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Microsatellite variation and genetic structuring in *Mugil liza* (Teleostei: Mugilidae) populations from Argentina and Brazil

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ABSTRACT

The mullet *Mugil liza* is distributed along the Atlantic coast of South America, from Argentina to Venezuela, and it is heavily exploited in Brazil. We assessed patterns of distribution of neutral nuclear genetic variation in 250 samples from the Brazilian states of Rio de Janeiro, São Paulo, Santa Catarina and Rio Grande do Sul (latitudinal range of 23–31°S) and from Buenos Aires Province in Argentina (36°S). Nine microsatellite loci revealed 131 total alleles, 3–23 alleles per locus, *He*: 0.69 and *Ho*: 0.67. Significant genetic differentiation was observed between Rio de Janeiro samples (23°S) and those from all other locations, as indicated by F_{ST}, hierarchical analyses of genetic structure, Bayesian cluster analyses and assignment tests. The presence of two different demographic clusters better explains the allelic diversity observed in mullets from the southernmost portion of the Atlantic coast of Brazil and from Argentina. This may be taken into account when designing fisheries management plans involving Brazilian, Uruguayan and Argentinean *M. liza* populations.

1. Introduction

The mullet *Mugil liza* Valenciennes, 1836 is a pelagic fish distributed along the Atlantic coast of South America, from Venezuela to Argentina (Menezes et al., 2010; Siccha-Ramirez et al., 2014). This mullet is commercially exploited in Brazil and Argentina, where peak catches can surpass 18,000 tons/year (González-Castro et al., 2009; MPA, 2011). Commercial catches in Brazil occur especially between May and August, following reproductive migration (Miranda and Carneiro, 2007; MMA, 2007; Vieira et al., 2008) and fishes are caught mainly during migration (Lemos et al., 2014). This resource, shared with Argentina and Uruguay, was declared overexploited by the Brazilian Ministry of Environment a decade ago (MMA, 2004). Although fishing rules do exist for regulating the exploitation of this species (IBAMA, IN N° 171/2008) there is still a lack of information on the dynamics and structure of mullet populations in Brazil (Garbin et al., 2014; Lemos et al., 2014) and no plan is available for responsible managing of this resource.

Mugil liza is a marine migrant estuarine-dependent fish (sensu Potter et al., 2013) marine single spawner (Albieri and Araújo, 2010; González-Castro et al., 2011; Lemos et al., 2014). The spawning areas for *M. liza* remains uncertain, but in South of Brazil evidences from artisanal and purse seine fisheries appoint the area between the North of Rio Grande do Sul State and North of Santa Catarina State (Brazil) as the main spawning area for the southern

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populations (Vieira and Scalabrin, 1991; Garbin et al., 2014; Lemos et al., 2014). After spawning, marine currents carry the juveniles to enter the estuaries (Vieira, 1991) and at 5–6 years old mullets are recruited to the adult population and reproduce once a year (Garbin et al., 2014; Lemos et al., 2014). Thus, given their reproductive and ecological characteristics are expected that mullets will be composed of discrete subpopulations of adults that exchange migrants through the pelagic larval phase (Durand et al., 2012).

Over recent years, there has been considerable debate on the taxonomy of *Mugil liza* (Cousseau et al., 2005; González-Castro et al., 2008, 2009, 2011, 2012; Menezes et al., 2010; Durand et al., 2012; Sicha-Ramirez et al., 2014). This debate is fueled with the increasing accumulation of ecological, genetic and morphological evidence supporting the classification of *M. liza* as a single species (Albieri and Araújo, 2010; Menezes et al., 2010; Sicha-Ramirez et al., 2014). However, as proposed by González-Castro et al. (2012), different populations of *M. liza* would occur along the species distribution range from Cuba to Argentina.

Aspects of population dynamics, evolutionary processes, migration, genetic drift, effective population size and sex-biased dispersal in fishes are described by both mitochondrial DNA (mtDNA) and nuclear markers (e.g. microsatellites) (Avise, 1994; Durand et al., 2013). Both types of markers differ in their mutational rates and inheritance mode; therefore, they could concurrently contribute to describe a complete picture of ongoing evolutionary processes and to better characterize the demographic history of natural fish populations (Durand et al., 2013). Mitochondrial DNA markers picture the more ancient history of populations and evolutionary processes (e.g. speciation), while nuclear markers are used to describe contemporary processes and resolving population structure on a finer scale (Goudet et al., 1996; González-Castro et al., 2012; Durand et al., 2013; but see Karl et al., 2012). To date, however, most genetic studies conducted in populations of *Mugil* spp. inhabiting the Atlantic coast of South America have primarily used mitochondrial markers (Fraga et al., 2007; Heras et al., 2007; Aurelle et al., 2008; Heras et al., 2009; Sicha-Ramirez et al., 2014). In addition, to the extent of our knowledge, and despite their utility, microsatellites have not been employed yet to study South American *Mugil liza*. There is, therefore, an apparent need of more studies using microsatellites to explore patterns of diversity at regional or fine geographic scales that could provide useful information on the dispersal abilities of mullets (Whitfield et al., 2012).

In this study, we investigate the patterns of distribution of nuclear genetic diversity in populations of *Mugil liza* inhabiting an area under strong fishing pressure at the southernmost distribution range of the species in South America. Our results indicate significant differentiation between Niterói (RJ) mullets and other samples from Southern Brazil and one from Argentina. We discuss our findings in the light of the species' dispersal and migratory abilities and of contemporary barriers to gene flow.

2. Material and methods

2.1. Sampling details

Fifty *Mugil liza* were sampled from May to September 2011 at each of four sites on the Brazilian Atlantic coast, Niterói (Rio de Janeiro State), Ubatuba (São Paulo State), Laguna (Santa Catarina State) and Rio Grande (Rio Grande do Sul State), and at one site on the Argentinean Atlantic coast, Lavalle, Bahía Samborombón (Buenos Aires Province) (Table 1; Fig. 1). Fishes were captured by commercial fishermen using gill nets (70–140 mm mesh size, opposing knots) and were transported on ice to the laboratory; a 5 mm^2 tissue sample was taken from near the caudal fin and stored in 100% ethanol at −4 °C until processing.

Table 1
Information on mullets (*Mugil liza*) analyzed in this study: names and abbreviation of sampling sites (four at the Brazilian Southern Atlantic coast and one in Argentina), geographical coordinates, number of specimens sampled (*N*), mean observed heterozygosity (H_O), mean expected heterozygosity (H_E), and average inbreeding coefficient (F_{IS}) overall nine microsatellite loci are shown.

Country	Sample (abbreviation)	Geographical coordinates	*N*	Mean H_O	Mean H_E	F_{IS}
Brazil	Niterói (RJ)	S22°58′23″; W42°49′41″	50	0.716	0.716	0.177
Brazil	Ubatuba (SP)	S23°27′53″; W44°59′12″	50	0.654	0.694	0.180
Brazil	Laguna (SC)	S28°27′14″; W48°49′01″	50	0.655	0.682	0.054
Brazil	Rio Grande (RS)	S31°58′34″; W52°7′26″	50	0.693	0.699	−0.115
Argentina	Lavalle (BsAs)	S36°10′37″; W57°1′36″	50	0.669	0.685	0.161

2.2. DNA isolation and microsatellites' amplification

Total genomic DNA was extracted from tissue samples using a standard phenol-chloroform procedure (Sambrook et al., 1989) and dissolved in 60 μL of TE buffer. A set of nine microsatellite loci previously described for the "*Mugil cephalus*" complex were used to genotype each sample: Muce-9 (Xu et al., 2010), Mcs17FM, Mcs16DM, Mcs2DM (Miggiano et al., 2005) Mce-4, Mce-11, Mce-14, Mce-24 and Mce-27 (Shen et al., 2010). Polymerase Chain Reaction (PCR) amplifications were performed in a final volume of 12.5 μl containing: 10–50 ng of DNA, 1X PCR buffer, 10 mM of each primer, 100 mM $MgCl_2$, 10 mM dNTPs and 0.5 U of *Taq* Platinum® (Invitrogen, São Paulo, Brazil). The PCR reaction profile included an initial denaturizing step at 95 °C for 5 min, 35 cycles of 94 °C for 30 s, annealing at the specific temperature of each primer for 30 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. Amplified products were separated on 6% denaturing polyacrylamide gels using silver staining (following Creste et al., 2001). Allele sizes were estimated by comparing bands to 10 bp and 50 bp DNA ladder standards (Invitrogen, Brazil).

2.3. Data analyses

Genotypes were inspected for the presence of null alleles, large allele drop-out and/or stuttering using Micro-Checker v2.2.3 (van Oosterhout et al., 2004). GenAlEx v6 (Peakall and Smouse, 2006) was used to estimate the number of alleles per locus, allele frequencies and expected (*He*) and observed (*Ho*) heterozygosities and to conduct assignment tests. The inbreeding coefficient (F_{IS}) (Weir and Cockerham, 1984) and genotypic linkage disequilibrium (*LD*) were computed using default settings in Genepop v4.0 (Rousset, 2008). The same program was used to test for the departure of genotypic proportions from those expected under Hardy–Weinberg equilibrium (HWE), adjusting the alpha level for multiple comparisons with the Bonferroni procedure (Rice, 1989). A possible recent reduction in population size was investigated using Bottleneck v1.2.02 (Piry et al., 1999), with 1000 replications, and a two-phase mutation model (TPM) with a 7:3 ratio of single-step: multi-step mutations and 30% of variance. The significance of results was evaluated with the Wilcoxon sign-ranked test. A Mantel test was carried out in IBDWS v3.23 (Jensen et al., 2005) to examine the correlation between genetic distance and geographic distance (measured in kilometers along the coastline).