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Looking for a home in foreign waters: population genetic structure of the introduced *Arapaima gigas* in Bolivia

Buscando un hogar en aguas foráneas: Estructura genética poblacional de *Arapaima gigas* introducido en Bolivia

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ABSTRACT

Establishment of invasive aquatic species is increasing globally due to factors related to globalization and accelerated trade between regions. Such invasions and subsequent establishment generally cause ecosystem disturbance with occasional local and/or regional socioeconomic impacts. The paiche (tentatively identified as *Arapaima gigas*), one of the largest fish in the Amazon, was introduced into Bolivia via Peru in the 1960s and has generated significant changes in Amazonian fisheries. In recent years, it has been proposed that the genus *Arapaima* is composed of different species distributed along the Amazon Basin. The present study evaluated the genetic variability of the paiche in the Bolivian Amazon Basin (sub-basins of the Orthon, Madre de Dios and Beni rivers) using nuclear (nDNA-microsatellites) and mitochondrial (mtDNA NADH and CO1) genetic markers to determine species identity and population structure. Microsatellite DNA analysis suggested that the three populations corresponding to geographic sub-basins are genetically distinct. The genetic distance between populations was not significantly related to the geographic distance between collection sites. We suggest that the

founder population in Bolivia was composed of a limited number of individuals that subsequently dispersed in search of environmental conditions similar as those habitats from which they were extracted. Planning for the sustainable use of the species by fisheries should consider the existence of different populations in the Bolivian sub-basins. Recruitment seems to depend on exchanges between nearby surrounding aquatic habitats rather than between sub-basins

Keywords: Invasive fish species, Osteoglossiformes, CO1, ATPase, microsatellites, Bolivian Amazon Basin, Madeira River basin.

RESUMEN

La introducción e invasión de especies acuáticas no-nativas está incrementando a nivel global debido a factores relacionados a la globalización, y el intercambio comercial acelerado entre regiones. Por lo general, las introducciones causan perturbaciones ecosistémicas, con subsecuentes impactos socioeconómicos locales y/o regionales. El paiche (tentativamente identificado como *Arapaima gigas*), uno de los peces más grandes del Amazonas, fue introducido a través del Perú en Bolivia en los años sesenta, y ha generado cambios significativos en las pesquerías y cadenas de valor de pescado amazónico. En años recientes, se ha propuesto que *Arapaima* se compone por diferentes especies distribuidas en la Cuenca del Amazonas. El presente estudio evaluó la variabilidad genética del paiche en la Cuenca del Amazonas en Bolivia (subcuencas de los ríos Orthon, Madre de Dios y Beni) para conocer su identidad (NADH y CO1 - DNAmt) y su estructura poblacional (microsatélites - DNAn). Los resultados de microsatélites sugieren que las tres poblaciones correspondientes a las subcuencas geográficas son genéticamente distintas. La distancia genética entre poblaciones no se relacionó significativamente con la distancia geográfica entre sitios de colecta. Se propone que el conjunto de individuos de la población fundadora en Bolivia estuvo constituido por un número limitado de individuos que subsecuentemente se dispersaron en búsqueda de condiciones ambientales similares a las presentes en los hábitats de donde fueron extraídos. La planificación del aprovechamiento sostenible de la especie por las pesquerías debe considerar que existen diferentes poblaciones en las subcuencas de Bolivia, y el reclutamiento parece depender de los intercambios entre hábitats cercanos circundantes más que entre subcuencas.

Palabras clave: Pez invasor, Osteoglossiformes, CO1, ATPase, microsatellites, Cuenca del Amazonas en Bolivia, Cuenca del río Madera.

INTRODUCTION

Human-mediated introduction of non-native species induces population founding events that generally involve a limited number of individuals. According to general theory (founder's principle - Mayr 1954), these individuals carry only a fraction of the genetic variation found in the source population(s), hence pass through generations of small effective population size (i.e. a bottleneck) (Nei *et al*. 1975). A founder population can originate from a single population or from multiple populations, which might lead to novel genetic combinations resulting from multiple independent introductions (Novak & Mack 2005). The effects of such novel combinations of genetic variants are yet poorly known (Colautti & Lau 2015; Sherpa & Després 2021), and this is a challenge for further studies in invasion biology (Kaňuch *et al*. 2021).

Most freshwater ecosystems have been affected to varying degrees by biological invasions or introductions. Such effects may cause food web disruptions, biodiversity loss, and economic harm at different scales (Parker et al. 1999, Thomaz et al. 2015). Aquatic invasion rates are increasing due to globalization and accelerated trade naturally differentiated regions. Concerns about the impacts of such invasions arise mainly from ecosystem disturbances with potential subsequent socio-economic harm (Thomaz *et al*. 2015). According to Salmenkova´s (2008) review and Havel et al. (2015), the term "introduction" in the context of biological invasions covers the introduction of living organisms into ecosystems located beyond their natural range. This notion is closely related to the terms "transplantation" (transport to a new location), "acclimatization" (transport of individuals to a new biome to form self-sustaining populations or new populations maintained by human populations), and "naturalization" (the establishment of alien species as stable self-sustaining populations in the new area).

In the 1960s, *Arapaima gigas* (paiche), one of the largest fish species in the Amazon, was transplanted and acclimatized for incipient aquaculture in Puerto Maldonado (Madre de Dios River, Upper Madeira Basin, Peru) (Carvajal-Vallejos *et al*. 2011). Precarious confinement and seasonal flooding allowed this species to escape and to spread downstream towards Bolivian rivers and oxbow lakes where it is non-native (Carvajal-Vallejos *et al*. 2011). The species successfully established and currently is one of the most important fishery resources in the area (Carvajal-Vallejos et al. 2011, Miranda-Chumacero *et al.* 2012, Van Damme et al. 2015). In its natural area of distribution in Brazil and Peru, where *Arapaima* was severely depleted by fisheries several decades ago, some genetic studies (mt- and nDNA) were conducted in the framework of conservation programs (Hrbek *et al*. 2005, 2007; Araripe et al. 2013, Vitorino et al. 2015, Farias *et al.* 2019, Torati *et al*. 2019, Nogueira *et al*. 2020 a, b). Individuals from the lower portion of the Amazon and some tributaries flowing into this area showed significant differentiation from fish from the upper portion. The general conclusions from these studies were that *A. gigas* is made up by a single taxonomic entity, with moderate spatial population structure, concordant with the

geographical distances (Western to Eastern). However, in contrast, some studies propose that different species co-exist in the Amazon basin and that new species should be described (Stewart 2013 a, b).

Our hypothesis is that an unknown number of individuals with low genetic variability spread in Bolivia following a single initial founding event. To test this hypothesis, we determined the genetic identity and population structure of extant Bolivian *A. gigas* based on mt- and nDNA. We evaluated whether the contemporary population structure and the genetic heterogeneity detected are concordant with a single introduction event based on one source population. Based on these results we provide recommendations for the sustainable use and/or control of the species in Bolivia.

METHODS

Study area

The study area is situated in the Upper Madeira River basin in Bolivia, comprising the Abuná, Orthon, Madre de Dios, Beni, Mamoré and Iténez (or Guaporé) River basins. It has a tropical climate, with the rainy season between October to March. The rivers and their floodplains are characterized by seasonal flood pulses (Molina Carpio 2011) and are species-rich (e.g., Maldonado & Carvajal 2005). Most of the rivers have meanders and are characterized by an unstable bed, structural heterogeneity, and high geomorphological complexity (Crespo & Van Damme 2011).

Samples were obtained from the Orthon, Madre de Dios and Beni river sub-basins. The Orthon River is formed by the confluence of a white (high water season) -black (low water season) mixed water river (Manuripi) (Navarro & Maldonado 2002) and a white-water river (Tahuamanu), which originate in lowlands of Peru and enter Bolivia in the extreme North-West. The Manuripi River has alluvial plains with seasonally flooded surfaces of fluvial terraces and internal deltas, associated with the river bed (Navarro & Maldonado 2002). The Orthon River has a denudation rate of 0.033 ± 0.004 mm/yr (Wittmann *et al.* 2011) and discharges its waters to the Beni River after the latter's confluence with the Madre de Dios River, approximately 33 km downstream from the city of Riberalta. The Madre de Dios River is a river-floodplain system characterized by its white-waters originating in the Peruvian Andes (> 6 000 m of elevation), and has a denudation rate of 0.28 ± 0.13 mm/yr (Wittmann *et al*. 2011). The Beni River flows from the Andes in Bolivia and runs through territories of La Paz and Cochabamba departments in its upper portion. Since its departure from the Andes, at the height of the city of Rurrenabaque, the fluvial landscape has the characteristics of a river-floodplain system in its middle portion of the basin, and is characterized by high turbulence and a large amount of suspended sediments (white-water) (Navarro & Maldonado 2002). Its denudation rate is 0.45 ± 0.07 mm/yr (Wittmann *et al.* 2011).

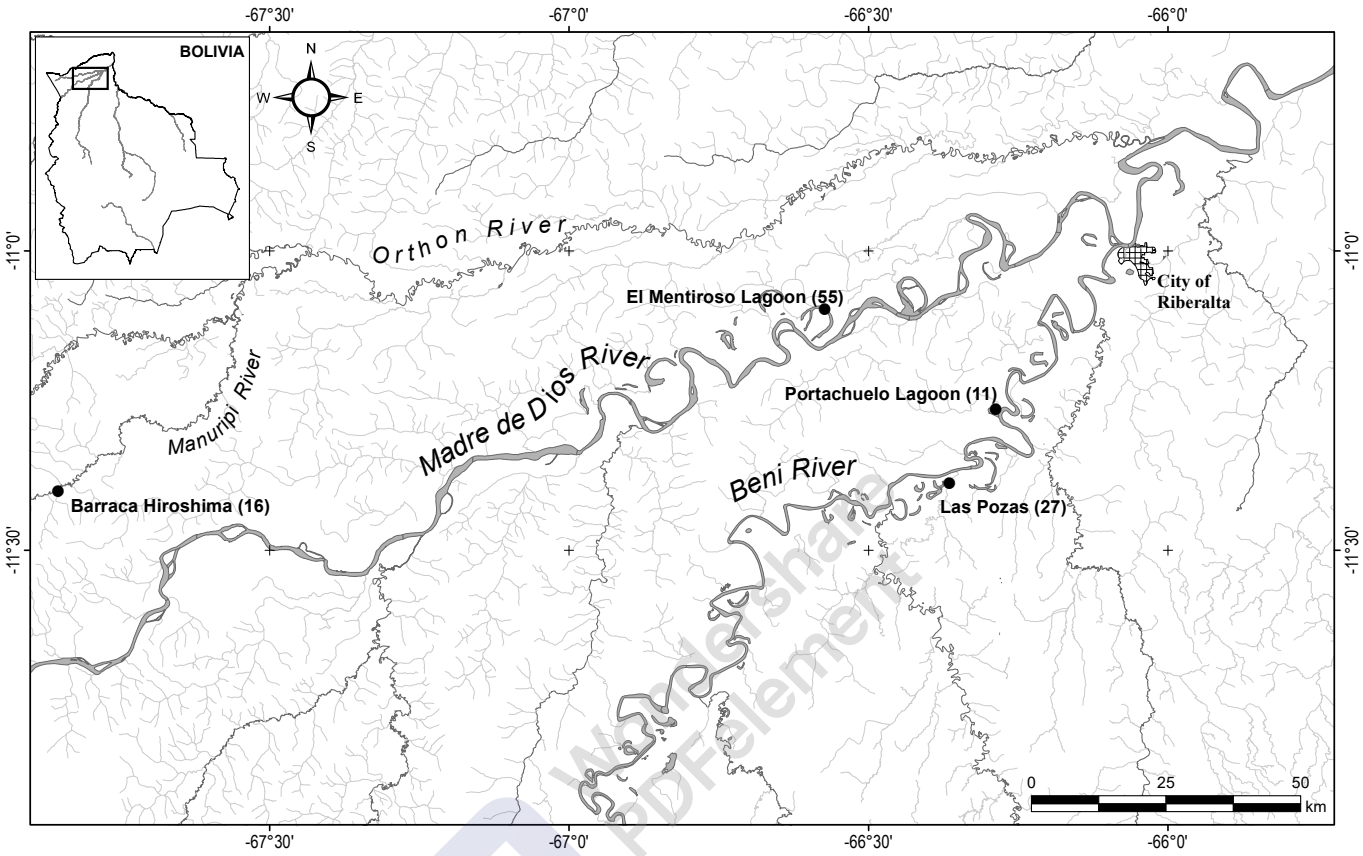

FIGURE 1. Representation of paiche *Arapaima gigas* samples collection sites in northern Bolivia between the years 2011-2013. Number in parentheses represent the sample size in the sites

Sample collection

Paiche were collected in the vicinity of the Barraca Hiroshima (latitude -11.40083, longitude-67.8525, elevation 175 m) in the Manuripi-Orthon river basin (16 individuals- ind); El Mentiroso lake (latitude-11.096981, longitude-66.572905, elevation 130 m), in the Madre de Dios River basin (55 ind); Las Pozas (latitude -11.38857, longitude -66.36494, elevation 130 m) (37 ind) and the Portachuelo lake (latitude -11.264171, longitude -66.288122, elevation 125 m) (38 ind), in the Beni River basin. A total of 119 individuals from the three basins were analyzed (Figure 1). The samples were obtained from commercial fishers or from indigenous fishers living in communities in the multi-ethnic Indigenous territory (TIOC) TIM II, all of them having resource and/or lake access rights. In particular, we received samples from commercial fishers in Puerto Rico that received permission to fish in the Manuripi River (Manuripi Amazonian Wildlife National Reserve), Trinidacito, a Tacana indigenous community fishing in El Mentiroso lake, Portachuelo Bajo, an Esse-Ejja indigenous community fishing in Portachuelo lake, and Flor de Octubre, a Tacana indigenous community fishing in Las Pozas, among other sites. A photographic record of the fish was made from and a sample of muscle tissue sample, taken from the dorsum of each fish, was preserved in a 15 ml tube with

95% ethanol. All samples were deposited in the fish collection of the Unidad de Limnología y Recursos Acuáticos (ULRA), of the University Mayor de San Simón (UMSS), Cochabamba, Bolivia (codes Madre de Dios sub-basin: AgMD 01-45, 47, 49-55; codes Beni sub-basin: AgBE 01-10, 12, 15, 17-18, 20-21, 24-26, 30-35; AgBEp 1-11, UMSS 11498, UMSS 11499; codes Orthon sub-basin: AgOR 01-16).

DNA extraction

DNA in muscle tissue was extracted by incubating a small sample piece in 100 µl/10% Chelex-100 (BIO-RAD, Bio-Rad Laboratories, Inc., Mississauga, Ontario Canada), 0.2% SDS and 0.4 mg Proteinase K/ml for two hours at 55° C, then at 95° C for 15 min. Total DNA extraction was checked in a 1% agarose gel.

Mitochondrial DNA (mtDNA) amplification

Two mtDNA loci were amplified. The cytochrome oxidase subunit 1 (CO1) was used for the taxonomic genetic identity of the paiche individuals in relation to sequences available in the literature and databases (GenBank). The DNA fragment encoding ATPase was used for population analysis and for comparisons with sequences previously obtained by Hrbek *et al.* (2005) for specimens caught in Brazil and Peru. The CO1 gene was amplified and sequenced with the FishF1 and FishR1 primers (Ward *et al.* 2005). ATPase was first amplified with the L8106 and H9264 primers, and then sequenced with primers L8537 and H8516 (Hrbek *et al.* 2005). The amplified ATPase fragment included the 3' cytochrome oxidase subunit two, lysine tRNA, ATPase subunit six, ATPase subunit eight, and the 5' of cytochrome oxidase subunit three. Only five individuals from each locality collected were sequenced for CO1, while all the individuals were sequenced for ATPase variation. The amplification of the fragments by polymerase chain reaction (PCR) was carried out using the whole extracted genome and negative controls were included in each reaction.

The cocktail for the amplification of CO1 totaled 25 µl, composed of 18.75 µl of ultrapure water, 2.25 µl of 10X buffer for PCR, 1.25 µl of $MgCl_2$ (50 mM), 0.25 µl of each primer (0.01 mM), 0.125 of each dNTP (0.05 mM), 0.625 U of Tap polymerase, and 1 µl of DNA extraction. The temperature profile of the reaction consisted of an initial step at 95° C for 2 min, followed by 35 cycles composed of 0.5 min at 94° C, 0.5 min at 54° C, one minute at 72° C, and a final cycle at 72° C for 10 min before being held at 4° C. The cocktail for the amplification of ATPase had a total volume of 25 µl composed of the same proportions of reagents used in CO1. The temperature profile for the reaction began with denaturation at 95° C for 2 min, followed by 30 cycles consisting of the sequence 94° C for 0.6 min, 50° C for 0.6 min, 90° C for 1.5 min, and one final cycle 72° C for 10 min before being held at 4° C.

Amplifications were carried out in a Mastercycler Eppendorf thermal cycler (Brinkmann Instrumments, Inc.). The PCR products were visualized on 1% agarose gels and the most intense products were selected for sequencing. Products were labeled using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Inc.) and bi-directionally sequenced using an ABI 3730 capillary sequencer following the manufacturer's instructions.

Nuclear DNA (nDNA) amplification

A total of 124 individuals were studied with microsatellite loci AgCTm7, AgCAm2, AgCAm15 identified by Farias *et al*. (2003). The reaction was carried out in 10 µl volume containing 5.5 µl of ultrapure water, 1.0 µl of 10X buffer (100 mM Tris-HCl, 500 mM KCl, 15 nM $MgCl_2$), 2.0 µl of each primer (0.2 µM each), 0.8 µl of a mixture of dNTPs (200 µM each dNTP), 0.2 U of Tap polymerase (AccuPrime, Invitrogen), and 1.0 µl of total DNA extraction. The temperature profile for the reaction began with a denaturation at 92° C for two minutes, followed by 35 consistent cycles of 94° C for 10 s, a specific temperature for each chosen primer (see Farias *et al*. 2003) by 10 s, 72° C for 30 s, and a final extension at 72° C for 30 min before being held at 4° C. The reactions were carried out in the same thermal cycler used for the mitochondrial fragments. The PCR products were visualized in 1% agarose gels and the most productive reactions were selected and subjected to 7% polyacrylamide gel electrophoresis of 1 mm thickness. The non-denaturing polyacrylamide was composed of acrylamide and bisacrylamide in a 19:1 ratio. The gels were prepared with 2X TAE buffer (Sambrook et al. 1989), the same that was used to immerse the chamber with the gels and close the electric field. The products were mixed with 3 µl of 10X running dye (50 mM EDTA [pH 8.0], 30% glycerol, 0.25% bromophenol blue) to conglomerate the products, and 10 µl of this solution was loaded into the polyacrylamide cells. After the fragments were subjected to the electric field and migrated overnight (12-14 h), they were stained in an aqueous solution of Ethidium Bromide (0.5 µg / ml) and the bands were visualized on an ultraviolet ray transluminator. The digital images of the gels were obtained with an Eagle-Eye system (Stratagene Corp., San Diego, California), and the gels were manually read and evaluated using the GelAnalyzer program (version 2010, www.gelanalyzer.com). Each electrophoretic run considered one column for the blank reactions and two for the molecular weight marker that varied by 100 base pairs (Promega) in the reading zone.

CO1 analysis

The CO1 sequences were aligned and edited with the BioEdit program (Hall 1999) with Clustal W. Comparison with other sequences available in the literature was carried out with the BLAST (Basic Local Alignment Search Tool) program (version 2017) in the base GenBank online data set (National Center for Biotechnology Information - http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?CMD=Web&PAGETYPE=BLASTHome). The publications with sequences available for comparisons were Hrbek *et al*. (2007) and Ardura *et al*. (2010). Haplotype and nucleotide diversity were calculated with the program DnaSP v. 5 program (Librado & Rozas 2009).

ATPase analysis

The ATPase sequences were edited and aligned following the same procedure as for CO1. The haplotypes identified were compared with the haplotypes identified

by Hrbek *et al*. (2005), who analyzed 110 samples from Brazil (different points along the main axis of the Amazon), 16 from Peru (Iquitos), and 13 from Bolivia (Madre de Dios River basin without specific localities).

Microsatellite (nDNA) analysis

The genetic variation was described by the classical parameters of population genetics such as allelic frequency per locus, level of heterozygosity observed (Ho) and expected without bias (Hn) (Nei 1978). The inbreeding index (F_{IS}) (Wright 1978), estimated by *f* (Weir & Cokerham 1984), was calculated to assess Hardy-Weinberg Equilibrium departure (HWE) within a defined population sample. The significance of the deviations was evaluated with 1 000 matrices generated at random by permutations between alleles. The general differentiation between geographic samples was evaluated considering all the loci together, using comparisons between the values of the fixation index F_{ST}, estimated by the value of theta - θ (Weir & Cokerham 1984). A genetic population is defined as a unit with no significant HWE departure (the value of F_{IS} not significantly different from zero), differentiated from the others by significant values of F_{ST}. All the above mentioned procedure and indices were generated with the program Genetix v. 4.05 (Belkhir *et al*. 2004).

To determine if the genetic differentiation between the geographic samples was related to their geographical location, the relationship between the genetic distance obtained (F_{ST} / 1-F_{ST}, Rousset (1997)) *vs*. the linear distance (km) between the studied localities and the distance along the (sinuous) course of the rivers was calculated. The degree of relationship was measured with the Pearson correlation coefficient (*r*) implemented in Excel (Microsoft Office Professional Plus 2016), and the geographical distances were obtained using Google Earth.

Genetic distance between populations when compared to the geographical distance following the course of the rivers (r = 0.14) or linearly (r = 0.16) between localities, did not show a significant relationship. This lack of relationship denotes that genetic differentiation does not depend on spatial distance but on other factors.

RESULTS

CO1 and ATPase

The obtained CO1 fragment consisted of 651 base pairs (bp). Haplotypes were identified in the samples from the three studied locations. The haplotype diversity of the 12 samples successfully sequenced (Hd) was 0.303 ± 0.147, and the nucleotide diversity (Pi) was 0.0005 ± 0.0002. These sequences varied at three sites when compared to a single sequence identified in two individuals by Ardura *et al*. (2010) and two individuals by Hrbek & Farias (2008) from the Brazilian Amazon Basin.

A single haplotype was identified in the 90 individuals from Bolivia screened at the ATPase locus. The sequence identified in Bolivia was different from the 13 haplotypes identified for 126 individuals from the main axis of the Amazon from Brazil to Peru, and Bolivia (Hrbek *et al*. 2005).

Microsatellites

The highest allelic variation observed was at the AgCAm2 locus with four alleles and the lowest at AgCTm7 with two alleles. The lowest allelic diversity across all loci was observed in the Orthon River basin, which showed the presence of six alleles; none of them exclusive to the sampling location. The other two locations were more diverse (8 alleles) but presented exclusive alleles. Exclusive alleles were identified, one in the Madre de Dios River basin at the AgCAm2 locus (310), and another in the Beni River basin at the Ag-CAm15 locus (238) (Table 1, Figure 2).

F_{IS} by locality as examined locus by locus and loci combined showed non-significant HWE departure ($p > 0.05$) (Table 1).

TABLE 1. Genetic variability of the paiche *Arapaima gigas* in three microsatellite loci for three localities of the Bolivian Amazon Basin. MD: Madre de Dios River basin; BE: Beni River basin; OR: Orthon River basin. N: number of individuals. Ho: observed heterozygosity; He: expected heterozygosity; SD: Standard deviation. F_{IS}: Inbreeding index; ns: not significant ($p > 0.05$).

Alleles	MD	BE	OR
N	55	38	16
Locus AgCAm2			
300	0.164	0.013	0.000
306	0.073	0.105	0.125
310	0.027	0.000	0.000
316	0.736	0.882	0.875
Ho	0.455	0.237	0.250
He	0.430	0.214	0.226
F_{IS} AgCAm2	-0.061 ns	-0.106 ns	-0.111 ns
Locus AgCTm7			
290	0.473	0.176	0.344
300	0.527	0.824	0.656
Ho	0.582	0.297	0.313
He	0.503	0.294	0.466
F_{IS} AgCTm7	-0.158 ns	-0.013 ns	0.336 ns
Locus AgCAm15			
228	0.827	0.763	0.531
238	0.000	0.197	0.000
244	0.173	0.040	0.469
Ho	0.346	0.395	0.313
He	0.288	0.382	0.514
F_{IS} AgCAm15	-0.200 ns	-0.034 ns	0.400 ns
All loci combined			

Alleles	MD	BE	OR
F_{IS}	-0.134 ns	-0.044 ns	0.281 ns
Ho	0.461	0.310	0.292
SD (Ho)	0.118	0.080	0.036
He	0.407	0.298	0.402
SD (He)	0.109	0.084	0.154

Examination of the F_{ST} estimator θ (Weir & Cockerham 1984) showed that there is significant differentiation ($p < 0.01$) between the localities; they can all be considered as genetically distinct from each other (Table 2). The least genetic distance, as defined by F_{ST} / 1-F_{ST} (Rousset 1997), was observed between the population of the Madre de Dios River and the population of the Orthon River; these sites were not the most distant geographically. On the other hand, the greatest genetic distance was observed between the Orthon and Beni River samples, which are the most distant from each other (Table 2).

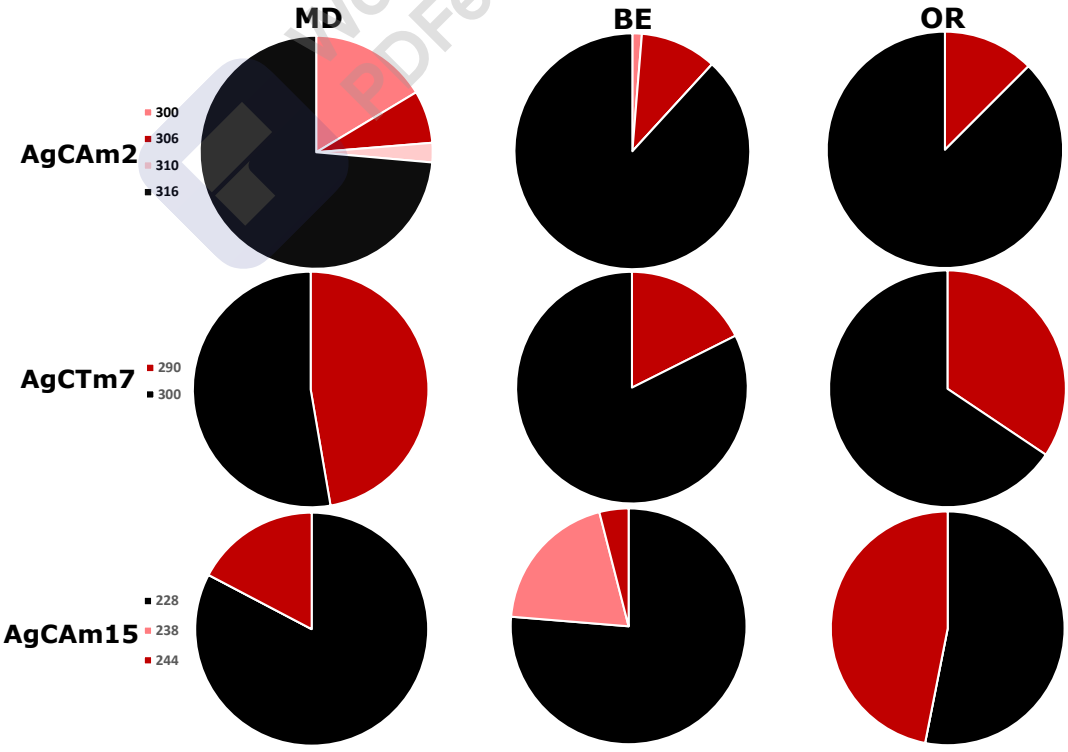

FIGURE 2. Genetic variability of the paiche *Arapaima gigas* in three microsatellite loci (AgCAm2, AgCTm7, AgCAm15) for three localities of the Bolivian Amazon Basin. MD: Madre de Dios River basin; BE: Beni River basin; OR: Orthon River basin.

D\F$_{ST}$	MD	BE	OR
MD		0.107**	0.080**
B E	0.120		0.127**
OR	0.087	0.146	

TABLE 2. Genetic differentiation of paiche *Arapaima gigas* samples from three localities in the Bolivian Amazon Basin. MD: Madre de Dios River basin; BE: Beni River basin; OR: Orthon River basin. F_{ST}: Fixation index (θ, Weir & Cockerham 1984) (upper diagonal); D: Genetic distance defined as F_{ST} / 1-F_{ST} (Rousset 1997) (lower diagonal). **: p <0.01 (highly significant)

Genetic distance between populations when compared to the geographical distance following the course of the rivers (r = 0.14) or linearly (r = 0.16) between localities, did not show a significant relationship. This lack of relationship denotes that genetic differentiation does not depend on spatial distance but on other factors.

DISCUSSION

CO1 information

The < 2% divergence of the CO1 of haplotypes of the present study and one haplotype identified for the Central Amazon in Brazil (Hrbek & Farias 2008, Ardura *et al*. 2010) suggests that the paiche present in Bolivia is likely the same species from the Central Amazon of Brazil. The benchmark for species differentiation using the CO1 is approximately 2% (Pereira *et al*. 2013). However, this benchmark is not absolute, and some species recognized as distinct species using morphology are not differentiated at this level. In general however, studies have shown that 93-98% of the known fish species can be differentiated through this methodology (Ward *et al*. 2009), although in some cases the accuracy may be lower. In the Amazon basin there are some cases in which the CO1 information does not differentiate fish species (e.g., Toffoli *et al.* 2008, Garcia-Davila *et al*. 2013, Machado et al. 2018)

ATPase information

The uniformity found in all the sequences obtained for the three geographical samples from Bolivia in the present study suggests that a bottleneck occurred when the paiche reached the natural environment in the Madre de Dios River basin in Peru or that the original source population was monomorphic for this marker. None of them were identical to the haplotypes identified by Hrbek *et al*. (2005), who found seven haplotypes in 17 individuals from the Peruvian portion (Iquitos) of the Amazon Basin. The sequences were differentiated by a substitution that was not present in any of the sequences obtained by Hrbek *et al*. (2005). The total number of polymorphic sites in Hrbek et al. (2005) was 18. The difference in the Bolivian sequences is due to a substitution of Thymine by Cytosine. The origin of this difference may be due to a mutation in the locus post colonization, but it is unlikely due to the mutation rate of 1.3% per million years for this locus (Bermingham *et al*.

1997). It may be the case that Hrbek et al. (2005) did not uncover all the potential source population haplotypes as they sequenced only 17 individuals and found seven haplotypes.

Microsatellite information

The analysis of the variation of the microsatellites showed that the analyzed samples are part of a large meta-population existing in three of the major systems of the Bolivian Amazon. This result is consistent with the unique escape of a small group of specimens which is reflected in the observed variation of CO1 and ATPase. These individuals were successful in their expansion and reproduction since their introduction and advancement towards water bodies in Bolivia. Conservation genetics readings suggest that reduced genetic variation due to gene drift or founder effects (bottlenecks) limits the adaptive capacity of populations, and reduced population sizes increase the risk of extinction due to inbreeding depression and reduced fitness (Frankham & Ralls 1998, Allendorf & Lundquist 2003). However, each geographic sample analyzed turned out to be a differentiated genetic unit (genetic population), with differences at the level of allele frequencies and allelic composition (exclusive alleles) .The presence of exclusive alleles could be the result of recent mutations in microsatellites by its hypermutable nature, and therefore hypervariable, but this is unlikely given the mutation rate that is known for this marker (1/100 to 1/1 000 000 per locus and per generation - Ellegren 2000, Sia et al. 2000), the recent presence of the paiche in Bolivia (around 50 years), and a four-year-old generation of the species. This result denotes the existence of a genetic microstructure where the resulting populations are not distributed randomly, they may have their own phenotypic characteristics and there are factors that are influencing population structure.

Rapid evolution of adaptive traits is known to sometimes occur in populations exposed to ecologically divergent environments (Reznik *et al*. 1997, Hendry & Kinnison 1999, Dlugosch & Parker 2008), and not necessarily with a loss of genetic variability (Roman & Darling 2007). The fact that the variation is not uniform coincides with the sedentary behavior that has been described for the species in its natural environment (Castello 2008, Arantes *et al.* 2013, Nuñez-Rodriguez *et al*. 2015). The degree of exchange between populations depends on the spatial distance and geomorphological and hydrological barriers and it is possible that there are phenotypic barriers to cross breeding among them. It has been seen that there is no relationship with geographical distances (linear or by the course of rivers), so other factors besides geographical distance are influencing the differentiation of populations. It is interesting to note that there is a correspondence between the observed population structure with the type of system where the populations are found. There are examples of successful invaders that may have developed traits associated with environmental fluctuations and bottlenecks, which allowed them to colonize new areas with relatively low levels of genetic diversity (Gelembiuk *et al*. 2006). Each system from which the populations come from has characteristics that differentiate them, for example, although the Madre de Dios and Beni rivers are

white water, the latter is characterized by transporting a large amount of suspended sediment and dissolved solids, almost four times higher in relation to the first (Guyot et al. 1996). In turn, the Manuripi-Orthon river system is known for having intermediate characteristics between the white water systems (dry season) and clear waters (rainy season), which is why it has been called a mixed water system (Navarro & Maldonado 2002). Founding events, likely performed by specimens from different stocks, and isolation mechanisms related to dominant factors of the systems underly the observed pattern. This means that the fish confined in Peru and transported to Bolivia (Carvajal-Vallejos *et al*. 2011) might have been gathered from different localities and/or tributaries, and therefore from different populations of the Peruvian portion of the main axis of the Amazon. These fish, once escaped, sought conditions similar to those of the environment from which they were taken from.

It is known that fish farming stocks in Peru were made up for several decades by specimens from white water systems of (e.g., Ucayalí, Marañón) and clear water systems (e.g., Nanay River), and no effort was made based on their origin to keep them together or separate (García-Dávila *et al.* 2011; personal observation in fish farming in Iquitos and Imiría lake 2006-2010). It is known that founder groups made up of individuals from different genetic populations have greater potential to expand and multiply, which makes them successful in the process of introduction and invasion (Kolbe *et al*. 2004). Another potential explanation for the identified population structure is that the species followed several bottle necks with rapid expansion and colonization, and subsequent genetic drift. The most aggressive individuals were at the forefront of the invasion line and the most sedentary were occupying the different habitats throughout the expansive wave, with a subsequent rapid change in allelic frequencies in their population constitution due to bottlenecks that occurred progressively and the sedentary lifestyle and territorialism they possess (at least during the reproduction season – Saavedra *et al*. 2005).

Management recommendations

The results of the present work show that the paiche present in the Madre De Dios, Orthon and Beni rivers likely originate from a stock in Perú, or from a mixture of stocks, that reached the natural environment as described in the literature (see Carvajal-Vallejos *et al.* 2011) and gave rise to several populations resulting from genetic bottlenecks throughout the invasion wave in the upper Madeira basin of Bolivia. This structuring is related to an important ecological feature of the species, which is the sedentary lifestyle and the short migratory movements carried out throughout its life (Castello 2008, Nuñez-Rodriguez *et al*. 2015), and which is described and commented on by fishers in Bolivia. The paiche does not perform large movements, and after water retreat at the end of the high water season it returns to its place of origin to feed and reproduce during the low water period. Therefore, the management and use of the species throughout the basin should not be considered as of a single stock. Some features of the systems may be related to the observed genetic variation. We suggest that future regional or local management initiatives

should consider that recruitment in lakes is sustained by stocks from the same lakes and/or surrounding nearby aquatic habitats, as a source-sink model. The specimens in these surrounding environments are less accessible for fishing, and likely have a close genetic and exchange relationship with the specimens present in permanent and temporal lakes and streams nearby, that are more accessible to fisheries.

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