PRIMARY RESEARCH PAPER

Elucidating a history of invasion: population genetics of pirarucu (*Arapaima gigas*, Actinopterygii, Arapaimidae) in the Madeira River

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Abstract The spread of non-native fish species is increasing globally and threatening aquatic ecosystems. Identifying the origins, the pathways, and vectors is crucial for managing biological invasions. We conducted a genetic characterization of *Arapaima gigas* from the Madeira River, considering native and non-native populations, to elucidate the invasion of the upper and middle Madeira River basin by *A. gigas*. We analyzed 9 microsatellite loci of 141 individuals from native (lower Madeira) and invasive (upper Madeira) populations, in addition to a locality from Peru outside the Madeira River basin drainage area, that previously was suggested to be one of the possible origins of the invasion. The results of discriminant analysis of principal components, Bayesian modeling of population structure, clustering and assignment tests (using microsatellite data from other Amazon locations) showed a clear separation between native and invasive populations and revealed a mixture of individuals from upper and middle Madeira and Peru. We confirm that the invasive population originates from Peru and was introduced by fish escapees from farms. Multiple secondary

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introductions may have advanced the invasion speed. Such a scenario represents a conservation paradox, because in its native habitat, *A. gigas* is endangered, whereas it has become invasive in non-native areas.

Keywords Introduced species · Invasion pathway · Microsatellites · Conservation paradox · Amazon

Introduction

Non-native species are species living outside their natural distributional range, as a result of human mediated introductions (Vitule et al., 2019). The increasing pressure on global biodiversity due to the increasing numbers of novel alien introductions, even in remote regions such as the Amazon (Doria et al., 2021), requires additional effort to prevent invasion success. This is especially true in the framework of Aichi target 9. '*By 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment*', which puts strong emphasis on the need to identify the major pathways taken by invasive populations (CBD, 1992; Hulme et al., 2018). The identification of pathways and vectors is crucial to better understand the genetic variability from original populations that can be key to explain or prevent invasion success (e.g., Chapple et al., 2012a, b; Carrete et al., 2012).

Human activities, especially those related to globalization and international trade, have facilitated and increased the spread of non-native species across the globe (Lockwood et al., 2005; Blackburn et al., 2011; Seebens et al., 2018; Vitule et al., 2019). In most cases, invasive species have a wide distribution and are not vulnerable to extinction in their natural range. However, scenarios exist where invaders are endangered within their natural range but well established and abundant in non-native areas; these are referred to as a biodiversity conservation paradox (Marchetti & Engstrom, 2016; Vellend, 2017). Often, this happens because species have been transported from their natural range and introduced in other regions around the world (Vellend, 2017), where there are niche opportunities (Blackburn et al., 2011; Lockwood et al., 2009; Ellis et al., 2012; Vellend et al., 2013). The pirarucu [*Arapaima gigas* (Schinz, 1822)] is an example of

this paradox while their native populations have been reduced and endangered due to fishing pressure, invasive populations have established and proliferated in non-native areas (Carvajal-Vallejos et al., 2011; Miranda-Chumacero et al., 2012; Doria et al., 2020; Marková et al., 2020).

The pirarucu is one of the largest freshwater fishes of the world (Nelson, 1994; Queiroz, 2000; Ferraris-Junior, 2003). The taxonomic status of the pirarucu is somewhat controversial. Stewart (2013a, b) suggested more than one species in the genus *Arapaima*, however, molecular studies do not find evidence of multiple species (Hrbek et al., 2005; Araripe et al., 2013; Farias et al., 2019; Torati et al., 2019). Therefore, we follow the traditional taxonomy; monotypic genus *Arapaima* with one species *Arapaima gigas*.

An apex predator, the pirarucu is preferentially not only piscivorous, but also feeds on insects, shrimps, and crabs when juvenile (Fontenele, 1952; Romero, 1960; Lüling, 1964). The variety of food items show that the species explores many components of the aquatic ecosystem, the adults being characterized as generalist piscivores. However, a recent study characterized the species as omnivorous (Watson et al., 2013), indicating its plasticity with respect to food resource use. Such traits, in combination with other life history strategies such as fast growth, nesting and parental care, are in general linked both to successful invaders and also invaders with high impacts, because they confer a per capita advantage, which is important to determine the impact of an invasive species on native communities (Parker et al., 1999; Lockwood et al., 2009; Bezerra et al., 2018). This shows that the pirarucu can be a voracious predator outside areas of its natural distribution and can disturb the local diversity when it occurs at large densities.

The natural distribution of *A. gigas* is in the floodplains of the Solimões-Amazonas and Essequibo river basins (Castello & Stewart, 2010; Castello et al., 2013), but it has also been introduced to aquatic systems in the north, northeast, and southeast of Brazil (Carvalho et al., 2015; Latini et al., 2016; Casimiro et al., 2018; Doria et al., 2020, 2021), as well as other countries such as Bolivia (Carvajal- Vallejos et al., 2011; Miranda-Chumacero et al., 2012; Van-Damme et al., 2015), India (Kumar et al., 2019) and Indonesia (Fadjar et al., 2019; Marková et al., 2020) as a consequence of aquaculture practices. The Madeira River basin represents a scenario of one of the most

successful *A. gigas* invasions (Carvajal-Vallejos et al., 2011; Coca Méndez et al., 2012; Miranda-Chumacero et al., 2012; Van-Damme et al., 2015; Carvajal-Vallejos et al., 2017; Lizarro et al., 2017; Doria et al., 2020). The species was introduced in the upper Madre de Dios basin by Peruvian initiatives, with the aim to introduce and repopulate it in environments considered favorable for its establishment (Carvajal-Vallejos et al., 2017). After its introduction in Bolivia, the species expanded its distribution, and is now found in the Beni and Mamoré basins (Lizarro et al., 2017; Carvajal-Vallejos et al., 2017). Currently, the invasion of pirarucu is also a reality in the Brazilian part of the basin, between the Santo Antônio Energia Hydroelectric Power Plant (UHE SAE) and the city of Guajará-Mirim (Doria et al., 2020; Catâneo, 2019; Doria & Souza, 2012). However, the origin, route and vectors of this invasion are uncertain, although testimonies from local fishermen suggest that these individuals originated from Peru, Bolivia and Brazil, having escaped from fish farms in these countries (Catâneo, 2019; Doria et al., 2020, 2021).

The pirarucu occurs naturally in the lower Madeira downstream of the now extinct Santo Antônio rapids—where the UHE SAE was built—which formed a natural barrier to colonization of the middle and upper Madeira River (Ferreira, 2013; Torrente-Vilara et al., 2011). The existence of invasive populations of *A. gigas* occurring so close to its natural distribution area and in the same hydrographic basin, raises questions about the native or invasive status of the population of the middle Madeira, mainly due to the lack of information about its origin and possible routes of invasion. Such difficulty in determining the origin of invasive species influences fishery management decisions adopted by local government agencies. *Arapaima gigas* is regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 1975) and the International Union for Conservation of Nature (IUCN, 1996), thus fishing of *A. gigas* was banned, including in the region of the middle Madeira, where the species is considered invasive. According to local fishermen, such a strategy increased the species abundance in the region, harming native species and fishery (Doria et al., 2020). Conserving native species and controlling invaders requires adequate management tools and information (Pyšek & Richardson, 2010; CBD, 1992). Determining the origin, and invasion pathways and

vectors of *A. gigas* populations in areas of new occurrence are essential to inform differentiated management strategies for native and invasive populations. In this context, the use of molecular tools has permitted significant advances, especially when combined with statistical methods (Kolbe et al., 2004; Colautti et al., 2005; Roman & Darling, 2007; Dlugosch & Parker, 2008; Reusch et al., 2010). In this study, we characterized native and invasive populations of *A. gigas* from the Madeira River basin, using microsatellite molecular markers to identify the origin, pathways and vectors of this invasion, and provide information that can be used for science-based management of this CITES II listed Amazonian species.

Materials and methods

Study area

The Madeira River Basin has an area of approximately 1,380,000 km^2 and extends throughout Brazil, Bolivia and Peru, draining the Andes Mountain ranges, the Brazilian Shield and the Amazonian floodplain via the Madre de Dios, Beni, Mamoré, Guaporé, Abunã and Madeira rivers (Guyot et al., 1999; Goulding et al., 2003). In addition to its considerable extension, the Madeira River Basin stands out for its flow, around 40 million liters of water per second in the rainy season, and also for the discharge of suspended solids, contributing 50% of the sediment load of the Amazon River (Sioli, 1968; Mortatti et al., 1989; Guyot et al., 1999; Latrubesse et al., 2005).

The sampling of the present study was done in the three stretches of the Madeira River basin (Fig. 1). The upper basin is cross-border and overlaps with Peruvian and Bolivian territory, and is drained by the Madre de Dios, Beni and Mamoré rivers; the middle Madeira includes the stretch between the confluence of the Beni and Mamoré rivers and Porto Velho city, characterized by the presence of 19 rapids, partly submerged by the Santo Antônio and Jirau dam reservoirs; and finally, the lower Madeira, which begins just downstream from the UHE SAE and extends to its mouth. The middle and lower stretches of the Madeira River, which is formed by the confluence of the Madre de Dios and Mamore Rivers, are in the Brazilian part of the basin.
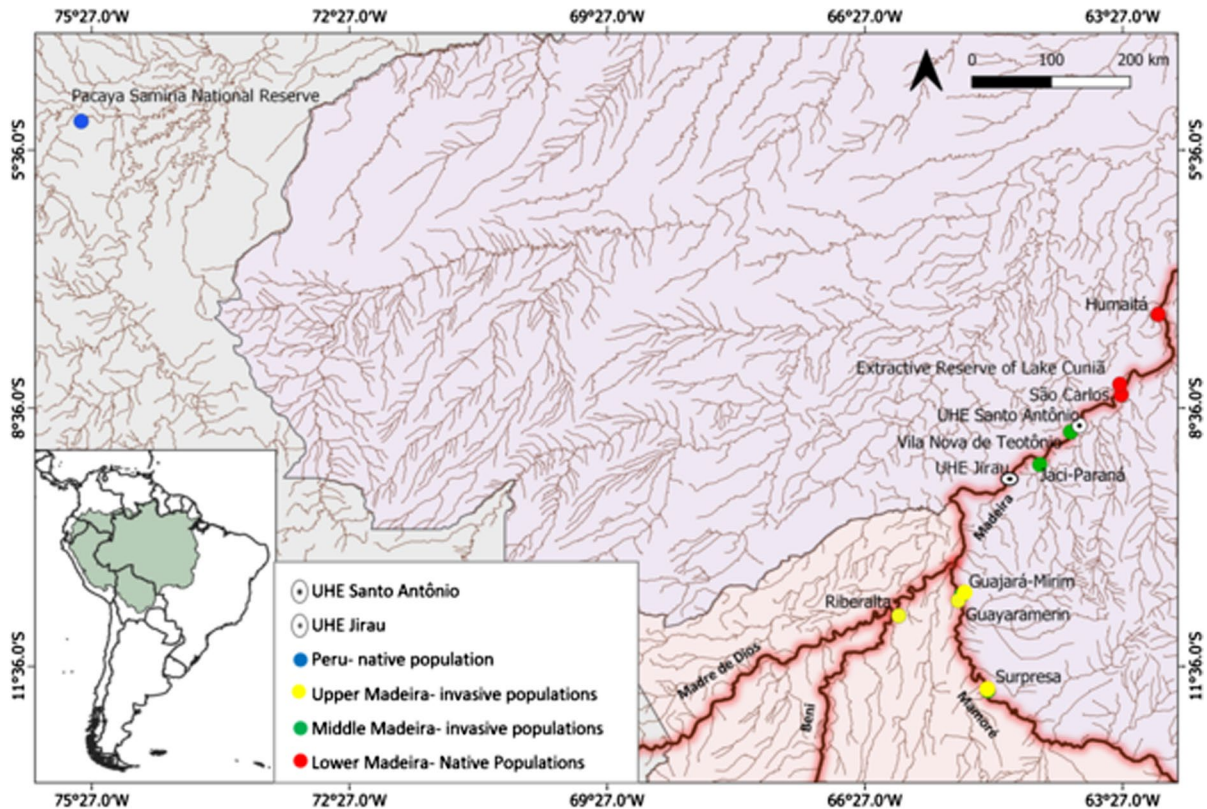
Fig. 1 Localities where *A. gigas* samples were collected for this study. Point colors indicate the areas where localities were grouped

Sampling

The tissue samples were collected with the support of fishermen, under authorization from SISBIO/ICM-BIO no. 60491-1, at fish landings and via expeditions to localities on the Madeira River where pirarucu occurrence is confirmed as native and invasive. We collected tissue samples from 141 individuals: 47 from upper Madeira, 49 from middle Madeira and 30 from lower Madeira, and 15 from Peru (Table 1). In the upper Madeira, we sampled two Bolivian locali-ties—Guayaramerín and Riberalta, and two Brazil-ian localities—Guajará-Mirim and Surpresa; in the middle Madeira, two localities from state of Ron-dônia were sampled—Jaci Paraná and Vila Nova de Teotônio—where the UHE SAE reservoir is located; and in the lower Madeira, where the species occur naturally, three locations were sampled—the Extrac-tive Reserve of Lago do Cuniã, where there is a man-agement plan for pirarucu, the district of São Carlos and Humaitá. We also sampled a locality in Peru,

the Pacaya Samiria National Reserve, which is out-side the drainage area of the Madeira River basin, but which was included in the study, because it is one of the possible areas of origins of the invasive pirarucu. Obtained samples were stored in 96% ethanol, kept in a freezer at −20 °C, and stored in the tissue collection of the Laboratory of Ichthyology and Fisheries of the Federal University of Rondônia, Brazil.

Genetic characterization

The mDNA was extracted using the cetyltrimethyl-ammonium bromide (CTAB) protocol described by Doyle and Doyle (1987). The extracted DNA was quantified using the NanoDrop 2000 spectrophotom-eter, and sample quality was observed in 1% agarose gel electrophoresis after being stained by GelRed®. We analyzed nine microsatellite loci – *CTm4, CTm5, CTm7, CAm2, CAm13, CAm15, CAm16, CAm20* and *CAm26,* developed by Farias et al. (2003).

Table 1 Localities of biological sample collections in each large sampling area, with the respective numbers of samples and geographic coordinates

Area	Locality	Number of samples	Coordinates (Long/lat)
Upper Madeira (invasive populations)	Riberalta	15	−66.076139/−10.995056
	Guayaramerim	10	−65.361360/−10.836760
	Guajará-Mirim	12	−65.348278/−10.792528
Middle Madeira (invasive populations)	Surpresa	10	−65.020963/−11.885942
	Vila Nova Teotônio	32	−64.062197/−8.861014
	Jaci Paraná	17	−64.395444/−9.260306
Lower Madeira (native populations)	Humaitá	6	−63.020028/−7.507889
	RESEX Cuniã	21	−63.489000/−8.319944
	São Carlos	3	−63.497303/−8.436014
Peru (native populations)	Pacaya Samiria	15	−74.96963/−5.31717
	Total	141	

Polymerase chain reactions were performed in a final volume of 15 µl, containing 6.2 µl ddH₂O, 0.48 mM DNTP, 1.5 µl 10X buffer (100 mM Tris–HCl, 500 mM KCl), 0.048 mM MgCl2, 1.5 µl reverse primer, 1.5 µl FAM or REX fluorescence-labeled forward primer, 0.4 units of Taq polymerase (1U/µl), and 0.5 µl bovine serum albumin (BSA). The reaction occurred under the following conditions: initial denaturation at 93 °C for 1 min, followed by 35 30-s cycles at 93 °C; primers pairing at 58 °C for 30 s, extension at 72 °C for 1 min, and a final extension of 72 °C for 30 min. Genotyping reactions were prepared in 1.0 µl diluted PCR product, 1.0 µl ROX size standard (DeWoody et al., 2004), and 8.0 µl formamide. The samples were genotyped in an ABI 3130XL (Applied Biosystems Inc.) automatic sequencer. Alleles size in base pairs were estimated using the GeneMapper™ v4.0 (Applied Biosystems Inc.).

Data analysis

Genetic diversity

Null alleles and possible genotyping errors were verified using Micro-Checker v2.2.3 (Van Oosterhout et al., 2004). The patterns of genetic diversity were characterized using observed and expected heterozygosities, number of alleles and allelic frequencies.

Population structure

The level of genetic differentiation among populations was estimated using the analysis of molecular variance (AMOVA—Excoffier et al., 1992). Additionally, pairwise *Fst* values and number of migrants (*Nm*) were estimated for all pairs of populations. All analyses were performed in Arlequin 3.5 (Excoffier & Lischer, 2010), and multiple comparisons were adjusted using the sequential Bonferroni correction (Rice, 1989).

To estimate the most likely number of biological populations in our dataset, we used the software Structure (Pritchard et al., 2000) and implemented the admixture model, which permits each individual to have ancestors from more than one population (Falush et al., 2003). Ten independent runs were done for each K of populations (K=1–10), each run consisting of 1,000,000 Markov Chain Monte Carlo (MCMC) simulations, with burn-in of 100,000 samples. Simulation results were extracted using Structure Harvester 0.6.92 (Earl & Vonholdt, 2012) and summarized in Clumpp 1.1.2 (Jakobsson & Rosenberg, 2007). The most likely number of population groups (K) was inferred using the method of Evanno et al. (2005) and confirmed through the posterior probability plot (log likelihood). The barplots of the best were made in Distruct 1.1 (Rosenberg, 2004).

We ran discriminant analysis of principal components (DAPC—Jombart & Collins, 2015) using the package adegenet v2.1.1 in R v4.0.4 (Jombart, 2008)

to quantify the discriminatory power of the predefined sampling groups. We also assessed the assignment probabilities of each individual to these *a priory* defined groups. Allelic frequency data were also used to generate a population cluster tree using the Web version of the Poptreew software (http://www.med.kagawa-u.ac.jp/~genomelb/takezaki/poptreew/) (Takezaki et al., 2014). The cluster tree was inferred by the Neighbor-Joining distance method (Saitou & Nei, 1987) and its statistical support was assessed by 1000 bootstrap replicates (Felsenstein, 1985).

Finally, we used Geneclass (Piry et al., 2004) to perform an assignment test, which based on the individual genotype and the allelic profiles of each population, identifies first-generation migrants by a Bayesian-based method described by Rannala & Mountain (1997) and a frequency-based method described by Paetkau et al. (1995). To expand the sampled area and the possibility of identifying the origins of the pirarucu in the Madeira basin, this analysis used microsatellite data from other populations of the southwestern portion of the Amazon basin, obtained from Farias et al. (2019). Included locations were Peru, Letícia, middle and upper Juruá, RDS Mamirauá, Coari, RDS Purus, Macapá, upper Purus (Manuel Urbano), Manacapuru, RESEX Unini, Manaus, and Madeira (Borba region).

Results

Genetic diversity of populations

The number of alleles (Na) per locus ranged from 3 (CAm26) to 11 (CAm13), totaling 56 alleles in *A. gigas* populations of the Madeira River Basin. When considering all populations as one—representing the Madeira River Basin population—observed heterozygosity (Ho) values ranged from 0.10 (CAm20) to 0.81 (CAm13) and the expected heterozygosity (He) from 0.15 (CAm26) to 0.85 (CAm13).

As for sampled areas, the CAm13 locus presented the highest values of Ho (0.87, Bolívia) and He (0.85, Peru) (Table S1). The CAm20 locus has the lowest value of Ho (0.07) and He (0.07) of the Peru population. Regarding upper Madeira, CAm26 was monomorphic and CAm20 showed the lowest value of Ho (0.04) and He (0.30). The lowest Ho and He values in the middle Madeira (0.01) were that of CAm20 and CAm26, whereas in the lower Madeira, it was that of CTm4 (Ho = 0.33; He = 0.28) (Online Resource 1). CAm20 was the only locus that showed Hardy–Weinberg disequilibrium in three populations studied, the other loci were in equilibrium. Including or excluding these loci in the analyses had no effect on the results, however, and therefore we opted to maintain them.

Considering all analyzed loci, genetic diversity by sampling area ranged from 0.443691 (middle Madeira) to 0.512116 (lower Madeira). The mean number of alleles ranged from 3.44 in populations from upper and lower Madeira to 4.667 in the middle Madeira. The inbreeding coefficient (F_{IS}) ranged from 0.03141 in the lower Madeira population to 0.10928 in the upper Madeira population. Expected heterozygosity (He) ranged from 0.43814 in the middle Madeira population to 0.50877 in the lower Madeira population (Table 2).

Population structure

AMOVA showed 27.62% of variance among populations, 4.12% among individuals within populations, and 68.26% within individuals ($Fst = 0.27620$, $P < 0.001$). Pairwise Fst showed no differentiation between and upper populations ($Fst = 0.07$, $P > 0.05$)

Table 2 Genetic characteristics of *A. gigas* populations in the areas of the sampled areas

Analyzed areas	Average genetic diversity	Na	Ho–He	Fis
Peru	0.475862 ± 0.269824	4.333	0.50370–0.47586	−0.06072*
Upper Madeira	0.448073 ± 0.252515	3.444	0.45437–0.50267	0.10928
Middle Madeira	0.443691 ± 0.247088	4.667	0.40987–0.43814	0.07752
Lower Madeira	0.512116 ± 0.282694	3.444	0.49770–0.50877	0.03141*

Na numbers of alleles, *Ho* observed heterozygosity, *He*: expected heterozygosity

*Indicates significant *P* value (*P* < 0.05)

and high geneflow ($Nm = 6.43$). As for upper Madeira x Peru ($Fst = 0.16$ and $Nm = 2.55$) and middle Madeira x Peru ($Fst = 0.20$ and $Nm = 1.94$), pairwise comparison indicated moderate differentiation. Conversely, we verified strong genetic differentiation between lower Madeira and the other populations, with high Fst and $Nm < 1$ per generation for Peru ($Fst = 0.36$ and $Nm = 0.88$), upper Madeira ($Fst = 0.36$ and $Nm = 0.90$) and middle Madeira ($Fst = 0.38$ and $Nm = 0.81$) (Table 3).

Analyses conducted in the program Structure identified two biological populations (K = 2, Fig. 2a). At K = 3 presents an interesting scenario of the species invasion history in the upper Madeira, whereby the population of Peru differs from that of upper and middle Madeira (in blue); this may be the consequence of being introduced many generations ago, and also because it potentially is an admixed population. The Structure graph also shows two populations within upper Madeira, where Riberalta (Beni River) individuals are grouped with those of Peru, and Guayaramerín, Guajará-Mirim and Surpresa (Mamoré River) individuals with those of the middle Madeira.

The DAPC revealed admixture between individuals from middle and upper Madeira, being partly mixed with those from Peru, in addition to showing the separation of lower Madeira individuals in relation to other areas (Fig. 3).

The Neighbor-Joining dendrogram showed that upper + middle and lower Madeira populations form separate groups (Fig. 4), where the upper + middle Madeira populations cluster more closely with those from Peru when compared to other populations from the central-western region of the Amazon basin.

Similarly, the Geneclass assignment test showed that upper + middle Madeira populations are highly likely to be derived from those from Peru. The test also showed more similarity between individuals from the middle Madeira and those of Mamirauá, Manacapuru, Letícia, and Coari (Online Resource 2).

Discussion

The native and invasive populations of pirarucu from the Madeira basin showed low genetic diversity. This has also been observed in other studied populations of the pirarucu and is generally attributed to bottlenecks related to the historic overfishing history of the species, which significantly reduced its native populations, including those of the lower Madeira (Hrbek et al., 2005; Araripe et al., 2013; Vitorino et al., 2015, 2017; Farias et al., 2019; Fazzi-Gomes et al., 2017). However, the low genetic diversity of the invasive populations can also be the result of founder events, since they established themselves from small groups of individuals introduced in this area. Reduced genetic diversity is disadvantageous in the invasion process, as it reduces the evolutionary potential to respond to new environmental pressures, impairing colonization (Keller & Waller, 2002; Allendorf & Lundquist, 2003; Schrieber & Lachmuth, 2017). In the Madeira River, the low genetic diversity of invasive populations of pirarucu was not enough to prevent the success of its establishment, which refers to the genetic paradox of invasions (Allendorf & Lundquist, 2003). Various authors proposed mechanisms allowing introduced populations mitigate the effects of low genetic diversity and establish themselves successfully (Colautti et al., 2005; Kelly et al., 2006; Perez et al., 2006; Roman & Darling, 2007; Dlugosch & Parker, 2008; Schrieber & Lachmuth, 2017). In the case of the pirarucu invasion in the Madeira River basin, the interaction between the characteristics of

Table 3 Number of migrants- *Nm* (diagonal below) and genetic differentiation between populations- *Fst* (diagonal above), obtained with microsatellite markers between sampled areas

Nm/Fst	Peru	Upper Madeira	Middle Madeira	Lower Madeira
Peru	0.00	0.16**	0.20**	0.36**
Upper Madeira	2.55	0.00	0.07**	0.36**
Middle Madeira	1.94	6.43	0.00	0.38
Lower Madeira	0.88	0.90	0.81	0.00

*Indicates significant *P* value ($P < 0.05$)

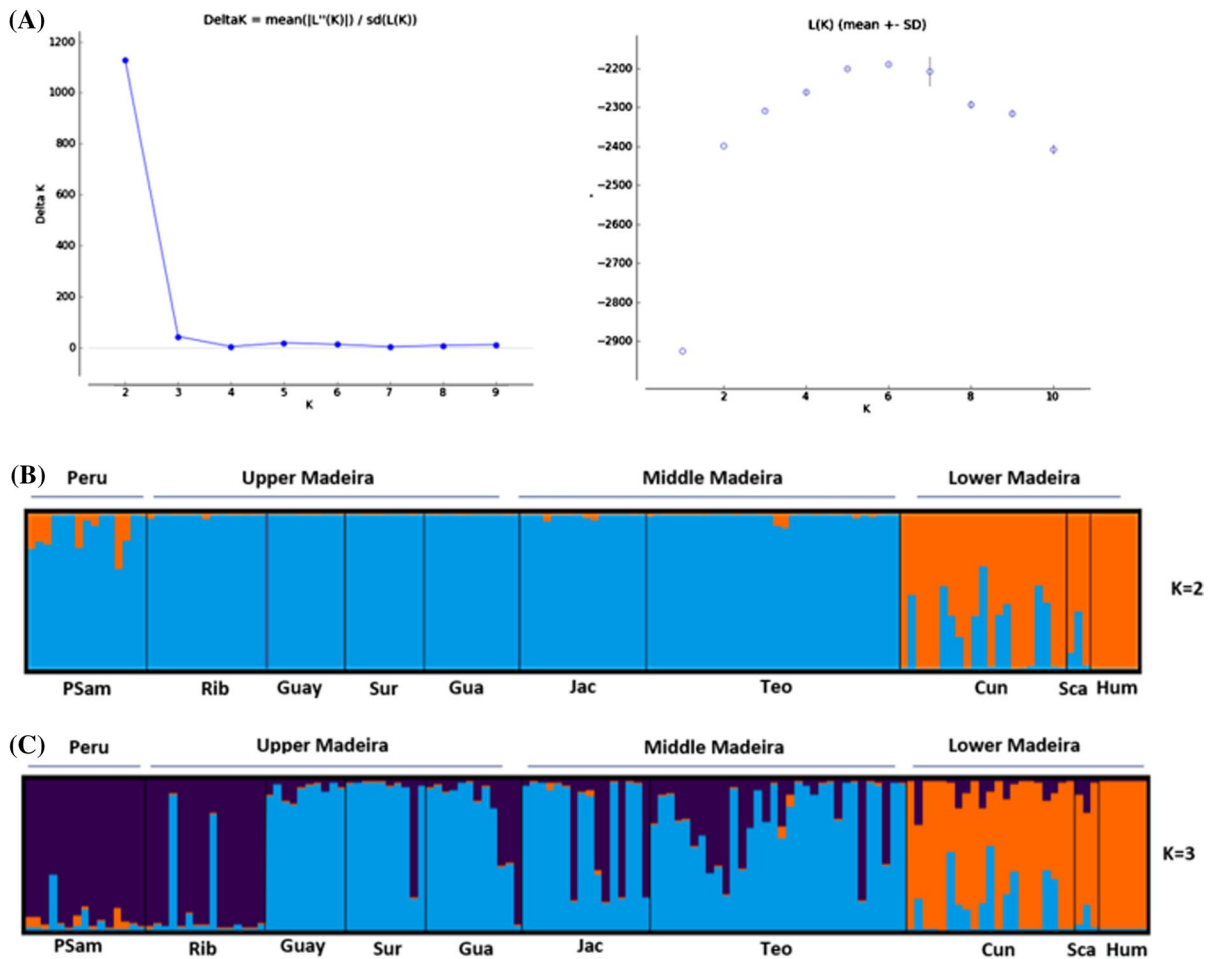**Indicates significant *P* value after Bonferroni ($P < 0.0125$ correction)

Fig. 2 Bayesian analysis of population structure using the S*tructure* software, based on the allelic frequencies of the nine microsatellite loci analyzed. **A** The best Delta K value (=2) and the mean likelihood Ln (K). **B** Population structure information K=2. Each vertical line represents an individual and colors represent the biological groups: Blue—PSam: Pacaya– Samiria National Reserve, Rib: Riberalta, Guay: Guayarame-rín, Sur: Surpresa, Gua: Guajará-Mirim, Jac: Jaci Paraná, Teo: Vila Nova Teotônio. Orange—Cun: Cuniã Lake Resex, SCa: São Carlos, and Hum: Humaitá—AM; **C** Population structure graph considering K=3

the species, environmental changes caused by the implementation of two hydroelectric plants and recurrent introductions may be the main factors related to the success of the invasion.

The results of Structure and DAPC analyses as well as the analyses of mitochondrial DNA in Hrbek et al. (2005) corroborate the colonization history of the upper Madeira basin in the Bolivian Amazon (Miranda-Chumacero et al., 2012; Catâneo, 2019; Doria et al., 2020), which proposes that the pirarucu (paiche in Bolivia) was introduced in the basin in the 1970s from the upper Amazon basin of Peru. Specifically, fishes escaped from aquaculture stations in

the upper Madre de Dios River in Peru and spread to Bolivia. Once the species has become established and expanded its distribution in the Madre de Dios and then the Beni aquatic systems, individuals eventually would have reached the middle Madeira River and the Mamoré and Guaporé rivers. This expansion of the distribution could have been favored by seasonal floods, mainly by the atypical flood of 2014 that connected many aquatic systems throughout the basin, as suggested by Carvajal-Vallejos et al. (2017) and Doria et al. (2020). We expected that individuals from the middle Madeira would be more closely related to individuals from Bolivia (upper Madeira) and Peru.
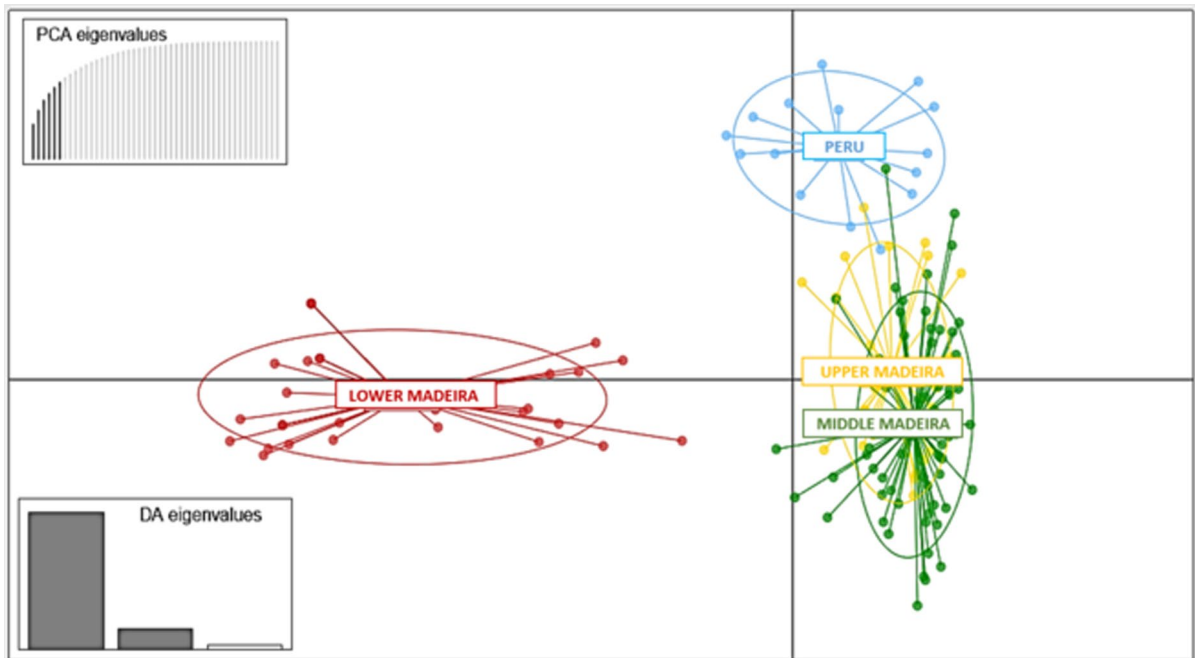
Fig. 3 Discriminant Analysis of Principal Components (DAPC). Each small colored circle represents an individual; each color represents a large area determined a priori: Blue—Peru, Yellow—upper M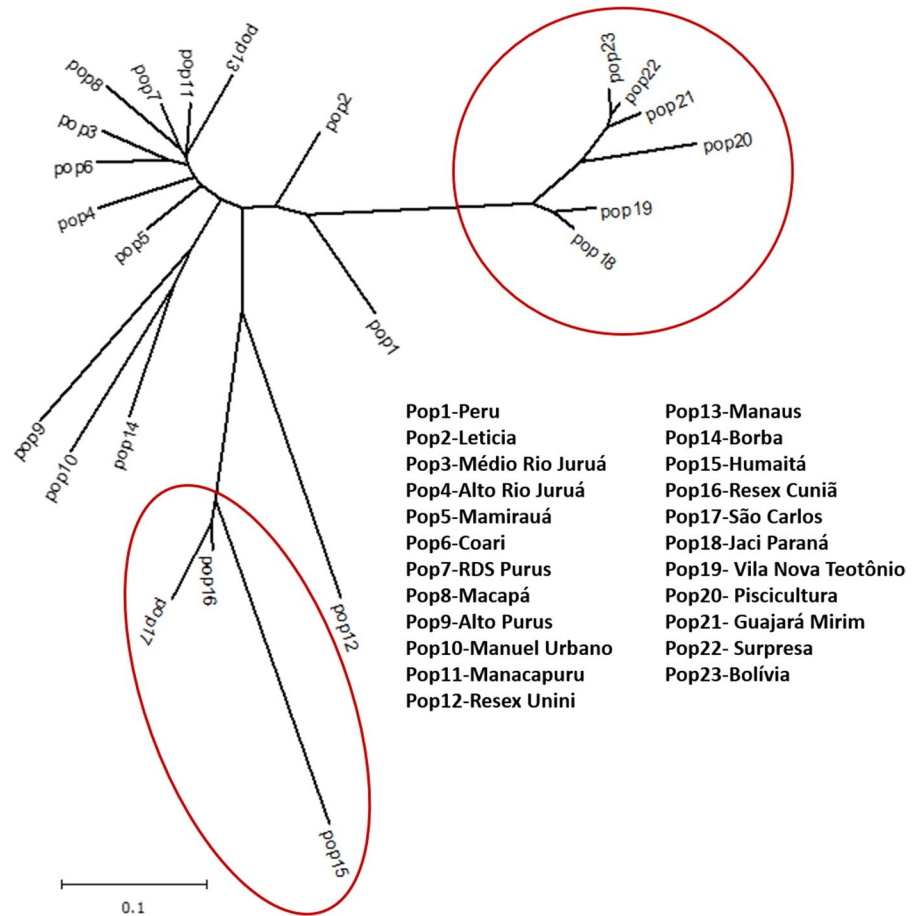adeira, Green—middle Madeira, and Red—lower Madeira. The number of principal components used for the analysis is at the upper left corner, and the number of discriminating factors is at the lower right corner

Our results showed a high number of migrants (Nm) and low genetic differentiation among populations (Fst) from the lower Madeira and upper Madeira and also Peru. Gene flow homogenizes allelic frequencies (Balloux & Lugon-Moulin, 2002; Oliveira et al., 2006), justifying the inclusion of the individuals of these localities within the same biological population in the Bayesian analysis of population structure (Fig. 2b, k=2), as well as the results found in the DAPC (Fig. 3).

Another hypothesis about the origin of the pirarucu from the middle Madeira is that it resulted from more recent escapes from fish farms in Rondônia and Bolivia, given that fingerling trade is a common practice across these regions (Carvajal-Vallejos et al., 2011, 2017; Van-Damme et al., 2015; Doria et al., 2020). This second hypothesis is supported by the assignment test results (Table, A2) and justifies the observed structuring identified in Bolivia, with one group in the Madre de Dios and Beni systems, and the other in the Mamore and Guapore systems and with individuals from the middle Madeira showing certain degree of mixture between these two groups (Fig. 2c).

Obtaining individuals from different regions to establish a captive stock is a common practice in Amazonian aquaculture (Oliveira et al., 2018), as the mixture of different population gene pools increases genetic variability (Kolbe et al., 2004) and consequently individuals' resistance to parasites and other diseases. When compared to other pirarucu populations of the Amazon basin, individuals from middle and upper Madeira areas were most likely to have primarily been derived from populations from the upper Amazon basin of Peru with additional input from geographically more distant areas, such as Manacapuru, Coari, Mamirauá, areas from which aquaculture individuals are commonly sourced. Aquaculture is one of the main vectors of species introduction in Brazil and the world (Orsi & Agostinho, 1999; Casal, 2006; Attayde et al., 2007; Britton & Orsi, 2012; Ortega et al., 2015). In the state of Rondônia, the number of fish farms is growing, and pirarucu is among the most cultivated species (Soares et al., 2020). Since few of these fish farms have adequate management systems, the risk of individuals escaping is inherent, and some fish farmers even admit the escape of aquacultured

Fig. 4 Clustering by the Neighbor-Joining method with Madeira River populations, grouped into distinct branches circled in red, and other populations of the central-western portion of the Amazon basin

Pop1-Peru
Pop2-Leticia
Pop3-Médio Rio Juruá
Pop4-Alto Rio Juruá
Pop5-Mamirauá
Pop6-Coari
Pop7-RDS Purus
Pop8-Macapá
Pop9-Alto Purus
Pop10-Manuel Urbano
Pop11-Manacapuru
Pop12-Resex Unini

Pop13-Manaus
Pop14-Borba
Pop15-Humaitá
Pop16-Resex Cuniã
Pop17-São Carlos
Pop18-Jaci Paraná
Pop19- Vila Nova Teotônio
Pop20- Piscicultura
Pop21- Guajará Mirim
Pop22- Surpresa
Pop23-Bolívia

species to the environment (Soares et al., 2020). Other records of *A. gigas* in areas outside its native distribution attributed to fish farm escapes were documented throughout diverse regions of the globe such as India (Kumar et al., 2019) and Indonesia (Fadjar et al., 2019; Marková et al., 2020).

The present data, confirmed by information obtained from local fishers (Doria et al., 2020), suggest that *A. gigas* populations from the middle Madeira were formed by more than one introduction event, probably from closely related founders. Multiple introductions favor invasion success, because they increase the number of individuals and, when coming from different sources, they can increase genetic diversity, making founder effects less pervasive (Simon-Bouhet et al., 2006; Roman & Darling, 2007; Crawford & Whitney, 2010; Barker et al., 2017). In this context, information on the propagules is equally important to understand the success of this invasion, as the propagule size, the

propagule number and temporal and spatial patterns of propagule arrival, i.e., the propagule pressure, can increase the probability for an introduced population to establish (Lockwood et al., 2005; Blackburn et al., 2009; Simberloff, 2009). Although we did not find high genetic diversity in the population of the middle Madeira, as expected in cases of multiple introductions and propagule pressure, it is possible that these factors contributed to maintaining the minimum viable population size (Reed et al., 2003; Blackburn et al., 2009). However, it is important to consider that other factors also favor invasion success, such as the absence of predators, competitors, pathogens, biotic and abiotic factors (Mitchell & Power, 2003; Latini et al., 2004; Callaway & Maron, 2006; Broennimann et al., 2007), as well as resource rich environment and the ability of the invader to either respond via phenotypic plasticity or adaptations to the new environment (Kowarik, 1995; Dietz & Edwards, 2006).

The scenario of the pirarucu represents a unique biodiversity conservation paradox (Vellend, 2017; Marková et al., 2020), whereby invasive and native populations of an endangered species inhabit the same watershed (CITES II; IUCN, 1996). Based on the history of decline in natural *A. gigas* populations arising from overfishing, authorities strive to protect and preserve wild stocks of this species throughout the Amazon River basin (Coca-Méndez et al., 2012; Castello et al., 2013; Oberdorff et al., 2015). However, considering that the pirarucu is an apex predator, this species represents a potential threat to native ichthyofauna is an invader and should be eradicated. This study reinforces the importance of confirming invasions and the need to implement appropriate management of native and invasive populations; otherwise, the damage could be irreversible. In this sense, adopting measures that reduce the impacts of this invasion in the upper and middle Madeira basin and developing public policies favorable to the conservation of the native biodiversity are key to avoiding new introductions and minimizing additional impacts. Brazil is a signatory of the Convention on Biological Diversity, whose eighth article dictates that each party must "prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species." Thus, fishing is considered an effective measure for managing aquatic invasive species, as it reduces the number of invasive individuals while allowing other species to be conserved (Weidel et al., 2007; Britton et al., 2009; Connelly et al., 2016; Santos et al., 2019). Based on this premise, our study results were presented to fisheries management organizations, suggesting the authorization of *A. gigas* fishing in the area of invasion. The organizations complied with the recommendations, allowing fishing in the UHE SAE upstream region, where the species is invasive (Normative Instruction No. 2 of May 10, 2019)—a good example of how science may contribute to fisheries management strategies in the Amazon region. In addition, the knowledge of the genetic diversity of the invasive population, the identification of invasion pathways and vectors constitute an important contribution to the knowledge about biological invasions in the Amazon, where records are scarce, but reveal advancement of a silent threat, especially to aquatic ecosystems (Doria et al., 2021).

Conclusion

Invasion science and biodiversity conservation need to consider the substantial impact of intra-country and intra-basin introductions (e.g., in different and isolated parts of the basin). Finer-scale data about the distribution and origin of invasive populations will enhance our policy advocacy and management ability. Our study adds important contributions to the existing knowledge about the genetic diversity of pirarucu from the Madeira River and determined the occurrence of both native and invasive populations in the basin, configured as a biodiversity conservation paradox. Such invasions enabled by fish farm escapes, invasive species expansion from Peru, and significant environmental changes, highlight the threat these practices constitute for local biodiversity and the urgent need for measures to control and minimize the impacts of these invasions.

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