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SHORT COMMUNICATION

Hidden genetic diversity and distinct evolutionarily significant units in an commercially important Neotropical apex predator, the catfish *Pseudoplatystoma corruscans*

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Abstract In the South American Neotropics, several fish taxa with distributions ranging over multiple river basins might actually represent cryptic species or distinct evolutionarily significant units (ESUs). Defining hidden genetic diversity within species is of great significance to inform on programs aimed at maintaining the evolutionary potential of natural populations and to conduct appropriate fisheries management. This is particularly important in commercially exploited species, such as the "surubim" catfishes (genus *Pseudoplatystoma*). Here, based on evidence of reciprocal mtDNA monophyly and significant nuclear divergence in eight microsatellite markers we report on two ESUs in the widely distributed *Pseudoplatystoma corruscans*. The implications of these results for the conservation management, traceability of fish products and for identifying breeders for restocking programs in this important apex predator are discussed.

Keywords mtDNA · Microsatellite · Cytochrome oxidase *c* subunit I · ESU · Restocking · Catfish

Introduction

A critical issue in conservation biology is to determine the units of species that have to be managed and conserved separately (Frankham 2010). Identifying Evolutionarily Significant Units (ESU) is an important step towards maintaining the evolutionary potential of natural populations (Moritz 1995). An ESU is recognized as a unit of conservation below the species level that is reproductively isolated (usually defined by reciprocal monophyly) and represents an important component in the evolutionary legacy of the species (Moritz 1994). Genealogical studies can provide extremely useful information for identifying ESUs and cryptic biodiversity (Moritz 2002). In addition, for species subjected to commercial exploitation (e.g. fisheries), such approaches can also be used to assign species and individuals to their population of origin, assisting management and enforcement (Martinsohn and Ogden 2009).

In the South American Neotropics, several fish taxa with distributions ranging over multiple river basins might actually represent cryptic species or distinct ESUs (e.g. Beheregaray et al. 2002; Carvalho et al. 2011). The catfishes of the genus *Pseudoplatystoma* (family Pimelodidea) are large, migratory predators that inhabit the major river basins of South America that drain into the Atlantic and Caribbean (Buitrago-Suarez and Burr 2007). *Pseudoplatystoma corruscans* (popularly known as *surubim* or

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pintado) is reported to the La Plata Basin and São Francisco River Basin (SFRB). The species represents an important fisheries resource, reaching up to 145 cm in size and weight up to 120 kg. A molecular systematic study provided evidence for monophyly of this taxon (Torrico et al. 2009), but at the population level, substantial nuclear differentiation has been reported within the La Plata Basin, with possible homing behavior (Pereira et al. 2009).

Anthropogenic activities such as the construction of dams for energy generation, overfishing, habitat degradation, pollution, introduction of exotic fish species, and climate change have negatively impacted on many Neotropical fish species. The conservation status of *P. corruscans* has not been assessed by the IUCN Red List (IUCN 2011). This taxon was not considered for inclusion on the Brazilian Red List of Threatened Species mainly because of the relatively abundant populations in the Pantanal wetlands (Monteiro et al. 2008), but the species is considered critically endangered in the São Paulo State, Brazil (Mello et al. 2009).

Herein, two ESUs for the Neotropical catfish *P. corruscans* are recovered based on mitochondrial and nuclear DNA markers. Considerations on the conservation management, traceability of fish products, and the genetic origins of breeders for restocking programs are discussed.

Methods

Sampling

Specimens of *P. corruscans* were captured on two sites in the middle São Francisco River in Brazil (Fig. 1) for tissue collection and released back to the river. Thirty fin clips samples were collected over a period of 3 years (2004–2007) from site 1 (S15°55′43.2114″, W44°51′6.0645″). Four previously described *P. corruscans* cytochrome oxidase subunit I (COI) haplotypes (Carvalho et al. 2011) collected at Pandeiros River (site 2; S15°40′18″, W44°38′123″) were included in the analysis. In the Paraguay River Basin, 23 samples were obtained from the Aquidauana River (site 3; S20°29′0.21″, W55°43′26.5063″). Four previously described *P. corruscans* COI haplotypes from the PRB (site 4; S25°25′11.2793″ W54°32′8.1555″) were also included in the analysis. All species were identified following (Buitrago-Suarez and Burr 2007).

Genetic analyses

Mitochondrial DNA (mtDNA)

A segment of the mtDNA COI marker was amplified using fish universal primers FishF1 and FishR1 as in Ward et al. (2005). The reactions were analyzed in an automated DNA sequencer ABI 310 (Applied Biosystems). Chromatograms were checked manually and aligned using the software CLUSTALX (Thompson et al. 1997). Each recovered haplotype was deposited in GenBank (Accession numbers JX462911–JX462945).

The number of variable sites, haplotypes and demographic history parameters such as Tajima's D and Fu's *F* tests were obtained by using DNASP 5.0 (Librado and Rozas 2009).

A Neighbor-joining (NJ; Saitou and Nei 1987) tree was obtained using PAUP v.4.0 b10 (Swofford 2003) specifying the K2P model (Kimura 1980). Maximum parsimony (MP) analysis was performed with 100,000 as the maximum number of trees to be analyzed with 5,000 random replications by stepwise addition of sequences. The permutation of sequences were computed by TBR (tree-bisection-reconnection) along both ACCTRAN and DELTRAN character optimizations. Confidence for branching supports were assessed by 10,000 bootstrap pseudo-replicates (Felsenstein 1985) for both NJ and MP analyses. Bayesian inference was conducted by using MRBAYES v. 3.1.1 software (Huelsenbeck and Ronquist 2001) in 1,000,000 generations of four Markov chains. *Pseudopimelodus* sp (ABY48091.1) and *P. reticulatum* were used as outgroups for all clustering methods.

Microsatellites

A set of eight microsatellite loci previously described for *P. corruscans* (Revaldaves et al. 2005) were amplified for all samples. Amplification followed the method described by Schuelke (2000) in which PCR products are fluorescently labeled through the inclusion of a third (fluorescent M13) primer in each reaction. Reactions were performed in a final volume of 10 µl following conditions described in Beheregaray et al. (2004). Amplification products were genotyped in an ABI 3130 genetic analyzer (Applied Biosystems). Resulting microsatellite profiles were examined using GENEMAPPER 4.0 (Applied Biosystems) and peaks were scored manually.

The fixation index between river basins was estimated using Fst (Weir and Cockerham 1984). Bayesian clustering methods were also applied to examine population genetic structure without allocating individuals to populations prior to analysis using STRUCTURE V.2.2 (Pritchard et al. 2000). To determine the number of populations (*K*) within the complete data set, five independent simulations for $K = 1$–9 with 100,000 burn in iterations and 500,000 data iterations were run. Analysis was performed using the admixture model of population structure and allele frequencies correlated among populations. The number of populations (*K*) was estimated using the protocol described by (Evanno et al. 2005).

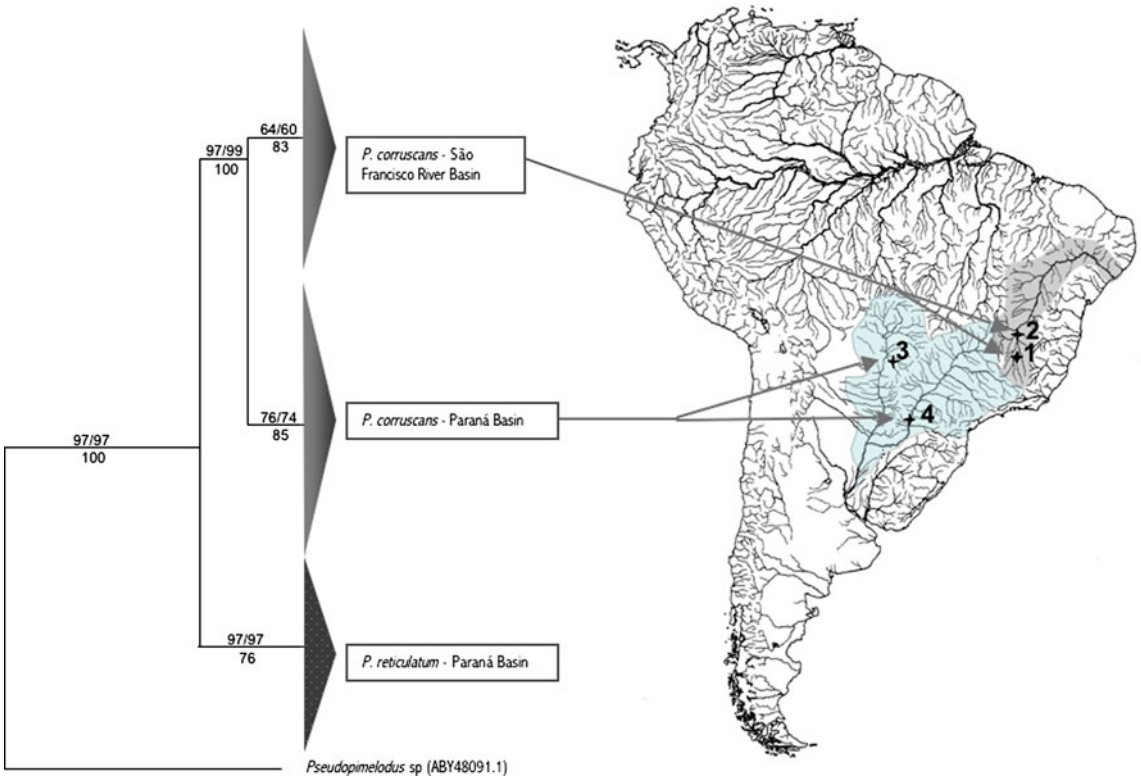

Fig. 1 Map showing the collecting sites within the SFRB(*sites 1 and 2*) and the Paraná-Paraguay River Basin (*sites 3 and 4*). A NJ tree of K2P distances based on COI region (534 bp) is presented. Numbers above the branches are NJ and MP bootstrap values respectively, numbers below the branches represent Bayesian posterior probabilities. Size of *triangles* is proportional to number of specimens sequenced

Results and discussion

A total of 34 COI sequences were analyzed from the four sampling sites encompassing the two river basins (Fig. 1). After trimming unclear ends, 534 bp were recovered. Every polymorphic site had a silence mutation, thus no changes to amino acid sequences were observed. When considering different river basins, two exclusive haplotypes were detected within *P. corruscans* from the SFRB, and seven for the PRB (Table 1, supplementary material).

The tree topologies obtained by NJ, MP and Bayesian analyses were identical therefore only the NJ tree is presented (Fig. 1). Three phylogroups were recovered: *P. reticulatum*, *P. corruscans* (SFRB), and *P. corruscans* (PRB). *P. corruscans* and *P. reticulatum* were clearly divergent (4.68 % K2P COI divergence). The *P. corruscans* from the SFRB grouped separately from *P. corruscans* from the PRB (Fig. 1).

For the nuclear DNA dataset, a total of 47 specimens of *P. corruscans* were genotyped using a set of eight microsatellite markers. A mean of 5.6 alleles was observed in the SFRB population, compared to 7.6 alleles in the PRB population of *P. corruscans*.

High fixation indexes values were detected between the SFRB and PRB for both genetic markers: Fst of 0.18 ($P = 0.01$) and ϕ-st of 0.89 ($P = 0.001$). Several private alleles were also observed for each River Basin. For instance, out of the five alleles recovered for Pcor7, four were diagnostic for each river system phylogroup (Table 2, supplementary material).

Analysis of population structure of the entire dataset using STRUCTURE indicated that the most likely value for K was two. The plot of estimated membership coefficient for each of the two clusters ($K = 2$) clearly shows a division between river basins SFRB and PRB (Fig. 2).

Tajima's D and Fu's Fs index were statistically significant for *P. corruscans* from the PRB [-2.034 ($P < 0.05$); -5.467; ($P < 0.05$)], but not for the SFRB [1.309 ($P > 0.10$); 1.247 ($P > 0.05$)], respectively. These results suggest population expansion of *P. corruscans* in the geologically recent foreland river system (i.e. the PRB). No such signals were recovered for *P. corruscans* from the geological older upland basin of the SFRB.

The river basins investigated here are geographically close to one other, despite few connections. Historical relationships between these two basins also appear to

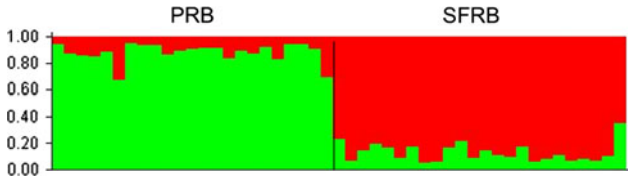

Fig. 2 Barplot of estimated membership coefficient (x axis) for each individual from *P. corruscans* populations of the two River Basins: Paraná-Paraguay (PRB-y, $n = 23$—*grey*) and São Francisco River (SFRB—*black*, $n = 24$) based on seven polymorphic microsatellite loci

account for the distribution of other migratory fishes, such *Prochilodus* (Sivasundar et al. 2001). Nonetheless, geological records indicate that rivers running over the Brazilian Shield might have an ancient geomorphological history compared to those from the lowlands in Central Brazil (Lima and Ribeiro 2011). Despite their geographical proximity, geological evidence suggests allopatric population differentiation, as detected herein for *P. corruscans*.

An ESU might be defined as a historically isolated set of populations with reciprocal monophyly for mtDNA, as well as significant divergence in frequencies of nuclear alleles (Moritz 1994). Despite the apparent morphological similarity of *P. corruscans* from the São Francisco and Paraná Basins, the overall nuclear and matrilineal DNA divergence, combined with historical separation in distinct hydrographical systems, strongly indicate that the two population groups have evolved independently from one another, warranting their classification as distinct ESUs.

Although *P. corruscans* is considered abundant in the Pantanal wetlands (Monteiro et al. 2008), a region that is part of the PRB, we have demonstrated here that populations from SFRB belong to a different ESU and as such should be prioritised in conservation management and environmental legislation. The latter is particularly important given the critically endangered status of some populations of *P. corruscans* are affected by habitat fragmentation (i.e. several hydroelectrical dams) and overexploitation (Mello et al. 2009).

Moreover, our results might prove useful as a traceability method helping fishery regulators to determine stock origins of *P. corruscans* in other river basins in Brazil. Traceability of overexploited fish stocks is an important issue that is currently been accessed for several commercial species in Europe, and is already helping to regulate fishery industry (Martinsohn and Ogden 2009). The findings of several microsatellite alleles diagnostic for *P. corruscans* from different river basin might be useful for traceability and certification purposes when applied together with mtDNA analysis. The combination of nuclear and mitochondrial markers is required since hybridization between *P. fasciatum* and *P. corruscans* is common in the aquaculture industry and hybrids have been reported in the wild (Bignotto et al. 2009). Therefore, we recommend that breeders of *P. corruscans* currently used in restocking programs of severely impacted river basins be genetically characterized. This characterization would probably avoid the spread of ESUs outside their natural range, ensuring the maintenance of local adaptations supporting the long-term conservation of this important Neotropical apex predator.

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