage was included in the legislation because introduced species are known to have negative impacts, in general, on native species, given their potential to provoke significant changes in local ecological communities. These invasive species are often voracious predators, and may be able to colonize environments with favorable conditions extremely rapidly (Ortega et al. 2015; Alho and Reis 2017; Almeida et al. 2022).

Lima (2017) referred to the occurrence of *Brycon amazonicus*, based on the evidence of introduced or escaped specimens, in other regions of Brazil, including the Corumbá River, a tributary of the Paranaíba River in the state of Goiás (Paraná basin); in Centro Nacional de Pesquisa e Conservação de Peixes Continentais – CEPTA (stocked specimens) in Pirassununga, São Paulo state; in artificial pond near Atibaia River in Campinas, São Paulo, and in the Paraíba do Sul River in Campos, Rio de Janeiro state (East basin). For the Upper Paraguay basin, this represents the first scientifically confirmed record of *Brycon amazonicus*. It is still unclear, however, whether

the *B. amazonicus* specimens collected in the present study represent an established population, although other non-native bryconid species (*B. hilarii* and *Salminus brasilienses*) have also been observed in the region.

The presence of *Brycon amazonicus* in the Santana River, Mato Grosso, is confirmed by solid scientific evidence, particularly molecular analyses. This invasive species is cause for concern due to its potential impacts on local aquatic ecosystems. It will be essential to monitor these introduced species to determine whether they have established resident populations. The impoundment of the river may facilitate colonization by these species, exacerbating their impacts on the local ecosystems, and affecting the conservation of both aquatic biodiversity and fishery resources (Agostinho et al. 2007).

The results of the present study confirmed the occurrence of *Brycon amazonicus* in central-southern Mato Grosso, and highlight the need to establish monitoring strategies to determine the extent of its dispersal and the potential establishment of resident populations in the rivers that form the Paraguay basin. These data will be crucial to the development of effective public policies for the control of invasive fish species in the region, and the mitigation of their impacts on the local aquatic ecosystems.

### Author contributions

PSSA: Laboratory analyses, data analysis, and manuscript preparation. DCF: Supervision and support of the molecular analyses, collaboration in the data analysis, and assistance in the preparation of the manuscript, and final revision. PCV: Support for the laboratory analyses of the morphological data, assistance in the preparation of the manuscript, and final revision. HPS: Collection of specimens, support for the laboratory analyses of the morphological data, and assistance with the final revision of the manuscript.

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### Supplementary material

The following supplementary material is available for this article:

**Figure S1.** Maximum Likelihood topology based on the analysis of the COI gene sequences.

**Table S1.** Details of the specimens analyzed in the present study, including their species, catalog numbers, locality, coordinates and BOLD and GenBank accession numbers.

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