Original article

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Spintherobolus papilliferus in the rio Ribeira de Iguape basin: implications for the biogeography and conservation of an endangered species (Ostariophysi: Characiformes)

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Spintherobolus papilliferus is an endangered characid endemic of the Atlantic Rainforest, known from sparse locations in the upper rio Tietê basin around the metropolitan area of São Paulo city, and from an affluent of rio Itapanhaú, a coastal stream in Bertioga, São Paulo State. In 2020, *S. papilliferus* was sampled from the rio Ribeira de Iguape basin in Juquitiba, São Paulo State, representing a new distributional record. We compared 17 morphometric and six meristic characters from all specimens with data from the rios Tietê and Itapanhaú. An overlap in the morphological data from the three populations was detected, except for five measure whose values are lower in rio Ribeira de Iguape. MANOVA and LDA revealed that this population differs significantly from the other two, showing shallower body and caudal peduncle, among other features. These morphological differences may be due to environmental selective pressures since rio Ribeira de Iguape drainage is marked by fast waters which can influence the shape of fish bodies over time. DNA-barcoding of all *Spintherobolus* species corroborate that the rio Ribeira de Iguape population belongs to *S. papilliferus*. We also present a hypothesis for the disjunct distribution of *S. papilliferus* involving headwater capture and discuss the implications for the conservation of this endangered species.

Keywords: Atlantic rainforest, Distributional pattern, DNA barcode, Morphometry.

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Spintherobolus papilliferus é um caracídeo endêmico da Mata Atlântica e ameaçado de extinção, conhecido de localidades esparsas na bacia do alto rio Tietê, região metropolitana da cidade de São Paulo, e de um afluente do rio Itapanhaú, um riacho costeiro em Bertioga, Estado de São Paulo. Em 2020, *S. papilliferus* foi amostrada na bacia do rio Ribeira de Iguape em Juquitiba, São Paulo, representando um novo registro de distribuição. Comparamos 17 caracteres morfométricos e seis merísticos dos exemplares com dados dos rios Tietê e Itapanhaú. Houve sobreposição nos valores dos dados exceto em cinco medidas cujos valores são menores no rio Ribeira de Iguape. MANOVA e LDA revelaram que esta população difere significativamente das demais populações, sendo estes exemplares caracterizados pelas menores alturas do corpo e pedúnculo caudal, dentre outras características. Estas diferenças morfológicas possivelmente decorrem de pressões ambientais, pois a drenagem do rio Ribeira de Iguape possui ambientes de corredeiras velozes que podem influenciar no formato do corpo dos peixes. DNA-barcoding de todas as espécies de *Spintherobolus* corroborou que a população do rio Ribeira de Iguape pertence à *S. papilliferus*. Apresentamos também uma hipótese para a distribuição disjunta de *S. papilliferus* envolvendo captura de cabeceiras e discutimos implicações para a conservação dessa espécie ameaçada.

Palavras-chave: Código-de-barras DNA, Mata Atlântica, Morfometria, Padrão de distribuição.

INTRODUCTION

Spintherobolus Eigenmann, 1911 was proposed over a century ago to include *Spintherobolus papilliferus* Eigenmann, 1911 from headwaters of the rio Tietê in Paranapiacaba Municipality, São Paulo State, Brazil (Eigenmann, 1911). Fourteen years later, the miniature *Spintherobolus broccae* Myers, 1925 was described from coastal streams in Rio de Janeiro State and Santos Municipality, São Paulo State (Myers, 1925). Weitzman, Malabarba (1999) reviewed the genus and described two more small species: *Spintherobolus ankoseion* Weitzman & Malabarba, 1999 from coastal creeks in Santa Catarina State, and *Spintherobolus leptoura* Weitzman & Malabarba, 1999 from the lower rio Ribeira de Iguape, the largest coastal river basin in southern São Paulo State (Malabarba, 2003). Weitzman, Malabarba (1999) and Bührnheim *et al.* (2008) also proposed the putative basal position of the larger species, *S. papilliferus*, sister to a clade with the remaining three species. All four species are endemic species of the Brazilian Atlantic Rainforest (Malabarba, 2003), listed as endangered at some level (Brasil, 2022) according to the International Union for Conservation of Nature (IUCN) categories and criteria (IUCN Standards and Petitions Subcommittee, 2019).

Spintherobolus papilliferus (Fig. 1) is currently classified as an Endangered (EN) species (Brasil, 2022) due to the small area of occurrence (approximately 8 km^2), decrease in habitat quality related to expansion of the city and pollution, and low number of recorded (n = 4) localities (Castro *et al.*, 2008; Oyakawa *et al.*, 2009; Akama *et al.*, 2018). Throughout

FIGURE 1 | *Spintherobolus papilliferus* sampled in an affluent of rio Juquiá, rio Ribeira de Iguape drainage, municipality of Juquitiba, São Paulo State, Brazil. **A.** Live specimen right after capture. **B.** LISO 426, fixed specimen, 33.0 mm SL. Scale bar = 10 mm.

most of the 110 years since its description, *S. papilliferus* was registered occasionally in the southern headwater streams of upper rio Tietê in Serra de Paranapiacaba, its type locality, and riacho Ipiranga (Weitzman, Malabarba, 1999), the former a mountain chain dividing rio Tietê drainage running inland towards the rio Paraná basin and the series of relatively short coastal systems draining into the Atlantic Ocean (*e.g.*, Abell *et al*., 2008), and the latter almost entirely supressed near São Paulo city centre (*e.g.*, Mattox, 2008). The species has not been found in the type locality since the 1980s (Castro *et al*., 2008), but has been sampled once in Parque Estadual da Cantareira in the northern headwater streams of rio Tietê and once in Estação Biológica de Boracéia, municipality of Salesópolis near the rio Tietê spring (Castro *et al*., 2008; Marceniuk *et al*., 2011). In 2015, *S. papilliferus* was found for the first time in coastal drainages of São Paulo State, specifically in rio Sertãozinho of the rio Itapanhaú basin (MZUSP 119089). In 2019 it was discovered in rio Juquiá, in the headwaters of rio Ribeira de Iguape basin (Cetra *et al*., 2020), a record that is explored in more detail herein.

The known distribution of *Spintherobolus papilliferus* is within the Atlantic Rainforest biome, one of the 33 biodiversity hotspots in the world, thus having priority in conservation efforts (Myers *et al*., 2000), but the second most threatened forest on Earth (Campanili, Prochnow, 2006). It is estimated that approximately 15% of the original 1.300.000 km^2 forested area remains today, with over 80% of the fragments with less than 50 ha, only 2–10% of which under protection (Menezes *et al*., 2007; Ribeiro *et al*., 2009; Fundação SOS Mata Atlântica, INPE, 2017; Wagner *et al*., 2020) and a rapid increase in deforestation in recent years (Fundação SOS Mata Atlântica, INPE, 2022). In light of the ongoing rapid loss of the Atlantic Rainforest, especially in Southeastern Brazil, and the fact that most endangered freshwater fishes in South America are small species restricted little streams (Castro, Polaz, 2020), the aim of this study is to provide new information regarding the geographic distribution of *Spintherobolus papilliferus*, the environmental conditions in which it was found in rio Juquiá, and discuss the expansion in the known distribution of the species for its biogeography and conservation.

MATERIAL AND METHODS

Study area. The rio Ribeira de Iguape basin is located between coordinates 23°45'S 46°45'W and 25°30'S 50°10'W in the states of São Paulo and Paraná. Sampling of the new population of *Spintherobolus papilliferus* occurred in the municipality of Juquitiba, SP, in the coordinates 23°59'13.20"S 47°00'10.8"W, in a stream with no name draining into the headwaters of rio Juquiá, itself the largest left affluent of rio Ribeira de Iguape (Fig. 2). The stream belongs to a dense ombrophile Atlantic Rainforest with a native plant coverage relatively well preserved. According to the Köppen scale, its climate is classified as Cfb with subtropical humid summers and mild winters (CBH-RB, 2012). The average precipitation is 1400mm/y and reaches 2000 mm/y in the slopes of Serra do Mar (CBH-RB, 2012). The area lies over a pre-Cambrian crystalline bedrock that suffered influence of neotectonisms during the Mesozoic and Cenozoic. As a result, the upper reaches of the basin are characterized by steep terrains along the hillsides with fast flowing rivers that drain into the traphogenic basin of rio Ribeira de Iguape (Ross, 2002; Riccomini *et al*., 2004). The sampling site in rio Juquiá, municipality of Juquitiba, São Paulo State, does not belong to a protected area.

The characterization of abiotic parameters followed Cruz *et al*. (2013) and Teshima *et al.* (2015). The area where the specimens were sampled (Fig. 2) was shallow with large patches of leaflitter and transparent waters. The average depth was 26 ± 12 cm and the average width was 6.80 ± 2.25 m. The average water flow was 0.100 ± 0.036 m/s under the influence of a close waterfall upriver. The substrate was composed of leaflitter (21.53%), wood debris (18.46%), sand (20%), silt (2.3%), pebble (20.77%), cobble (0.8%), boulder (3.84%) and rock outcrops (12.3%). Water pH was 6.19, conductivity 0.037 mS/cm and temperature 17.66 °C.

Sampling. Fish was caught in two fieldtrips (September 12 and November 22, 2019) using handnets and an electrofishing gear (LR-24 Electrofisher, Smith Root). Immediately after capture, some specimens were placed in a photograph tank to capture their live coloration with a Nikon D3200 and Mikro lenses of 60 mm. Specimens were then euthanized with eugenol and preserved in 4% formalin (n = 6) or kept in 100% alcohol for DNA analyses (n = 2). Formalin specimens were transferred to 70% alcohol and deposited in the collection of Departamento de Ciências Biológicas, UNESP, câmpus São José do Rio Preto (DZSJRP 23020), Laboratório de Ictiologia de Sorocaba (LISO 426) and Museu de Zoologia da Universidade de São Paulo (MZUSP 127727). Specimens for molecular analyses were deposited in Laboratório de Biologia de Peixes (LBP 31056).

Morphological analyses. Specimens were identified according to Weitzman, Malabarba (1999). Seventeen measures and six meristic characters were collected from the specimens of rio Ribeira de Iguape following Fink, Weitzman (1974). Measurements were taken point-to-point with a digital calliper on the left side of the body whenever possible. Morphometric data is presented as percentages of standard length (SL), except for subunits of the head which are given as percentages of head length (HL). Simple

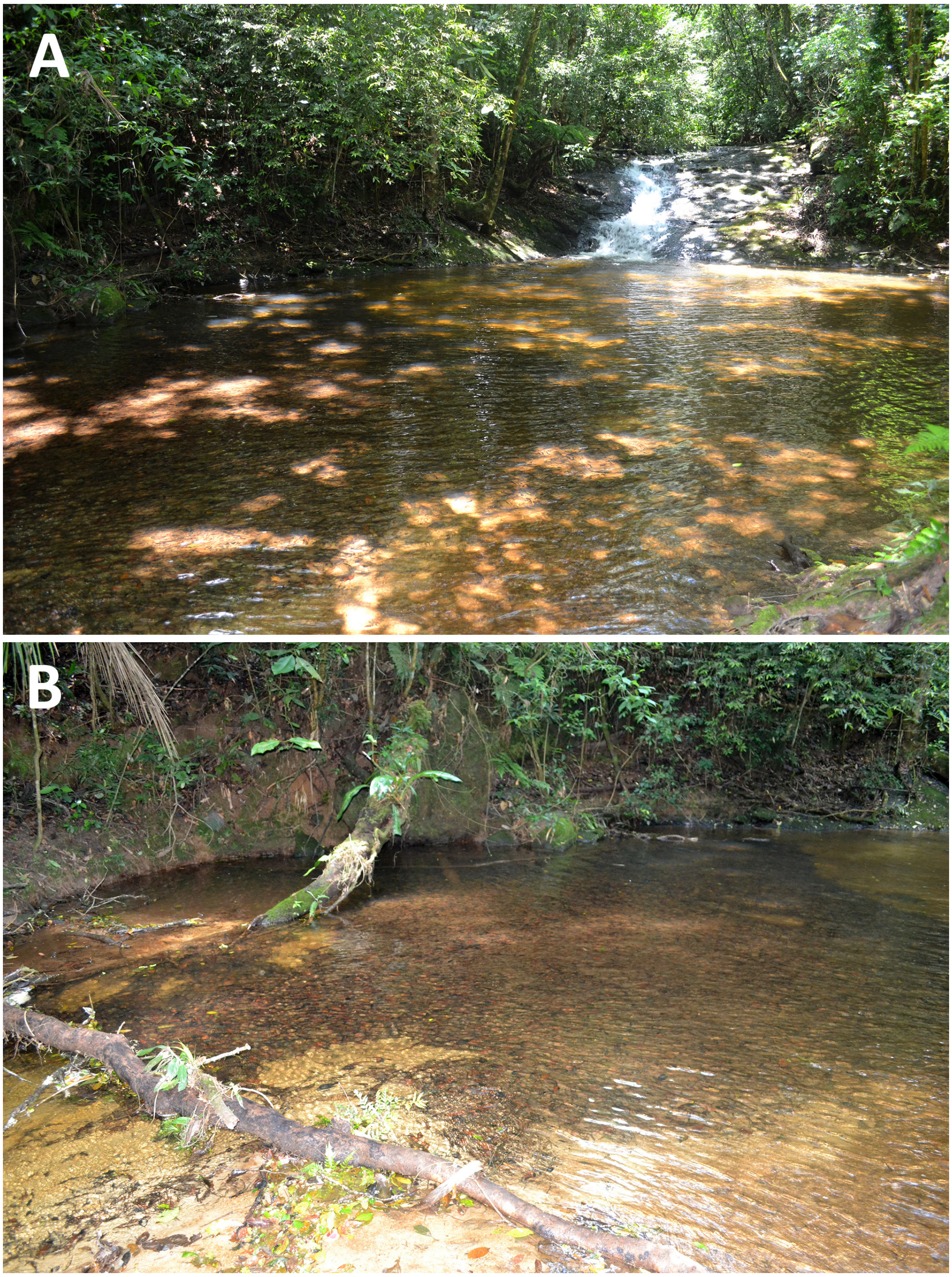

FIGURE 2 | Stream stretch affluent of rio Juquiá, rio Ribeira de Iguape, municipality of Juquitiba, São Paulo State, where specimens of *Spintherobolus papilliferus* were sampled for this study. **A.** General view showing rapids in the background. **B.** Rapids pool where specimens were caught.

statistical descriptors (*i.e*., sampling number, mean, standard deviation and range) were calculated for each of the measurements. Counts were made under a Zeiss Discovery V20 stereomicroscope and presented as their range associated with the absolute frequency of each count between parentheses. Photography of the preserved specimen was made with the above-mentioned stereomicroscope connected to a digital Zeiss AxioCam.

For comparative purposes, ten specimens from the upper rio Tietê and seven specimens from rio Itapanhaú were also examined, measured and had their meristics counted following the methods described above. Data are presented in a table with each column representing a different drainage (Itapanhaú – IT, Ribeira de Iguape – RI, upper Tietê – UT). We used multivariate analysis of variance (MANOVA) to detect patterns between multiple dependent variables [(body depth (BD), predorsal distance (PD), preanal distance (PA), caudal-peduncle depth (CPD), and upper jaw length (UJL)] and statistically test the differences between three drainage means (RI, IT, UT). The null hypothesis is that the mean drainage vectors are the same for all drainages or don't differ significantly. We selected the dependent variables that showed a statistically significant correlation with body depth (BD). When dependent variables are correlated, the MANOVA has greater statistical power and limits the joint error rate, and we can assess patterns between multiple dependent variables (Frost, 2019). We used Linear Discriminant Analysis (LDA) as a posthoc test. The LDA finds a linear combination of features that separates the drainages. We used a scatter plot with the two linear discriminants on the X and Y axes to match the independent variable, *i.e*., the drainage (Radečić, 2022). Percentage data received arcsine square root transformation. All the above analyses were carried in the R environment (R Development Core Team, 2020) and RStudio Team (2020). We used "manova" and "lda" functions.

The list of comparative material examined follows museum acronym, number of specimens, locality, date of collection, and collectors. The material is deposited in MZUSP, all from São Paulo State, Brazil: *Spintherobolus papilliferus*: MZUSP 39080, 6, 18.5–61.1 mm SL, ribeirão Curiango, affluent of rio Claro, Estação Biológica de Boracéia, Salesópolis, 11 Sep 1988, Otávio Froehlich; MZUSP 63135, 1, 21.4 mm SL, riacho da Cachoeira do Engordador, Parque Estadual da Cantareira - Núcleo do Engordador, São Paulo, 20 Jun 2020, MZUSP/Uniban Team; MZUSP 72837, 3, 48.7–57.4 mm SL, last stream crossing the road before city of Paranapiacaba, 20 Mar 1980, Ricardo M. C. Castro; MZUSP 119089, 7, 20.9–46.5 mm SL, affluent of rio Sertãozinho, rio Itapanhaú drainage, Bertioga, 29 Aug 2015, LAPEC Team.

Molecular analyses. Fifteen specimens were included in molecular analyses. Fourteen sequences of *Spintherobolus* were generated herein (Tab. 1) and a single sequence of *Triportheus guentheri* (Garman, 1890) was retrieved from GenBank (HM404957.1) to root the tree. DNA extraction followed Ivanova *et al.* (2006) and partial sequences of the *cytochrome c oxidase subunit I* (COI) gene were amplified by polymerase chain reaction (PCR) with primers FishF1/FishR1 and FishF2/FishR2 (Ward *et al*., 2005), L6252-Asn/H7271-COXI (Melo *et al*., 2011) or FishF6 and FishR7 (Jennings *et al*., 2019). PCR amplifications were performed in a total volume of 12.5 µl, with 1.25 µl of 10X buffer, 0.25 µl of MgCl 2 (50 mM), 0.2 µl dNTPs (2 mM), 0.5 µl of each primer (5 mM), 0.1 µl of PHT Taq DNA polymerase (*Phoneutria*), 1.0 µl of genomic DNA (200 ng) and 8.7 µl ddH 2 O. PCR conditions consisted of an initial denaturation (5 min at 94 °C), followed by 30 cycles of chain denaturation (50s at 94 °C), primer hybridization (45s at 50–54°C) and nucleotide extension (1 min at 68 °C), and a final extension (10 min at 68 °C). All PCR products were checked on 1% agarose gels and then purified with ExoSap-IT (USB Corporation) following the manufacturer's instructions. The purified PCR products were subjected to sequencing procedures with the BigDye Terminator v. 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and purified by ethanol precipitation. The PCR products were loaded onto an ABI 3130 DNA Analyzer automatic sequencer (Applied Biosystems).

Raw sequences were assembled to consensus using Geneious v. 7.1.9 (Kearse *et al.*, 2012) and posteriorly aligned with Muscle (Edgar, 2004) under default parameters. Substitution saturation was determined using Xia *et al.* (2003)'s approach in DAMBE v. 5.3.38 (Xia, 2013). Nucleotide variation, substitution patterns and best-fit model of nucleotide evolution were estimated in MEGA v. 10 (Kumar *et al.*, 2018). The overall mean distance, the intraspecific distances and interspecific distances were estimated using the Kimura-2-parameter model (K2P; Kimura, 1980) and maximum likelihood (ML) analysis was conducted with the Hasegawa-Kishino-Yano (HKY)+G model and 1,000 bootstrap replicates in MEGA v10.

TABLE 1 | Lots, vouchers, locality information, and GenBank accession numbers of the analyzed specimens of *Spintherobolus*.

Species	Lot	Voucher	Basin	Locality	Coordinates	GenBank n.
Spintherobolus papilliferus	LBP 31056	101792	Rio Ribeira de Iguape	Affluent of rio Juquiá, Juquitiba, São Paulo, Brazil	23º59'13.20"S 47º00'10.8"W	OQ657210
	LBP 31056	101793	Rio Ribeira de Iguape	Affluent of rio Juquiá, Juquitiba, São Paulo, Brazil	23º59'13.20"S 47º00'10.8"W	OQ657211
	LBP 20540	61600	Coastal drainage	Affluent of rio Sertãozinho, Bertioga, São Paulo, Brazil	23°41'44.3"S 46°03'10.7"W	OQ745939
Spintherobolus broccae	LBP 14312	53286	Coastal drainage	Affluent of rio Itaguaré, Bertioga, São Paulo, Brazil	23°45'51.8"S 45°57'13.6"W	OQ745942
	LBP 14350	54731	Coastal drainage	Caraguatatuba, São Paulo, Brazil	23°41'20.3"S 45°27'46.1"W	OQ745943
	LBP 14350	54732	Coastal drainage	Caraguatatuba, São Paulo, Brazil	23°41'20.3"S 45°27'46.1"W	OQ745941
	LBP 14350	54733	Coastal drainage	Caraguatatuba, São Paulo, Brazil	23°41'20.3"S 45°27'46.1"W	OQ745940
	LBP 14350	54734	Coastal drainage	Caraguatatuba, São Paulo, Brazil	23°41'20.3"S 45°27'46.1"W	OQ745944
Spintherobolus ankoseion	LBP 11448	52274	Coastal drainage	Itapoá, Santa Catarina, Brazil	26°02'23.8'S 48°38'09.4"W	OQ745945
	LBP 11448	52275	Coastal drainage	Itapoá, Santa Catarina, Brazil	26°02'23.8'S 8°38'09.4"W	OQ745946
Spintherobolus leptoura	LBP 7544	36094	Rio Ribeira de Iguape	Affluent of rio Momuna, Iguape, São Paulo, Brazil	24°42'57.8'S 47°41'28.3"W	OQ745950
	LBP 7544	36095	Rio Ribeira de Iguape	Affluent of rio Momuna, Iguape, São Paulo, Brazil	24°42'57.8"S 47°41'28.3"W	OQ745951
	LBP 7544	36096	Rio Ribeira de Iguape	Affluent of rio Momuna, Iguape, São Paulo, Brazil	24°42'57.8"S 47°41'28.3"W	OQ745949
	LBP 7544	36097	Rio Ribeira de Iguape	Affluent of rio Momuna, Iguape, São Paulo, Brazil	24°42'57.8"S 47°41'28.3"W	OQ745948

RESULTS

Identifying the new specimens from rio Ribeira de Iguape basin using the most comprehensive key (Weitzman, Malabarba, 1999) leads to *Spintherobolus papilliferus* (Fig. 1). Our samples have four of the five autapomorphies for this species: (i) number of branched anal-fin rays (9 or 10); (ii) eye-diameter smaller than in the remaining *Spintherobolus* species (23.2–32.7% of HL in our samples, 18.4–26.0% of HL in Weitzman, Malabarba, 1999); (iii) long caudal peduncle (26.1–30.5% of HL in our samples, 21.3–27.0% of SL in Weitzman, Malabarba, 1999); and (iv) adult body size larger than remaining *Spintherobolus* species (39.6 mm SL in our samples, up to 60.8 mm SL in Weitzman, Malabarba, 1999). The fifth autapomorphy, number of vertebrae, was not accessed in the present study.

Morphometric and meristic data are presented in Tabs. 2 and 3, respectively. While almost all data greatly overlap between drainages, five measures were different between *Spintherobolus papilliferus* from the rio Ribeira de Iguape and the other two populations. Values of ranges and means for these two characters are lower in the population from rio Ribeira de Iguape than those of the remaining drainages. The MANOVA detected a pattern between these five dependent variables (body depth, pre-dorsal distance, pre-anal distance, caudal-peduncle depth, and upper jaw length) which were significantly different between the three drainage means (rio Ribeira de Iguape, rio Itapanhaú, and upper rio Tietê basins) (Fig. 3A). As body depth increases, all other dependent variables tend to increase. Moreover, for any given body depth, specimens of *S. papilliferus* from the rio Ribeira de Iguape drainage tend to have lower values of these measures than specimens from Itapanhaú and upper Tietê basins, *i.e.*, specimens with equal body depth tend to have smaller measures in rio Ribeira de Iguape. We can conclude that there is an association between drainage and the relationship among the morphometric variables. The group mean vector of rio Ribeira de Iguape is significantly different from the other drainage means (Fig. 3B). We assumed it was a crucial factor for rejecting the null hypothesis.

The molecular dataset resulted in a matrix of 531 bp, with 146 variables sites. The nucleotide frequencies were 25.4% adenine, 15.5% guanine, 30.8% thymine, and 28.3% cytosine. DAMBE indicated no saturation for either transitions or transversions in both asymmetrical (Iss.cAsym) and symmetrical (Iss.cSym) topologies. The best nucleotide model selected for the matrix was HKY+G. The maximum likelihood (ML) tree showed high bootstrap values supporting each of the analyzed species (Fig. 4). The overall mean of genetic distances among *Spintherobolus* species was 6.9%. The values of interspecific distances ranged from 1.5% between *S. leptoura* and *S. ankoseion* to 16.2% between *S. papilliferus* and *S. ankoseion* (Tab. 4). Interestingly, the genetic distances between the three smaller species (*S. ankoseion*, *S. broccae*, and *S. leptura*) range from 1.5–3.5%, while these distances are much higher (14.8–16.2%) when any of these three species are compared to *S. papilliferus*.

TABLE 2 | Morphometric data of *Spintherobolus papilliferus* collected in three different drainages: rio Ribeira de Iguape (RI), rio Itapanhaú (IT), and upper rio Tietê (UT). Measures 2–13 are percentages of SL; measures 14–17 are percentages of HL. N = number of sampling units; SD = standard deviation.

	RI (N = 8)			IT (N = 7)			UT (N = 9–10)		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
1.Standard length (SL) (mm)	26.7–39.3	34.4	–	20.9–46.5	30.3	–	18.5–61.1	41.1	–
2. Body depth	23.7–28.9	25.5	1.8	28.1–33.4	29.4	1.9	26.8–32.3	29.6	1.7
3. Predorsal distance	51.1–54.3	52.7	1.1	53.1–57.9	55.4	1.8	49.6–56.4	54.0	1.8
4. Prepelvic distance	42.8–49.2	46.4	2.2	46.5–47.9	47.3	0.5	42.2–47.7	45.1	1.7
5. Preanal distance	56.8–63.5	60.2	2.0	64.5–65.7	64.9	0.5	57.5–66.9	62.7	2.7
6. Dorsal-fin length	23.4–27.4	25.4	1.2	21.2–28.3	25.0	2.6	21.9–25.3	23.5	1.3
7. Dorsal-fin base length	10.8–15.3	12.9	1.7	11.4–15.2	12.9	1.2	12.5–17.1	13.8	1.3
8. Pelvic-fin length	12.9–17.3	15.7	1.5	14.2–18.9	16.8	1.6	13.6–18.6	16.4	1.6
9. Anal-fin length	20.5–25.1	22.5	2.0	20.3–24.2	22.8	1.3	18.2–23.4	20.3	1.5
10. Anal-fin base length	10.7–16.1	14.0	1.7	8.6–13.6	10.3	1.8	13.1–15.0	13.6	0.6
11. Caudal-peduncle depth	9.8–12.7	11.0	0.9	12.4–16.2	14.0	1.3	12.8–15.4	14.1	1.0
12. Caudal-peduncle length	26.1–30.5	28.1	1.8	26.5–31.9	29.2	2.1	24.5–33.0	27.0	2.3
13. Head length (HL)	29.4–33.7	31.1	1.7	29.6–33.1	31.4	1.1	28.5–32.7	30.3	1.4
14. Orbital diameter	23.2–32.7	27.4	2.6	22.2–30.4	26.6	2.9	18.6–28.8	22.6	2.7
15. Interorbital width	27.4–33.8	30.1	2.4	29.1–35.8	32.1	2.7	27.9–32.0	29.6	1.2
16. Snout length	11.7–15.1	12.9	1.4	11.6–18.9	16.5	2.4	13.2–18.0	15.8	1.6
17. Upper jaw length	20.0–26.5	22.4	2.3	28.7–34.1	30.5	2.0	23.1–39.7	28.4	4.4

TABLE 3 | Meristic data of *Spintherobolus papilliferus* collected in three different drainages: rio Ribeira de Iguape (RI), rio Itapanhaú (IT) and upper rio Tietê (UT). Number in parentheses is the absolute frequency of a count. For variable 5 (number of branched caudal-fin rays), numbers before slash are rays in the dorsal lobe, and number after slash are rays in the ventral lobe.

	RI	IT	UT
1. Number of branched dorsal-fin rays	9(8)	9(7)	9(10)
2. Number of branched pectoral-fin rays	10(1), 11(4), 12(2) or 13(1)	11(3) or 12(3)	10(2), 11(5), 12(1) or 13(1)
3. Number of branched pelvic-fin rays	6(8)	5(1) or 6(6)	6(8) or 7(2)
4. Number of branched anal-fin rays	9(7) or 10 (1)	8(1), 9(5) or 10(1)	9(10)
5. Number of branched caudal-fin rays	9/8(7) or 10/7(1)	9/8(6) or 10/8(1)	9/7(1) or 9/8(7)
6. Number of perforated lateral-line scales	4(1), 5(5) or 6(2)	3(1), 4(2) or 5(4)	3(1), 4(3), 5(3) or 6(1)

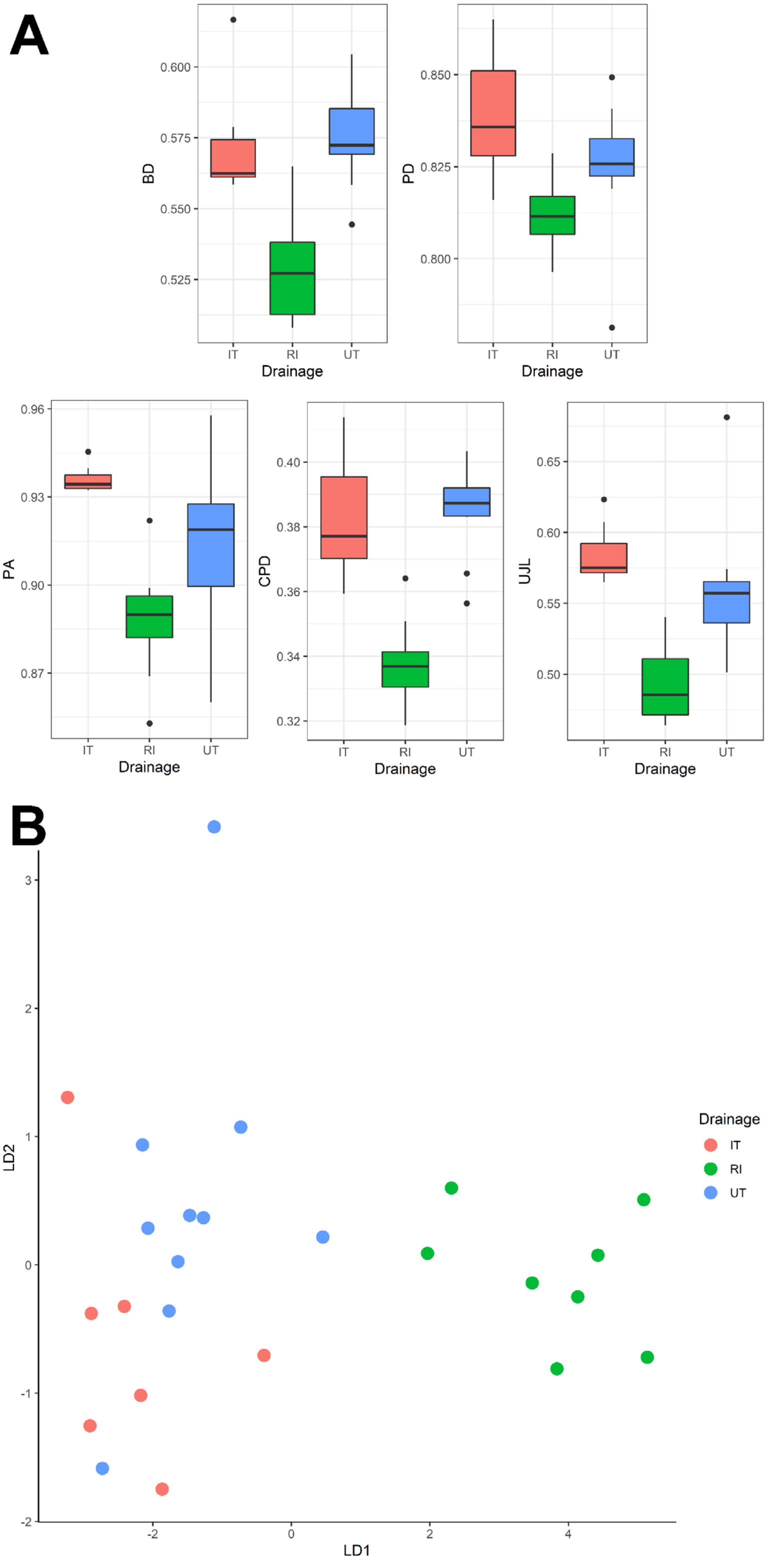

FIGURE 3 | **A.** Boxplot for dependent variables body depth (BD), predorsal distance (PD), preanal distance (PA), caudal-peduncle depth (CPD), and upper jaw length (UJL) of *Spintherobolus papilliferus* from the three drainages: Itapanhaú (IT), Ribeira de Iguape (RI), and upper rio Tietê (UT). **B.** Scatter plot of LDA features for distinct drainages Itapanhaú (IT – red dots), Ribeira de Iguape (RI – green dots), and upper rio Tietê (UT – blue dots).

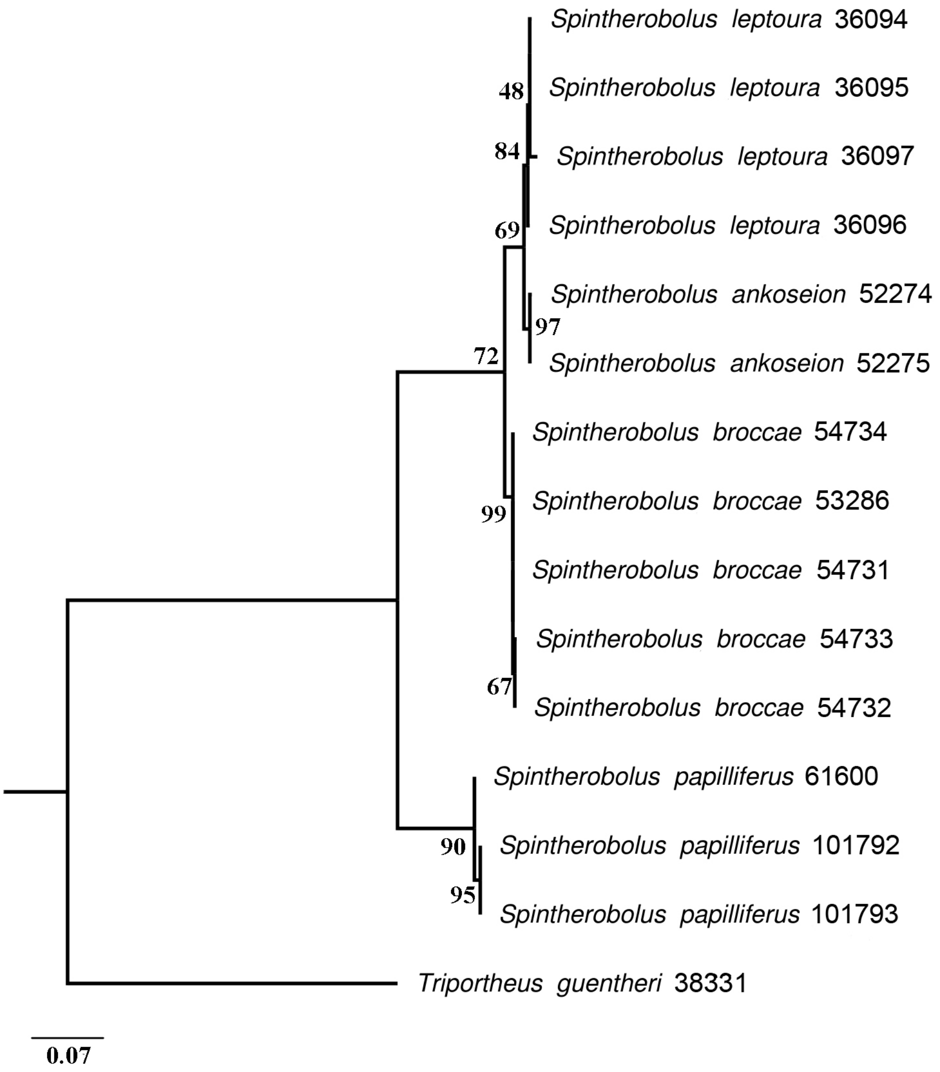

FIGURE 4 | Maximum likelihood tree of species of *Spintherobolus* based on partial sequences of the COI gene (531 bp). Numbers represent voucher specimens deposited in LBP (Tab. 1). Vouchers LBP 101792 and 101793 are from rio Ribeira de Iguape drainage, municipality of Juquitiba, and voucher LBP 61600 is from rio Sertãozinho, rio Itapanhaú drainage, municipality of Bertioga, both São Paulo State, Brazil.

TABLE 4 | Pairwise K2P genetic distances among species of *Spintherobolus*. Intraspecific genetic variations are highlighted in bold. Numbers below diagonal are values of interspecific distances. Values are shown in percentages, followed by standard deviation.

	1	2	3	4
1- *S. papilliferus*	**0.4 ± 0.2**			
2- *S. broccae*	14.8 ± 2.1	**0.2 ± 0.1**		
3- *S. ankoseion*	16.2 ± 2.3	3.4 ± 0.8	**0.2 ± 0.2**	
4- *S. leptoura*	15.8 ± 0.2	3.5 ± 0.8	1.5 ± 0.5	**0.6 ± 0.2**

DISCUSSION

The populations of *Spintherobolus papilliferus* from the rio Ribeira de Iguape, rio Itapanhaú, and upper rio Tietê present all meristic data and almost all morphometric data greatly overlapped. The exceptions are body and caudal-peduncle depths, predorsal and preanal distances, and upper jaw length, which are relatively smaller in samples from rio Ribeira de Iguape basin (Tab. 2). The MANOVA and LDA confirmed these differences as statistically significant (Fig. 3). Despite these five morphometric differences, DNA barcoding corroborates samples from both coastal drainages (*i.e.*, Ribeira de Iguape and Itapanhaú) as belonging to the same species, with the other three species apart in their own group (Fig. 4). It is unfortunate that we did not have access to molecular samples from the upper rio Tietê to verify if the coastal samples would indeed group with the crystalline shield, but our meristic data and the majority of our morphometric data confirm that our three samples belong to the same species. Moreover, several other fish species have a similar distribution pattern in both upper rio Tietê and coastal basins (*e.g.*, Ribeiro, 2006; Oyakawa, Menezes, 2011), some of which will be further discussed below.

It is interesting that specimens of *S. papilliferus* from rio Ribeira de Iguape have a more streamlined body reflected by their shallower body and caudal-peduncle, and this could be interpreted as a populational variation from the river as our sample was caught in a rapids pool, therefore with high water flow, a typical environment of this region (Ross, 2002). Streamlined body in fishes is usually associated with faster and more agile forms (*e.g.*, Webb, 1984; Astudillo-Clavijo *et al*., 2015), and a similar pattern of shallower body and caudal-peduncle associated with lotic environments was demonstrated by Dunn *et al.* (2020) with species of *Galaxias* Cuvier, 1816. Indeed, McLaughlin, Grant (1994) demonstrated that juveniles of *Salvelinus fontinalis* (Mitchill, 1814) raised in faster flowing waters tend to develop shallower bodies and caudal peduncles, illustrating a clear case of phenotypic plasticity. A similar experiment with *S. fontinalis* in the lab also demonstrated phenotypic plasticity in juveniles raised in fast-flowing and slow-flowing conditions, however found out that fish raised in faster currents tend to have a deeper caudal peduncle probably due to swimming muscle increase in the tail (Imre *et al*., 2002). Our hypothesis is that *S. papilliferus* from the fast-flowing waters of rio Ribeira de Iguape basin are under a selective pressure which makes their body more streamlined. Their body is also shorter with lower values of preanal and predorsal distances, in addition to a shorter upper jaw (Fig. 3A).

Until recently, *Spintherobolus papilliferus* was restricted to the highlands of the upper rio Paraná basin, where rio Tietê empties, while all the other species of the genus are lowland coastal river species: *S. broccae* from coastal rivers in Rio de Janeiro and São Paulo states, *S. ankoseion* from coastal rivers of Santa Catarina and Paraná states, and *S. leptoura* from lower rio Ribeira de Iguape basin. This led Ribeiro (2006) to classify *Spintherobolus* in his pattern B of distribution of species defined as "sister-group relationships between endemic ichthyofauna of the Brazilian coastal drainages and adjacent shield that includes intermediate degree of inclusiveness, in which, in some cases, both sister-clades underwent some subsequent radiation". Also, according to this comprehensive work of Ribeiro (2006), this cladogenetic events would have happened in the Tertiary. The presence of *S. papilliferus* in coastal drainages (Fig. 5) indicates that this interpretation needs to be updated. We propose that diversification of *Spintherobolus* species fits best Ribeiro's (2006) pattern C, that "reflects the most recent vicariant events between upland crystalline shield rivers and the adjacent coastal drainages that lead to the recognition of shared species between them".

Ribeiro (2006) provided examples of species that fit his pattern C, including Characiformes, Siluriformes, Gymnotiformes, Cichlidae and Poeciliidae fishes along the entire Brazilian coast. Specifically in Southeastern Brazil, Ribeiro (2006) described the pattern in which the upper rio Tietê shares species with the headwaters of rio Ribeira de Iguape and other adjacent coastal rivers such as *Hollandichthys multifasciatus* (Eigenmann & Morris, 1900), *Pseudocorynopoma heterandria* Eigenmann, 1904, *Hyphessobrycon bifasciatus* Ellis, 1911, *Hyphessobrycon reticulatus* Ellis, 1911, and *Gymnotus pantherinus* (Steindachner, 1908). However, these are examples of species widespread in the coastal drainages that were also found in the upper rio Tietê system, suggesting a recent colonization of the latter basin by species originally from the coastal drainages (Ribeiro, 2006), which is not the case of *Spintherobolus papilliferus* with a restricted area in coastal drainages (*i.e.*, so far, only found in two localities).

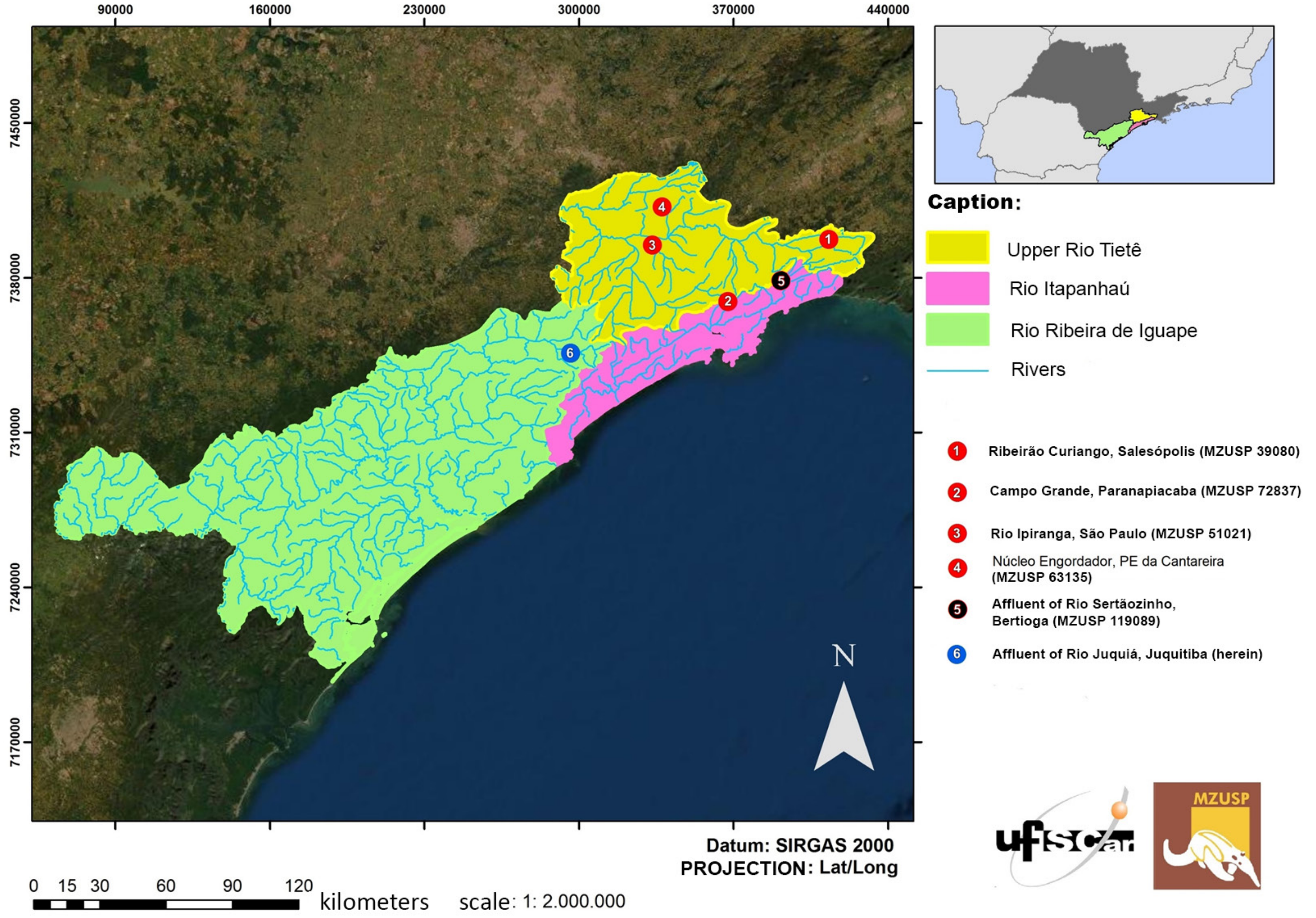

FIGURE 5 | Map of southeastern South America showing a detail of the map of São Paulo State (dark grey in small map) and the three drainages discussed in this study: upper rio Tietê (yellow), rio Itapanhaú (pink) and rio Ribeira de Iguape (green). Localities 1–4 belong to the upper rio Tietê, locality 5 belongs to rio Itapanhaú and locality 6 belongs to rio Juquiá, rio Ribeira de Iguape drainages. PE = Parque Estadual.

Alternatively, the distribution of *Spintherobolus papilliferus* in both upper rio Tietê and costal drainages such as rios Ribeira de Iguape and Itapanhaú could be explained by headwater capture from the crystalline shield towards the ocean as the Serra do Mar mountain chain that divides both systems suffered erosional retreat since its initial formation in the Paleogene (Almeida, Carneiro, 1998; Ribeiro, 2006). A similar model was proposed for the headwaters of rio Guaratuba, a coastal river whose upper reaches are close to rio Claro, a tributary of the upper rio Tietê (Ribeiro *et al*., 2006). As a matter of fact, †*Megacheirodon unicus* (Travassos & Santos, 1955) is a fossil in the clade including *Spintherobolus* from the Tremembé Formation and dated as Oligocene (35 to 23 million years ago), therefore within the boundaries of the Paleogene (Malabarba, 1998). †*Megacheirodon unicus* was considered as sister to *Spintherobolus* until the description of *Amazonspinther dalmata* Bührnheim, Carvalho, Malabarba & Weitzman, 2008 a miniature fish from the rios Madeira and Purus in the Amazon basin (Bührnheim *et al*., 2008). Therefore, the most recent relatives of *Spintherobolus* are from the Amazon and crystalline shield and the distribution of *Spintherobolus papilliferus* in coastal drainages could be explained by headwater captures due to retreat of the Serra do Mar (*e.g*., Camelier *et al*. (2018)), a hypothesis that must be tested with a phylogeographic approach when pertinent material is available.

Serra *et al.* (2007) studied the ichthyofauna of rio Itatinga, an affluent of rio Itapanhaú, and found four species that were shared between this system and upper rio Tietê with relatively restricted geographic distributions: *Coptobrycon bilineatus* (Ellis, 1911), *Glandulocauda melanogenys* Eigenmann, 1911 [= *Glandulocauda melanopleura* (Ellis, 1911)], *Pseudotocinclus tietensis* (Ihering, 1907), and *Taunayia bifasciata* (Eigenmann & Norris, 1900), three of which endangered species except for *Glandulocauda melanopleura* (Akama *et al*., 2018). Interestingly, *Spintherobolus papilliferus*, another endangered species, is added to this list as it also occurs in a headwater affluent of rio Itapanhaú, in addition to rio Ribeira de Iguape basin.

Recently, Camelier *et al*. (2018) presented an integrative approach to *Glandulocauda melanopleura* in which disjunct populations of the upper rio Tietê, and headwaters of coastal drainages such as Guaratuba, Itatinga, Itanháem, and Ribeira de Iguape have some morphological variation albeit monophyletic according to molecular data. In addition to confirming the monophyly of *G. melanopleura*, the genetic data showed two interesting results: (i) there are two distinct and well corroborated lineages, one from upper rio Tietê and rio Itanháem, and another exclusive of rio Guaratuba; (ii) there is a single haplotype endemic of rio Guaratuba, and two other haplotypes shared by upper rio Tietê and rio Itanháem indicating a recent event of river capture between these two latter drainages. *Spintherobolus papilliferus* seems to have a similar diversification history to that of *G. melanopleura*, with disjunct populations resulting from river capture from the uplands towards the coastal drainages that yields to intraspecific morphological variation.

Although the updates in the geographic range of the species, *Spintherobolus papilliferus* might not change to a less strict conservation status according to the criteria and standards of the IUCN (IUCN Standards and Petitions Subcommittee, 2019). *Spintherobolus papilliferus* was found in only four locations, one of them without records after the 1980s (Castro *et al*., 2008) and another almost entirely suppressed with the growth of the city of São Paulo (*e.g*., Mattox, 2008). The other two locations are protected by Conservation Units (Parque Estadual da Cantareira and Parque Estadual da Serra do Mar). The estimated area of occupancy was 8 km^2. We report herein two additional locations where the species can be found, one in the rio Sertãozinho, an affluent of rio Itapanhaú system whose preservation status is not known to us, and another in an affluent of rio Juquiá, rio Ribeira de Iguape basin, which is relatively well preserved although it does not belong to conservation units. This raises the number of locations and will likely increase the estimated area of occupancy of *S. papilliferus*, but will probably be not enough to change the classification of the species as Endangered.

Sampling efforts should be done in places where *Spintherobolus papilliferus* might still survive (*e.g.*, Parque Estadual da Cantareira, Estação Biológica da Boracéia) to investigate if it is still present in these locations. It is of uttermost importance that the few sites where *S. papilliferus* occurs are kept under protection. This is especially relevant due to the apparent co-occurrence of *S. papilliferus* with other endangered species such as *Coptobrycon bilineatus*, *Pseudotocinclus tietensis*, *Taunayia bifasciata*, and *Cambeva paolence* (Eigenmann, 1917), all of which have restricted distributions in the upper rio Tietê and coastal rivers such as the headwaters of rio Ribeira de Iguape, rio Itapanhaú and rio Guaratuba (*e.g.*, Ribeiro *et al*., 2006; Serra *et al*., 2007). Creation and monitoring of conservation units are important measures to mitigate our impact on freshwater ecosystems such as the Atlantic Rainforest streams, but these need to take into consideration the connectivity of aquatic habitats to fulfil their aims (Albert *et al*., 2020).

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The author declares no competing interests.

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