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RESEARCH ARTICLE

Basin-scale distribution and haplotype partitioning in different genetic lineages of the Neotropical migratory fish *Salminus brasiliensis*

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Abstract

1. Four valid species are currently recognized in the Neotropical migratory genus *Salminus*: *Salminus brasiliensis*, *Salminus franciscanus*, *Salminus hilarii* and *Salminus affinis*. However, molecular evidence strongly suggested that two different species might be contained under the taxonomic denomination *Salminus brasiliensis*. Therefore, the geographical distribution of each entity was evaluated in order to understand their contribution to the different stocks of major river networks in South America.
2. Major river networks of the La Plata River basin were explored to characterize the geographical distribution of the two genetic lineages. To characterize further the genetic partitioning within each lineage of *S. brasiliensis*, a haplotype analysis was conducted. The 5′ region of the mitochondrial COI gene was used as the molecular marker. In total, 45 fish samples of *S. brasiliensis* from 19 sites in Argentina, Brazil and Paraguay were sequenced. Additional COI sequences of *S. brasiliensis*, *S. franciscanus* and *S. hilarii* were gathered from public databases.
3. All samples of *S. brasiliensis* comprised two different mitochondrial lineages. Accordingly, phylogenetic tree topologies segregated the complete set of sequences into two disparate clusters. One of these clusters was far closer phylogenetically to *S. hilarii* than to other *S. brasiliensis*.
4. While one of the genetic lineages of *S. brasiliensis* seemed mostly restricted to the upper Paraná River, the other showed a widespread distribution along major river networks of the basin.
5. Fifteen unique haplotypes were identified and collapsed. *Salminus hilarii* and *S. franciscanus* have private haplotypes. In *S. brasiliensis*, each mitochondrial lineage also hosts a set of unshared haplotypes.
6. The sympatry of two different putative species within *S. brasiliensis* together with their unshared haplotypes present a difficult situation for management and conservation that calls for timely solutions.

KEYWORDS

fish, fishing, floodplain, genetics, hydropower, river

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1 | INTRODUCTION

The genus *Salminus* (family Bryconidae) contains species of large, migratory and piscivore fish that exclusively inhabit freshwater ecosystems of South America. Early in the 20th century, Eigenmann (1917) recognized four valid species of *Salminus*: *Salminus maxillosus*, *Salminus brevidens*, *Salminus hilarii* and *Salminus affinis*. A later revision by Géry and Lauzzane (1990) made drastic changes in the nomenclature of *Salminus*. These authors demonstrated that *Hydrocyon brevidens* and *Salminus cuvieri* were objective synonyms of *Salminus brasiliensis*, since both species were described based on the holotype of *Hydrocynus brasiliensis*. Gery and Lauzzane (1990) also proposed that *S. maxillosus* and *Salminus orbignyanus* were junior synonyms of *S. brasiliensis*. After Géry and Lauzzane (1990), the name of the large-sized *Salminus* species from the São Francisco River was left out and subsequently was formally described as *Salminus franciscanus* (Lima & Britski, 2007). In summary, four valid species of *Salminus* are currently recognized: *S. brasiliensis*, *S. franciscanus*, *S. hilarii* and *S. affinis*. However, mitochondrial and nuclear molecular evidence supported the presence of eight distinct lineages in *Salminus* and suggested the existence of two potentially undescribed taxa in *S. hilarii* (Machado, Ishizuka, Freitas, Valiati, & Galetti, 2016). This hidden diversity will certainly pose problems for management and conservation. Underestimation of species richness in a given environment will not only present an inaccurate picture of its biodiversity and community structure, but will result also in inadequate policies and regulations for the protection of natural resources (Bortolus, 2008).

Although large international efforts have been made to increase the rate of species discovery and description (Stockle & Hebert, 2008) and to improve the portrayal of phylogenetic relationships (Letunic & Bork, 2007), comparatively little attention has been devoted to intraspecific variation and population differentiation. This is particularly relevant for freshwater migratory species such as *S. brasiliensis*, considering the wide area that they inhabit and the high level of exploitation to which they are exposed. Among species of *Salminus*, *S. brasiliensis* has the widest geographical distribution, ranging from the La Plata River Basin and Lagoa dos Patos drainage in southern Brazil to the Chaparé and Mamoré Rivers in the upper Amazon Basin (Gómez, 2016; Reis, Kullander, & Ferraris, 2003). Throughout this geographical range, *S. brasiliensis* plays an important role in the regional economies of South America owing to its extensive harvest by commercial and recreational fisheries as well as in aquaculture and stocking programmes (Agostinho, Gomes, Suzuki, & Ferreira Julio, 2003). Accordingly, aspects of the ecology (Rossi, 2008), phylogeny (Calcagnotto, Schaefer, & DeSalle, 2005; Javonillo, Malabarba, Weitzman, & Burns, 2010; Lima, 2006; Mirande, 2010; Ortí & Meyer, 1997; Roberts, 1969) and population genetics (Lopes et al., 2007; Ramella et al., 2006; Ribolli et al., 2017; Rueda, Amavet, Brancolini, Sommer, & Ortí, 2011) of this species have been well studied.

Recently, molecular evidence strongly suggested that two different species might be contained under the taxonomic denomination *Salminus brasiliensis* (Rosso, Mabragaña, González-Castro, & Díaz de Astarloa, 2012). According to these results, Machado et al. (2016) also recognized the existence of two well-defined molecular operational taxonomic units within the nominal species *S. brasiliensis* by means of DNA barcoding. However, their analysis of one nuclear marker (rag2) failed to recognize the COI haplogroups for this species. Overall, these results clearly showed two different mitochondrial lineages within *S. brasiliensis*.

This hidden diversity within *S. brasiliensis* introduces uncertainty over current knowledge about phylogenetics, fisheries, aquaculture, stocking and ecological aspects of this species. Conservation methods should address well-defined taxonomic or evolutionary units to be successfully applied (Moritz, 1994). Therefore, the current taxonomic uncertainty surrounding both lineages of *S. brasiliensis* precludes the development of effective management activities designed to enhance conservation. Adding uncertainty to this problem is the migratory behaviour of *S. brasiliensis*. Species of the genus *Salminus* are large predatory fishes that undertake extensive main-channel migrations for reproduction in large rivers of South America (Bonetto & Pignalberi, 1964; Sverlij & Espinach Ros, 1986). Therefore, the actual distribution of both mitochondrial lineages may cover a much wider geographical area than currently known (Machado et al., 2016; Rosso et al., 2012). Within this context, it is important to characterize the degree of geographical distribution of each intraspecific entity in order to understand their contributions to the different stocks of major river networks in South America.

In this study, the major river networks of the La Plata basin were sampled to gain confidence and robustness in relation to the geographical distributions of these two genetic lineages. Owing to the migratory behaviour of *S. brasiliensis*, our hypothesis was that both mitochondrial lineages would be present in all large river networks of this basin. In an attempt to further characterize the genetic partitioning within each lineage of *S. brasiliensis*, haplotype analysis was conducted.

2 | MATERIALS AND METHODS

2.1 | Study area

The La Plata river basin drains large areas of Argentina, Bolivia, Brazil, Paraguay and Uruguay. It is the second largest drainage system in South America and the fourth largest in the world, covering an area of 3.2 million km^2. The La Plata river basin consists mainly of three sub-basins: the Paraná, the Paraguay, and the Uruguay river basins. The Paraguay River extends 2670 km southwards from its sources in the western hills of the Brazilian Shield to its confluence with the Paraná River. The Paraguay River is scarcely disturbed by human activities, and fisheries are still at a low level of exploitation when compared with those of the Paraná and Uruguay rivers (Quirós, Bechara, & Resende, 2007). The Uruguay River runs 1858 km from Southern Brazil to its confluence with the Paraná River draining a basin of 365 000 km^2 (Di Persia & Neiff, 1986).

Downstream of the Yacyretá dam, the Paraná River forms a wide valley that opens in several arms. It flows towards the west, progressively widening and branching into several channels that form a large number of fluvial islands. After its confluence with the Paraguay River, the Paraná River abruptly turns to the south. The floodplain of the Paraná River is influenced by variable hydrometric levels, contributing to habitat complexity. However, the increasing number of dams,

dredging and artificial dikes disturbs the connectivity between the ecological units of the floodplain (Tockner & Stanford, 2002). This hampers the natural disturbances that maintain high levels of diversity and connectivity through the fluvial landscape. Overall, this regulation reduces the availability of environments for fishes, both juveniles and adults, which leads to a decline of their populations (Scipioni, Casciotta, Almirón, Santinón, & Ruiz-Díaz, 2016). The *Esteros del Iberá*, located in the lower Paraná River drainage, constitutes one of the most important wetland systems in South America. This wetland, covering more than 13 000 km^2, is located in the province of Corrientes, Argentina, and consists of a groundwater reservoir exclusively of rainfall origin (Casciotta, Almirón, & Bechara, 2005). Its only relationship with the nearby river basins is through the Corrientes River, which acts as the sole effluent connecting this wetland with the lower Paraná River.

The Juramento–Salado river basin is an important fluvial system, whose headwaters are located in north-western Argentina in the Calchaquíes Valleys in Salta and Catamarca provinces, flowing in a south-west direction to the Paraná River in Santa Fe province (Monasterio de Gonzo, 2003). The basin can be divided into three different areas: the upper basin that receives water from several mountain rivers, the middle basin that has an intermittent flow regime, and the lower basin in which the regime becomes permanent and water flows into the Paraná River basin (Venturini & Krepper, 2012). Biological sampling was carried out in the upper reaches of the Juramento-Salado river basin.

2.2 | Fish and tissue sampling

Nineteen locations were visited to collect specimens and tissues of *S. brasiliensis* from representative drainages of all large rivers of the La Plata River basin. In addition, selected DNA sequences of *S. franciscanus*, *S. hilarii* and *S. brasiliensis* were gathered from GeneBank and BOLD (Barcode of Life Database) (Table S1, Supporting information).

A small piece of white muscle or fin was excised from each specimen. Tissue samples were preserved in ethanol 96% at −18°C until their further analyses. Before fixation, all specimens were labelled, photographed and preserved as vouchers for further taxonomic studies in the fish collections of the Instituto de Ictiología del Nordeste, Fundación Miguel Lillo and Instituto de Investigaciones Marinas y Costeras (IIMyC, UNMDP-CONICET). When keeping vouchers was not possible, photographs were retained as e-vouchers (Monk & Baker, 2001).

2.3 | DNA extraction, amplification and sequencing

DNA extraction and amplification of a fragment of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) were performed at the Argentine International Barcode of Life Laboratory reference (IIMyC, CONICET, Mar del Plata, Argentina) as well as the Genetics Laboratory (FHUC-UNL, Santa Fe, Argentina). DNA extraction and polymerase chain reaction (PCR) were performed in accordance with standard DNA barcoding protocols (Ivanova, de Waard, & Hebert, 2006). Different sets of primer cocktails, including those designed for fishes (Ivanova, Zemlak, Hanner, & Hebert, 2007) were used to amplify sequences.

Each amplification reaction contained 2 μL DNA template, 6.25 μL 10% trehalose, 2 μL molecular biology grade water, 1.25 μL 10× reaction buffer, 0.625 μL $MgCl_2$ (50 μM), 0.0625 μL dNTP (10 mM), 0.0625 μL of each primer (10 μM) and 0.0625 μL Invitrogen's Platinum Taq. polymerase (5 U μL^{-1}) producing a total reaction volume of 12.375 μL. The PCR profile comprised an initial step of 2 min at 95°C, and 35 cycles of 30 s at 94°C, 40 s at 52°C and 1 min at 72°C, with a final extension at 72°C for 10 min. E-Gels (Invitrogen) were used to screen for amplification success.

Sequencing of the COI gene was carried at the Canadian Centre for DNA Barcoding (CCDB) in Ontario and Macrogen, Inc. (Korea).

2.4 | Molecular data analysis

Original and downloaded DNA sequences were combined into a single sequence alignment to perform molecular analyses. Sequences were aligned using MAFFT version 7 (Katoh & Standley, 2013) and edited using AliView (Lassmann, Hayashizaki, & Daub, 2009). ALTER (Alignment Transformation Environment; Glez-Peña, Gómez-Blanco, Reboiro-Jato, Fernández-Riverola, & Posada, 2010) was used to obtain unique haplotypes of the aligned sequences. The COI alignment was tested to find the best-fitting nucleotide substitution model using Bayesian Information Criterion (BIC) implemented in jModel Test v2.1.7 (Darriba, Taboada, Doallo, & Posada, 2012). Phylogenetic relationships among haplotypes were estimated by maximum likelihood (ML) analyses using MEGA 7 (Kumar, Stecher, & Tamura, 2016) and 1000 bootstrap pseudoreplicates were conducted to estimate node support values for the resulting phylogeny. A Kimura 2 parameters (K2P, Kimura, 1980) distance matrix was built for all possible pairwise comparisons of aligned sequences. A neighbour-joining (NJ) tree of K2P distances was created to provide a graphic representation of divergences between species. The discontinuity between the mean intraspecific divergence and the smallest interspecific divergence between species (Barcode gap) was reported (Meier, Zhang, & Ali, 2008; Meyer & Paulay, 2005).

The Barcode Index Number (BIN) was used to estimate the number of species directly from the barcode sequences (Ratnasingham & Hebert, 2013). The concordance of BIN estimates with the NJ and ML tree topologies was addressed.

Genetic diversity was quantified in terms of number of haplotypes (n), haplotype diversity (*h*) and nucleotide diversity (π) using DnaSP (Librado & Rozas, 2009). A haplotype network was built with software NETWORK v. 5 using the median joining algorithm (http://www.fluxus-engineering.com/sharepub.htm, Bandelt, Forster, & Röhl, 1999).

3 | RESULTS

In total, 45 specimens or tissues of *S. brasiliensis* were collected in the major river networks of the La Plata basin, comprising 19 sites from Argentina, Brazil and Paraguay (Table 1). The geographical distribution

TABLE 1 Collecting and sequence sources sites for species of *Salminus*. Number and diversity of haplotypes and nucleotide diversity are discriminated at the main basin level. BZ = Brazil; AR = Argentina; PY = Paraguay; NGR = no geographical references in GeneBank; h = haplotype number; Hd = haplotype diversity; π = nucleotide diversity

SPECIES	BASIN	SITE	N	h	Hd	π	State/ (country)	LAT	LONG	Map code
Salminus brasiliensis	Upper Paraná	Tieté River	7	8 (H1, H2, H3, H4, H5, H6, H7, H15)	0.78947	0.00996	Sao Paulo (BZ)	−21.927321	−47.368234	1
		Ivinhema River	1				Matto Grosso do Sul (BZ)	−22.241850	−53.574056	2
		Lagoa Finado Raimundo	2					−22.799333	−53.544171	3
		Upper Paraná (GB)	10					NGR	NGR	
	Lower Paraná	Paraná River	20	5 (H1, H2, H6, H13, H14)	0.53000	0.02249	Corrientes (AR)	−27.460856	−57.300556	4
		Carambolas Stream	1					−28.120722	−57.394666	5
		Paraná River	2				Entre Ríos (AR)	−31.716111	−60.582222	6
		El Pescado Lake	1					−32.655000	−60.157000	7
		Paraná River	1				Santa Fe (AR)	−31.49400	−60.446000	8
		Rojas River	5	4 (H1, H2, H13, H14)	0.64286	0.02912	Buenos Aires (AR)	−34.170000	−60.910000	9
		Delta of Paraná	3					−34.025600	−58.491400	10
		Parana de las Palmas	1					−34.253000	−58.695500	11
	Iguazú	Iguazú River	2	2 (H13, H15)	1.00000	0.00225	Misiones (AR)	−25.696389	−54.444147	12
	Pilcomayo	"La Estrella" swamp	1	1 (H13)	-	-	Formosa (AR)	−24.026167	−60.835833	13
	Paraguay	Corumbá River	1	1 (H13)	0.00000	0.00000	Corumbá (BZ)	−19.008169	−57.651565	14
		Paraguay River	3				Asunción (PY)	−25.279892	−57.636994	15
			1				Formosa (AR)	−26.241120	−58.127754	16
	Uruguay	Uruguay River	2	3 (H2, H13, H14)	0.80000	0.02748	Entre Ríos (AR)	−31.377186	−57.982317	17
		Bonito River	4				Misiones (AR)	−27.443000	−54.941000	18
	Juramento	Juramento River	1	1 (H13)	-	-	Salta (AR)	−25.225854	−64.471120	19
Salminus franciscanus	San Francisco	Urucuria River	1	2 (H11, H12)	0.18182	0.00036	Mina Gerais (BZ)	−16.152000	−45.694000	20
		Pandeiros River	7					−15.672000	−44.637000	21
		Upper San Francisco	3					NGR	NGR	
Salminus hilarii	San Francisco	Verde Grande River	2	3 (H8,H9,H10)	0.68889	0.00675	Mina Gerais (BZ)	−15.323000	−43.66500	22
	Upper Paraná		4				Sao Paulo (BZ)	−23.333300	−48.566700	23
		Paraná River	4					NGR	NGR	

of both entities throughout the lower La Plata Basin is shown in Figure 1. The newly obtained sequences were deposited in GenBank (accession numbers: pending).

All these specimens exhibited two different BIN numbers, AAZ3803 and AAD2790. New sequenced COI fragments ranged from 460 to 669 BP. Additional sequences of *S. brasiliensis* from different river drainages were gathered from GenBank (n = 19) and BOLD (n = 29) and were grouped into these two entities. The NJ K2P and ML algorithms clearly segregated the complete set of sequences of *S. brasiliensis* into two disparate clusters (Figure 2). Moreover, one of these clusters is far closer phylogenetically to *S. hilarii* than to the other *S. brasiliensis* cluster. This tree also showed that all sequences of *S. franciscanus*, a morphologically close species to *S. brasiliensis*, clustered tightly together and distant from both BINs of *S. brasiliensis*. There was a perfect match between the BIN assigned by BOLD and phylogenetic clusters in both NJ and ML topologies.

Interestingly, the geographical distribution of each cluster of *S. brasiliensis* was largely uneven. Whereas individuals bearing BIN AAD2790 seemed to be mostly restricted to the upper Paraná River, specimens exhibiting BIN AAZ3803 showed a widespread distribution along major river networks of the basin (Table S1 and Figure 3). For instance, in the Pilcomayo and Juramento rivers, only one specimen was collected in each river and both were assigned to BIN AAZ3803. The geographical distribution of individuals within this BIN also includes aquatic ecosystems of the lower Paraná River, upper and middle Uruguay River as well as the Iguazú and Paraguay rivers.

Mean genetic distance between BINs of *S. brasiliensis* was extremely large (7.01%), leading to a marked barcoding gap (21.92) between these mitochondrial lineages (Table 2). Moreover, both entities were more similar to *S. hilarii* (BIN ABY7701) than to each other. Indeed, mean congeneric distance between *S. brasiliensis* AAD2790 and *S. hilarii* ABY7701 was only slightly more than 2% (2.17%). The nearest neighbour between these two entities was at 1.68%. In *S. brasiliensis*, BIN AAZ3803 showed a more cohesive cluster with a mean intra-specific genetic distance of 0.07%.

3.1 | Phylogeography of *Salminus* spp.

Among the 89 COI sequences (comprising *S. brasiliensis*, *S. hilarii* and *S. franciscanus*), both new sequences and those obtained from BOLD and GenBank, 15 haplotypes defined by 57 polymorphic sites were recovered (Table 3). Overall, the haplotype network obtained (Figure 3) closely reflected the phylogenetic structure detected by tree analyses. *Salminus hilarii* (H8-H9-H10) and *S. franciscanus* (H11-H12) have private haplotypes. The other 10 haplotypes were found in *S. brasiliensis*, where each BIN also hosts a set of unshared haplotypes. Haplotype H13 was the most common and was shared by 31 individuals of *S. brasiliensis* of BIN AAZ3803, collected from several localities, mostly from the Lower Paraná and Paraguay basins as well as in some individuals of the Uruguay basin and Iguazú River. Individuals from the Pilcomayo and Juramento localities also were included in this group. Two other haplotypes of this BIN were observed, H14 in the Lower Paraná and Uruguay basin, and H15 from the Iguazú River. The most

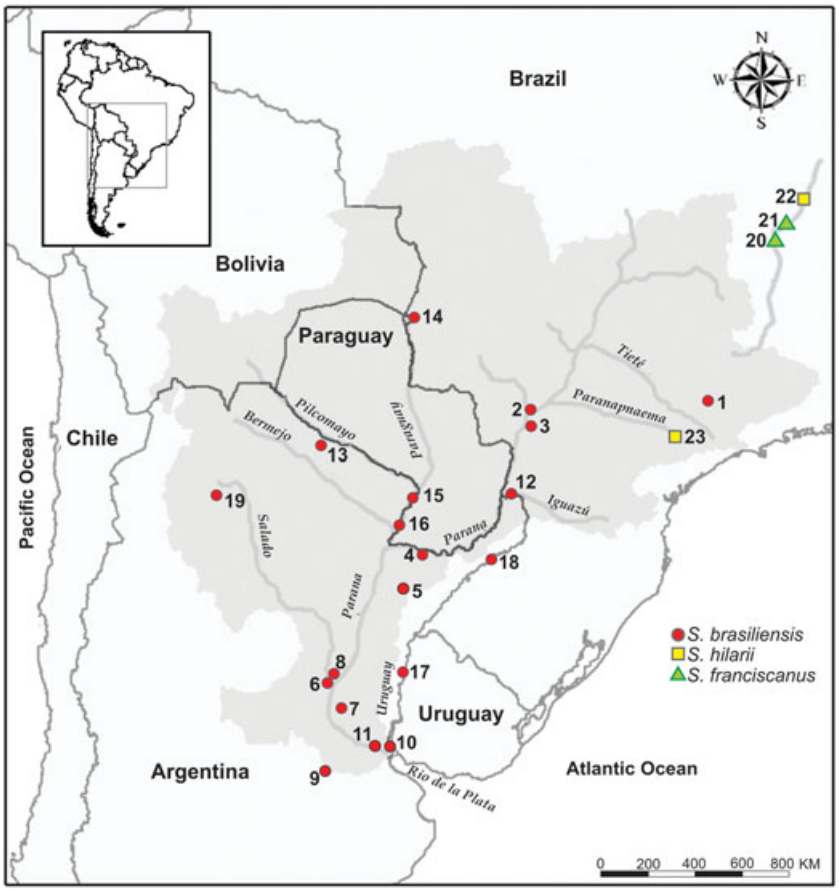

FIGURE 1 Map of the study area showing approximate locations of sampling sites for *S. brasiliensis*. Each single point may represent more than one sampling event and site. References to *S. franciscanus*, *S. hilarii* and some *S. brasiliensis* correspond to the geographical location of tissues or DNA sequences (see Table S1)

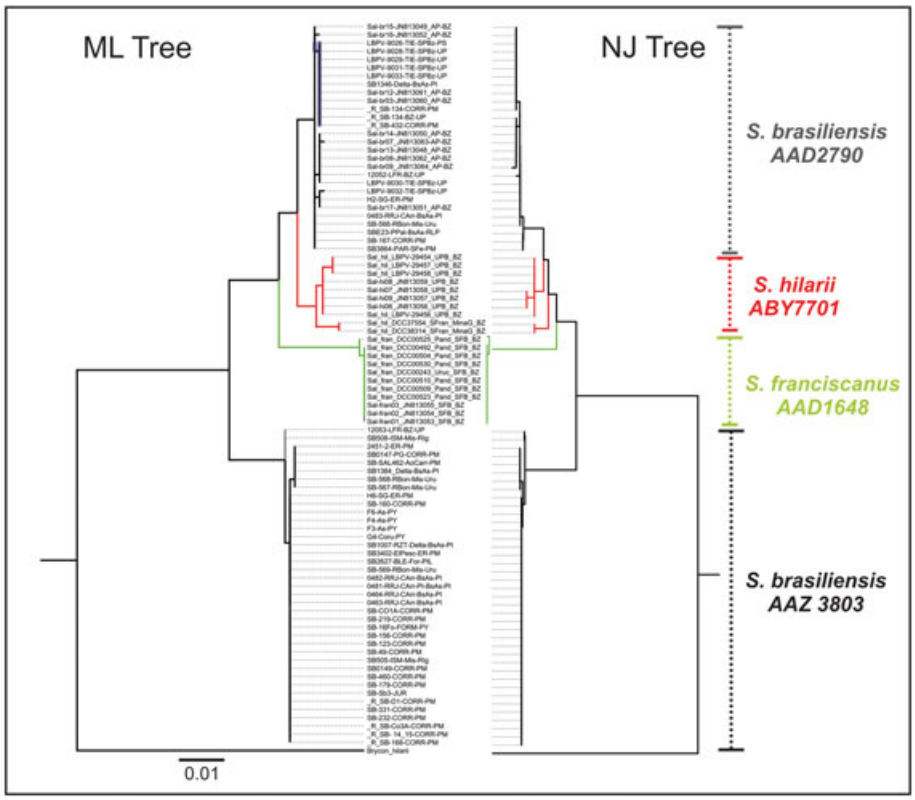

FIGURE 2 The K2P/NJ and ML trees displaying topologies for 89 sequences of *Salminus* from La Plata River basin. *Brycon hilarii* was used as an outgroup. Tree branches are highlighted following the BIN assigned to each cluster of sequences

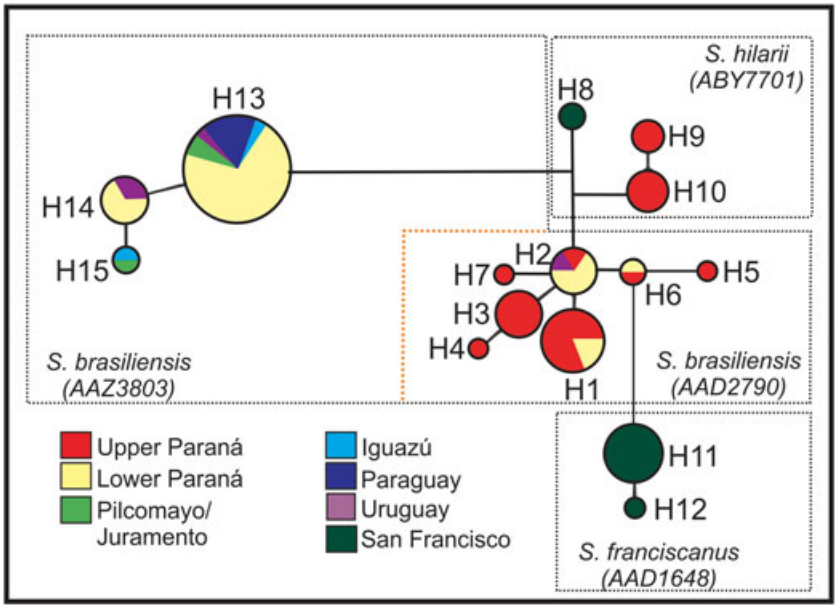

FIGURE 3 Haplotype network of COI sequences inferred for all species of *Salminus*. Size of the circle is proportional to the number of individuals for each haplotype and different colours represent different river basins. Dashed lines enclose haplotype partitioning within each BIN

frequent haplotype of BIN AAD2790 was H1, represented by 12 individuals of *S. brasiliensis*, nine from the Upper Paraná and three from the Lower Paraná basin. Haplotypes H3, H4, H5 and H7 were shown only by *S. brasilinesis* of BIN AAD2790 from the Upper Paraná basin. Individuals from the Lower Paraná were represented also in the haplotypes H2 and H6. Haplotype H2 was observed in one individual from the Uruguay River.

4 | DISCUSSION

4.1 | Taxonomy

Owing to the large genetic distance between the two mitochondrial lineages of *S. brasiliensis*, a further detailed taxonomic examination of specimens from both putative species is needed. Previous taxonomic

TABLE 2 Summary of genetic distances (K2P) for mitochondrial lineages of *Salminus* from the La Plata River basin. Distances to the nearest neighbour (NN) and barcoding gaps are also reported. BIN: Barcode index number

Species	BIN	Mean intra-Sp	Max intra-Sp	Nearest species	Distance to NN	Barcoding gap
Salminus franciscanus	AAD1648	0.000328	0.001976	AAD2790	0.045728	139.4146341463
Salminus cf. *brasiliensis*	AAD2790	0.002701	0.008350	ABY7701	0.016811	6.2239911144
Salminus cf. *brasiliensis*	AAZ3803	0.000768	0.005030	ABY7701	0.055221	71.90234375
Salminus hilarii	ABY7701	0.006625	0.015978	AAD2790	0.016811	2.537509434
	Species 1	**Species 2**	**Mean K2P**	**Min K2P**		
	AAD1648	AAD2790	0.049738	0.045731		
	AAD1648	AAZ3803	0.085880	0.077531		
	AAD2790	AAZ3803	0.070170	0.059231		
	AAD1648	ABY7701	0.058501	0.052991		
	AAD2790	ABY7701	0.021751	0.016811		
	AAZ3803	ABY7701	0.061729	0.055221		

examination of specimens from the lower La Plata basin did not find any conclusive evidence about the likely presence of two different species. In a detailed pioneer taxonomic study analysing specimens from the Paraguay, Paraná, Uruguay and La Plata rivers, Fuster de Plaza (1950) concluded that all individuals belonged to a unique nominal species, *Salminus maxillosus* (currently a junior synonym of *S. brasiliensis*). Later, Rosso et al. (2012) noted that specimens from the Pampa Plain shared meristic and morphometric characters with type material of *S. brasiliensis*, *S. maxillosus* and *S. orbignyanus*. The existing taxonomy in *Salminus* is not adequate to reflect the underlying genetic diversity observed within *S. brasiliensis*. In the light of recent genetic evidence (Machado et al., 2016), it is important to determine whether the likely cryptic species within *S. brasiliensis* ultimately represents a new taxonomic entity, or alternatively whether one of the former synonymized species must be resurrected. Since traditional morphological and meristic features are in many cases insufficient to delimit species, landmarks-based morphometric approaches have been successfully used to solve complex boundaries among sibling species (González-Castro, Rosso, Mabragaña, & Díaz de Astarloa, 2016). In addition, molecular evidence is especially important because it increases knowledge about the genetic diversity within the group (Vanlerberghe-Masutti, 1994). This knowledge is fundamental in conservation biology, since it constitutes the basis of organic variation and generally has a close correlation with the adaptation of species to the environment (Allendorf & Leary, 1986).

4.2 | Genetics of *Salminus* spp.

The large distance (7.01%) between both genetic entities within *S. brasiliensis* almost perfectly matched the mean intraspecific distance reported by previous authors (Machado et al., 2016; Rosso et al., 2012) for this species. The magnitude of genetic segregation between these lineages (8.11%) parallelled the average congeneric differentiation reported in a revision of barcoding in freshwater fishes (Ward, Hanner, & Hebert, 2009). Interestingly, the *S. brasiliensis* from the Upper Paraná River (BIN AAD2790, *S. brasiliensis* 2 in Machado et al., 2016) was more similar to *S. hilarii* than to the *S. brasiliensis* of the Paraguay, Uruguay and Lower Paraná rivers (BIN AAZ3803, *S. brasiliensis* 1 in Machado et al., 2016). Nonetheless, both the average (2.17%) and minimum (1.68%) genetic distances between them were well above the empirical threshold (1.1%) for the transition between intraspecific to interspecific distances calculated for the genus (Machado et al., 2016). High genetic divergence within a single Neotropical freshwater fish species is no longer a surprising result. For instance, it has been shown that 14 species displayed high intraspecific genetic divergence (>2%), pointing to at least 23 strong candidates for new species (Pereira, Hanner, Foresti, & Oliveira, 2013). Indeed, cryptic diversity seems to be the rule rather than the exception in several fishes of the Neotropical region. Recently, cryptic diversity doubling the original number of valid species was detected within the genus *Curimatopsis* (Melo, Ochoa, Vari, & Oliveira, 2016). The *Hoplias malabaricus* species complex has been receiving particular attention during recent years (Mattox, Bifi, & Oyakawa, 2014) and two new species (Azpelicueta, Benítez, Aichino, & Mendez, 2015; Rosso et al., 2016) have been formally described recently. Cryptic diversity in the freshwater pufferfish genus *Colomesus* (Amaral, Brito, Silva, & Carvalho, 2013), and in pencilfishes of the genus *Nannostomus* (Benzaquem, Oliveira, da Silva Batista, Zuanon, & Porto, 2015) have also been revealed. The first molecular phylogeny of Chilodontidae also unveiled cryptic biodiversity (Melo, Sidlauskas, Hoekzema, Vari, & Oliveira, 2014). The small characid *Piabina argentea* (Pereira, Pazian, Hanner, Foresti, & Oliveira, 2011), the cichlid *Crenicichla mandelburgeri* (Piálek, Rícan, Casciotta, Almirón, & Zrzavy, 2012) and the two spot *Astyanax bimaculatus* (Rossini et al., 2016) also contain hidden diversity.

If two populations have significantly different allele frequencies, they are expected to be demographically independent, meaning that they have differences in demographic parameters such as age structure, fecundity, survivorship, growth rate and perhaps sex ratio (Helfman, Collette, Facey, & Bowen, 2009). Haplotype diversity analysis showed that further partitioning of genetic structure is present within each BIN of *S. brasiliensis*. Therefore, in addition to the widespread distribution of two disparate genetic entities within *S. brasiliensis*, these results also highlight the existence of likely demographically distinct populations. Although haplotypes H13 and H1 were the most frequent within each respective BIN, it seems that H2 could be the most ancestral because of the high number of branches which also connect either *S. hilarii* or *S. franciscanus*. Two unshared

TABLE 3 Data matrix of polymorphic sites derived from the original 669-bp-alignment of 89 mtDNA COI sequences of *Salminus* spp. Dots (.) denote the nucleotide identical to that of reference sequences (haplotype 1). The top three rows of numbers represent the concrete polymorphic positions and should be read from up to down. H1-H15: Haplotypes

	117	147	156	159	168	192	196	198	204	213	216	219	222	225	240	252	258	294	312	327	330	333	342	345	354	363	381	408	414	420	423	426	438	441	444	447	453	456	462	474	477	483	489	492	501	502	504	507	510	513	516	522	528	534	537	540	549
H 1	T	T	G	G	C	G	T	A	T	A	T	A	T	T	C	A	A	G	A	G	C	A	A	A	C	A	C	T	G	A	A	C	T	T	G	T	C	A	C	T	G	G	T	A	G	G	G	A	A	T	A	C	G	T	C	G	A
H 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.
H 6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.
H 7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.
H 8	.	.	.	A	.	.	C	G	.	.	.	.	.	.	T	.	.	.	.	.	.	C	.	.	.	.	T	.	.	G	G	.	.	.	.	.	.	G	.	.	.	.	.	.	A	G	.	C	.	.	.	.	.	.	G
H 9	.	.	.	A	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	C	.	.	.	.	T	.	.	.	G	.	.	.	.	.	.	G	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	G
H 10	.	.	.	A	.	.	.	.	C	.	.	.	.	.	T	.	.	T	.	.	.	C	.	.	.	.	T	.	.	.	G	.	.	.	.	.	.	G	.	.	.	.	.	.	.	G	.	.	.	T	.	.	.	.	G
H 11	C	.	A	A	T	.	C	G	.	.	C	G	C	C	.	.	G	.	G	.	.	C	G	.	.	G	.	.	.	.	G	.	C	.	A	C	.	.	.	.	.	.	G	A	.	.	.	G	.	G	.	.	.	.	.	.	.
H 12	C	.	A	A	T	.	C	G	.	.	C	G	C	C	.	.	G	.	G	.	.	C	G	.	.	G	.	.	.	.	G	.	C	.	A	C	.	.	.	.	.	.	G	A	.	.	G	G	.	G	.	.	.	.	.	.	.
H 13	.	C	.	A	.	A	C	G	.	T	C	.	.	.	T	.	.	.	.	.	.	T	C	.	G	T	G	T	.	A	.	G	T	.	C	.	.	T	.	A	.	A	A	C	G	.	.	.	G	.	.	.	.	A	C	T	A	G
H 14	.	C	.	A	.	A	C	G	.	T	C	.	.	.	T	.	.	.	.	.	.	T	C	.	G	T	G	T	.	A	.	G	T	.	C	.	.	T	.	A	C	A	A	C	G	.	.	.	G	.	.	.	.	A	C	T	A	G
H 15	.	C	.	A	.	A	C	G	.	T	C	.	.	.	.	.	.	.	.	.	.	T	C	.	G	T	G	T	.	A	.	G	T	.	C	.	.	T	.	A	.	A	A	C	G	.	.	.	G	.	.	.	.	A	C	T	A	G

haplotypes were recovered from *S. hilarii* for the Upper Paraná and Sao Francisco rivers. Both phylogenetic (Lima, 2006) and molecular (Abe, Mariguela, Avelino, Foresti, & Oliveira, 2014) approaches have already suggested that the two lineages may represent different biological entities. Conversely, the headwaters of the Sao Francisco River and two of its tributaries (the Pandeiro and Urucuria rivers) hosted two haplotypes for *S. franciscanus*, suggesting a genetic structure for this species at a lower level of hydrographic hierarchy than explored in this study.

Haplotype network analysis detected private haplotypes for samples of *S. brasiliensis* BIN AAD2790 from the Upper Paraná River basin. This section of the Paraná River at present is isolated from the Middle and Lower Paraná reaches by more than 40 reservoirs spread throughout the major river networks, such as the Grande, Parapanema, Paranaiba and Tiete rivers (Agostinho, Pelicice, & Gomes, 2008). It could be anticipated that downstream displacement of these unique haplotypes would be minimal since large reservoirs function as barriers to downstream movements (Pelicice, Pompeu, & Agostinho, 2015). For instance, spatial segregation caused by a dam where there are long periods with no efficient connection by fish passageways has led to fragmentation and interpopulational structuring of *S. hilarii* (Esguicero & Arcifa, 2010). Indeed, four different genetic lineages of *S. hilarii* are known in South America (Machado et al., 2016). Similarly, Ribeiro et al. (2016) also pointed out a strong pattern of genetic differentiation between populations of *S. brasiliensis* downstream and upstream of a natural barrier. The effects of artificial barriers on genetic structure of fish populations in the Neotropical region have been reported for migratory species other than *Salminus* (Garcez, Calcagnotto, & Almeida-Toledo, 2011; Sekine, Prioli, Prioli, & Júlio, 2002). In a recent perspective analysis, Winemiller et al. (2016) noted that without a more careful planning of projected dams, species extinctions and basin-wide declines in fisheries and other ecosystem services are certain to accompany new hydropower development in the world's mega-diverse tropical rivers.

4.3 | Implications for conservation and management

Historically, *Salminus brasiliensis* sustained several artisanal and recreational fisheries throughout South America. In Brazil, where it was once common, the species was only caught sporadically in rivers such as the Paranaíba, Grande, Tietê and Paranapanema (Carolsfeld, Harvey, Ross, & Baer, 2004). In Argentina, although commercial fishing for *S. brasiliensis* has been severely restricted, catches have been decreasing since the late 1940s throughout the Lower Paraná basin (Quirós, 1993). This had led to conflicts between sport and commercial fishermen, while the trophy size of *Salminus* steadily decreases (Sverlij & Espinach Ros, 1986) and sport fishing licences increase. For instance, licences for sport fishing almost doubled (33 622 to 53 515) during the last 8 years in Corrientes, Argentina (S. Sanchez, pers. com., from data provided by the Dirección de Recursos Naturales de Corrientes). Similarly, the recreational fishery for this species in the Juramento River (Salta, north-western Argentina) is gaining importance. A recent study indicates low mortality rate in *S. brasiliensis* after release when individuals are captured by fly-fishing methods (Gagne et al., 2017). As the individual recovery from catch and release angling can vary among species (Cooke & Suski, 2005), the particular response of each BIN of *S. brasiliensis* to angling deserves further research. Beyond these results, little is known about the current level of exploitation of *S. brasiliensis* in different reaches of major rivers of the La Plata River basin. Accordingly, the partitioning of such activity between both genetic lineages is also unknown. The correct species identification on which a fishery operates is a basic requirement for effective management.

The high degree of sympatry detected between the two entities of *S. brasiliensis* in the lower reaches of the La Plata River basin suggested that both putative species might eventually share feeding and nursery grounds. Whether both putative species manage to thrive isolated under such circumstances is a matter of future research. An alternative hypothesis is that reproduction of these entities is temporally segregated. Data from seven variable microsatellite loci showed that eels from across Europe do not, as traditionally thought, form a single panmictic population in the Sargasso Sea, possibly because migrating eels from different latitudes reproduce at different times (Wirth & Bernatchez, 2001). In *Salminus*, three different populations of *S. brasiliensis* that reproduce at different time periods in the Uruguay River in Brazil were recognized by means of 11 polymorphic microsatellite loci, resulting from an isolation-by-time mechanism (Ribolli et al., 2017). Whatever the segregation mechanism, knowledge of the genetic structure within each putative species is essential, as it allows priorities to be defined for management and conservation (Moritz, 1994). In particular, genetic studies are relevant in defining both Evolutionary Significant and Management Units. By definition, Evolutionary Significant Units (ESUs) should be reciprocally monophyletic for mtDNA alleles and show significant divergence of allele frequencies at nuclear loci. The two mitochondrial lineages detected in this study have been proved to be phylogenetically distinct and also showed a large genetic divergence. However, their divergence was not supported by a nuclear locus of low rate of nucleotide substitution (Machado et al., 2016) and therefore the ESU concept does not fully apply. In this respect, these BINs do not totally align with some species concepts (Wheeler & Meier, 2000). Conversely, the existence of private haplotypes within each BIN of *S. brasiliensis* for different river reaches in South America certainly should be considered as potentially different Management Units (MUs). Management units are the logical unit for monitoring since they are usually defined as demographically independent populations (Palsbøll, Berube, & Allendorf, 2007). However, true integration of management, population dynamics, and genetic theory must occur before genetic data can be fully used to define MUs (Taylor & Dizon, 1996). Indeed, defining MUs using genetic data that do not consider either the specific management objectives or the anthropogenic threats facing the populations being studied can easily result in a management failure by losing local populations (Taylor & Dizon, 1999).

Conservation or fishery management plans with no prior knowledge of genetic structure could result in the overexploitation or segmentation of populations, and consequently gene pools could be extirpated or genetic diversity within populations could be reduced (Laikre, Palm, & Ryman, 2005). Particularly in fisheries management, the importance of genetic criteria for maintaining biologically sustainable fisheries and stocking operations are well known (Ryman & Utter,

1987). Many programmes for population recovery include translocation of fishes from hatcheries without adequate concern about genetic diversity, sometimes resulting in displacement of native species (Rueda et al., 2017). For *S. brasiliensis*, it is highly probable that the breeding stocks of existing stocking programmes were obtained from natural populations of the region where both genetic lineages are sympatric. For that reason, the correct genetic characterization of specimens of *S. brasiliensis* used for culture and population recovery purposes should be a priority. Without this, individuals from different haplotypes may be crossed, resulting in hybrid fish, with the consequent introgression caused by backcrossing with wild fish. Evidence of crossbreeding among fertile hybrids that have escaped from fish farms or caused by stocking has been reported for many freshwater species, such as *Pseudoplatystoma* sp. (Hashimoto, Senhorini, Foresti, Martínez, & Porto-Foresti, 2014; Vaini, Grisolia, Prado, & Porto-Foresti, 2014) and *Odonthestes* sp. (Conte-Grand, Sommer, Ortí, & Cussac, 2015). As the perils of a careless stocking programme seem high, stock enhancement for conservation purposes should be considered only when factors limiting stock recovery have been removed or reduced, such as by improvements in water quality, habitat rehabilitation or removal of barriers to migration (Collares-Pereira & Cowx, 2004).

In addition to fisheries aspects, conservation actions must also consider the ecological importance of *S. brasiliensis*. This species is the top predator in the fish communities of the La Plata River basin. Top predators can alter the abundance and distribution of prey populations, and these predation effects have been shown to influence many aspects of community ecology (Paine, 1969). For example, sites occupied by top predators are in many cases consistently associated with high biodiversity (Sergio et al., 2008). Indeed, local loss of a top predator can trigger a cascade of secondary extinctions (Ebenman & Jonsson, 2005). These aspects may have strong implications for the conservation of *S. brasiliensis*. In particular, given a defined number of river reaches within a river network, the number of sites needed in a conservation plan to maintain high biodiversity might be lower when using sites occupied by *S. brasiliensis* than when using any other sites. The role of *S. brasiliensis* in promoting and maintaining biodiversity still needs to be tested as well as its position as a potential keystone species (Mills, Soulé, & Doak 1993). In this respect, functional response may vary between BINs and even among populations. Difference between BINs may be sustained by species-specific patterns in feeding and behaviour. Differences among populations may be sustained by private haplotypes that are restricted to particular river reaches and particular environmental conditions.

In summary, the results of this study suggest that the existence of two disparate putative species in *S. brasiliensis* hampers management and conservation actions, and calls for timely solutions. This study makes an important contribution by clarifying the distribution of these putative species within major river networks of the La Plata River basin. Data on occurrence of fish species together with environmental and landscape information are crucial elements commonly used to build probability-of-occurrence models (MacKenzie et al., 2006), which in turn leads to an estimate of the conservation value of aquatic ecosystems. These results, therefore, represent a first step towards planning the assessment of critical reaches along the La Plata River Basin for conservation of these putative distinct species. Meanwhile, managers need to be aware that they are probably dealing with two different species within *S. brasiliensis*. Special attention should be given to the design of stocking programmes in order to improve conservation actions while avoiding further adverse impacts such as a reduction of genetic diversity or outbreeding depression (Frankham, Ballou, & Briscoe, 2004; Laikre, 2010). Similarly, biologists and fishery scientists should consider these results in order to reduce the bias that more than one species would introduce to the analysis and management of *S. brasiliensis*. Moreover, once units for conservation (Moritz, 1994) are defined, the success of this approach relies upon whether the basic ecology of this species, including its habitat preferences and environmental tolerances are known (Cowx & Collares-Pereira, 2002). Once sufficient data have been acquired, adopting the 'do nothing' option would adversely affect populations of *S. brasiliensis*.

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SUPPORTING INFORMATION

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