

## Deep genetic divergence in the Southern kingcroaker *Menticirrhus americanus* in its southernmost distribution

Alejandro Márquez<sup>1</sup>\*, Antonio Mateo Solé-Cava<sup>2</sup>, Alfredo Nicolás Pereira<sup>3</sup>, Anderson Vasconcellos<sup>4</sup>, Enrique Pablo Lessa<sup>5</sup>

<sup>1</sup> UMEB-NSAT. Facultad de Ciencias, Universidad de la República (Iguá 4225 Esq. Mataojo C.P., 11400 Montevideo - Uruguay)

<sup>2</sup> Laboratório de Biodiversidade Molecular, Universidade Federal do Rio de Janeiro (Av. Carlos Chagas Filho, 373, Prédio do CCS, Bloco K, 2º andar, sala 20, Ilha do Fundão, Cidade Universitária, Rio de Janeiro, RJ - 21944-970)

<sup>3</sup> Laboratorio de Genética - DINARA-MGAP (Constituyente N° 1476, Código Postal: 11.200, Ciudad Montevideo, Uruguay)

<sup>4</sup> Laboratório de Genética Pesqueira e da Conservação, Universidade do Estado do Rio de Janeiro (Pavilhão Haroldo Lisboa da Cunha Rua São Francisco Xavier, 524 - Rio de Janeiro, RJ - 20550-900 - Brazil)

<sup>5</sup> Departamento de Ecología y Evolución. Facultad de Ciencias, Universidad de la República (Iguá 4225, CP 11400, Montevideo. Uruguay)

\*Corresponding author: [ale@fcien.edu.uy](mailto:ale@fcien.edu.uy)

The Southern kingcroaker *Menticirrhus americanus* (Linnaeus 1758), is distributed from Cape Cod (USA) to Argentina (MENEZES; FIGUEIREDO, 1980). It is considered to be estuarine-dependent as it uses estuarine areas primarily as a nursery (HARDING; CHITTENDEN, 1987). This species is fished throughout a large section of its distribution, so that knowledge of its genetic structure is important for management (BEAUMONT; HOARE, 2003; HARDING; CHITTENDEN, 1987; LEWIS et al., 1999; MENEZES; FIGUEIREDO, 1980). Although morphological differences have not been reported, the discrete distribution of estuaries along the southwestern Atlantic coast could have generated different evolutionary units throughout the vast species distribution. In the region, estuarine conditions were proposed as promoting population differentiation in fishes (BEHEREGARAY; SUNNUCKS, 2001; D'ANATRO et al., 2011; D'ANATRO; LESSA, 2011; RODRIGUES et al., 2014) and marine mammals (COSTA-URRUTIA et al., 2012; MENDEZ et al., 2010). We used mitochondrial DNA sequences to investigate whether individuals of *M. americanus* found between the Río de la Plata (36°S) and Espírito Santo (20°S) belong to a single population or are subdivided into distinct populations that should be treated as independent units that could be associated to the discrete distribution of estuaries. We also assessed the influence of paleoclimatic events in the distribution of the genetic variability of *M. americanus*.

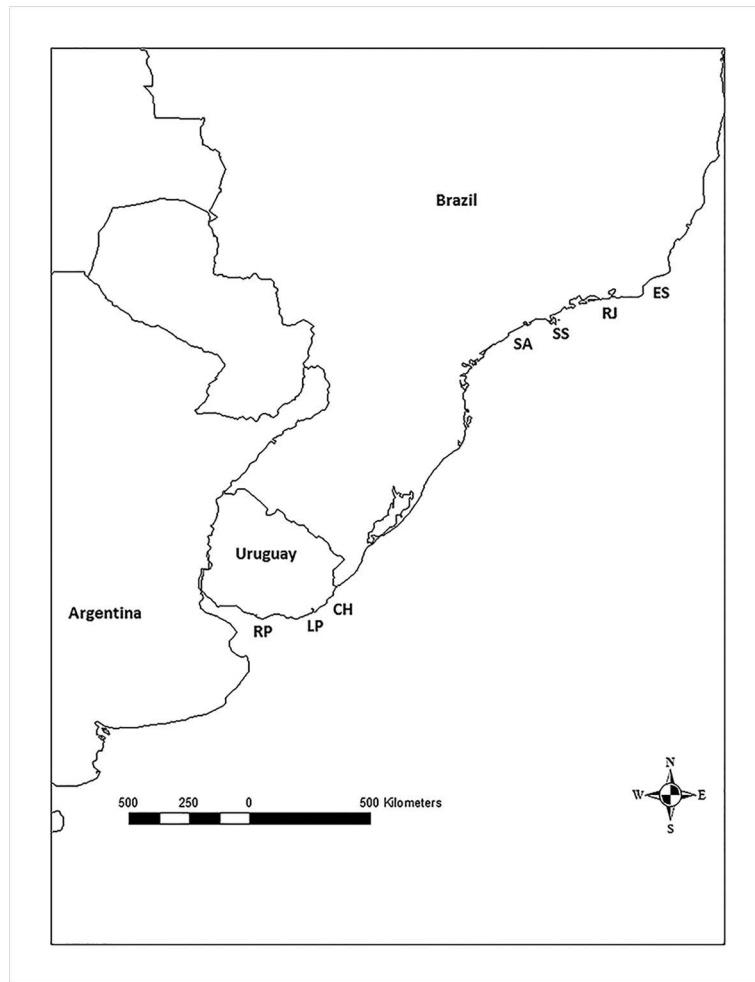
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A fragment of the mtDNA control region (CR) was sequenced from 107 individuals of *M. americanus* collected from 7 localities between Río de la Plata (Uruguay) and Espírito Santo (Brazil): Río de la Plata (n=30), La Paloma (Uruguayan Atlantic Ocean) (n=2), Chuy (Uruguayan coast of the Atlantic Ocean) (n=4), Santos-Cananéia (Southern São Paulo, Brazil) (n=24), São Sebastião (Northern São Paulo, Brazil) (n=21), Rio de Janeiro (Brazil) (n=21) and Vitória (Brazil) (n=5) (Figure 1). Sequences of individuals of *Menticirrhus littoralis* from Rio Grande do Sul, Cananéia and Rio de Janeiro, were used as outgroup. There are no studies evaluating the phylogeny of the genus *Menticirrhus* but SANTOS et al. (2013) showed that the two species are monophyletic, justifying the use of *M. littoralis* as an outgroup. The primers used were ProCor1 (forward) and PhePer (reverse), from PEREIRA et al. (2009), following the PCR conditions described by those authors.

A neighbour joining tree was constructed in PAUP\* 4.0b10 (SWOFFORD, 2001) with 1000 bootstrap pseudo-replicates. A median-joining haplotype network (BANDELT et al., 1999) was constructed using Network (FLUXUS TECHNOLOGY, 2008). Fu's  $F_s$  test (FU, 1997), which is particularly sensitive to demographic fluctuations, was used to test departures from neutrality. When departures from neutrality were detected, demographic history was inferred from the distribution of mismatches between pairs of sequences (ROGERS; HARPENDING, 1992; SCHNEIDER; EXCOFFIER, 1999), and using an Extended Bayesian Skyline Plot approach (DRUMMOND et al., 2005). These tests were carried out with Arlequin 3.5 (EXCOFFIER; LISCHER,

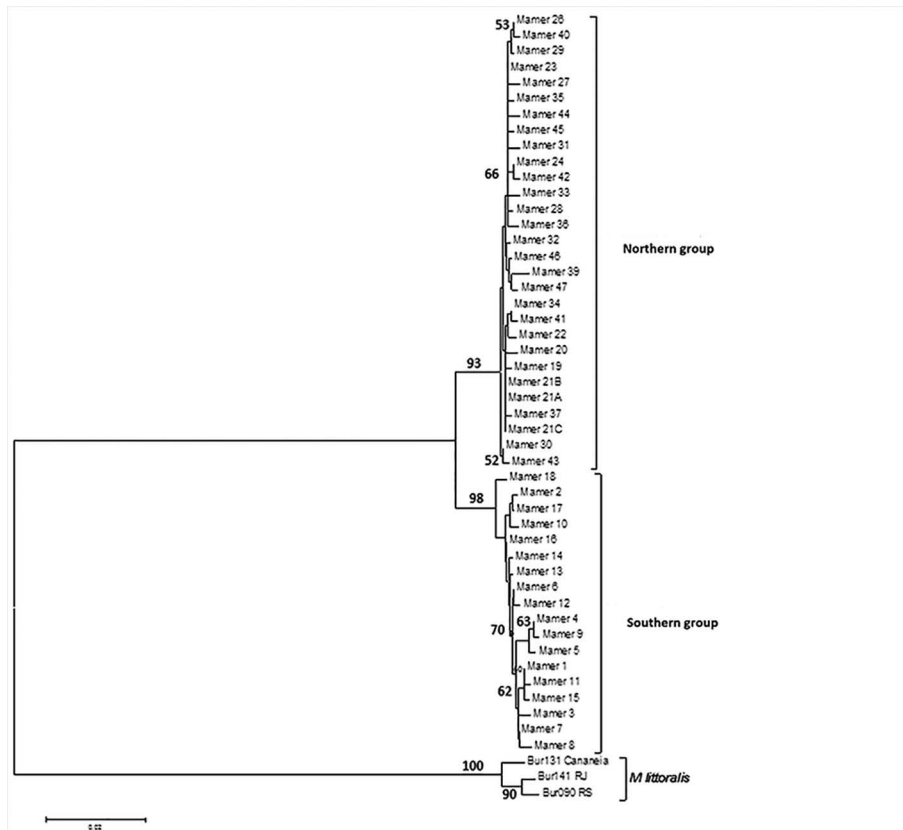


**Figure 1.** Sample localities. RP, Río de la Plata; LP, La Paloma; CH, Chuy; SA, Santos-Cananéia (Southern São Paulo); SS, São Sebastião (Northern São Paulo); RJ, Rio de Janeiro; ES, Espírito Santo.

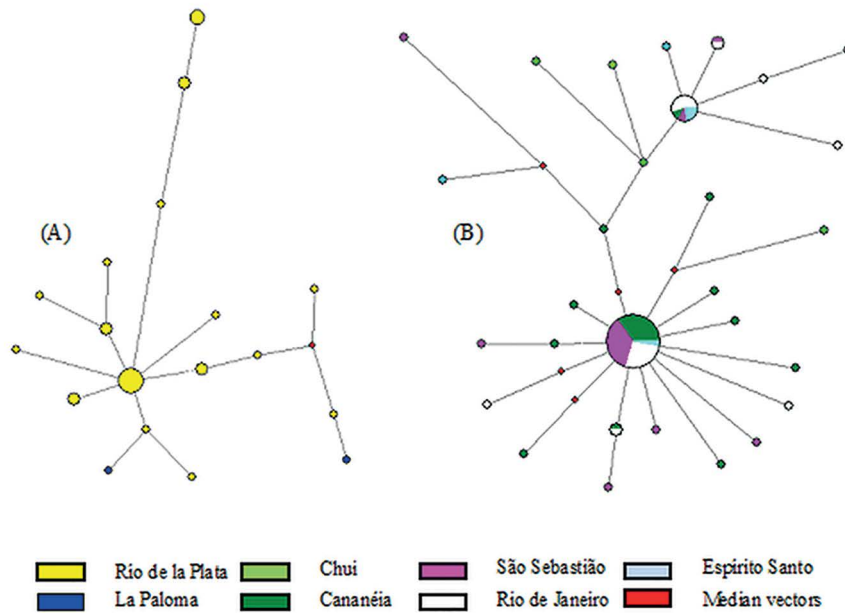
2010). To choose an approximation of the mutation rate for control region sequences, we compared the divergence observed in the control region with that observed with cytochrome b, a genetic marker with a relatively constant rate among taxonomic groups. To do this, we used the distance observed with the mitochondrial cytochrome b, whose substitution rate in fishes is less variable (approx.  $2\% \text{ MA}^{-1}$ ) (BOWEN et al., 2006; BROWN et al., 1993). A divergence of around 10% was calculated using sequences of cytochrome b of *M. americanus* and *M. littoralis*, while the difference observed with the control region was of about 20%. Therefore, an approximated substitution rate for the CR within *Menticirrhus* was estimated as  $4\% \text{ MA}^{-1}$ .

An aligned fragment of 806 bp of the CR resulted in the identification of 47 haplotypes (Accession numbers: JQ322702-JQ322748). The distance tree showed two

reciprocally monophyletic groups with significant bootstrap support (Figure 2). One group is composed of sequences of individuals from the Río de la Plata and La Paloma (bootstrap 96%), that we called the “*Southern group*”. The second monophyletic group (*Northern group*) contained haplotypes found from Chuy (Uruguay) to Espírito Santo (Brazil) (bootstrap 90%). Within these two main clusters, the majority of sub-clusters showed low bootstrap support, probably because of a recent radiation of the matriline. The same phenomenon was observed in the star-like topologies in the haplotype network (Figure 3). The two most common haplotypes in the network separated the clades by 13 mutational steps. The mean genetic distance between the main groups was 0.026 whereas the mean genetic distance between this species and the sympatric species *M. littoralis* was 0.21.



**Figure 2.** Neighbour-joining tree of *Menticirrhus americanus* control region haplotypes. Numbers represent bootstrap supports above 50%.



**Figure 3.** Unrooted haplotype network of the two clades of *Menticirrhus americanus*. (A): Southern group; (B) Northern group. Median vectors are hypothetical not sampled haplotypes.

Both groups had significantly negative Fu's indices. The historical analyses based on the mismatch distribution indicate that the Northern group expanded more recently (1.500; time since expansion = 23,200 years before present, YBP) than the Southern group (2.676; time since expansion = 41,500 YBP). The extended Bayesian skyline plot gives a similar result: the Northern group expanded around 25,000 YBP, coalescing sequence around 41,000 YBP, whereas the Southern group expansion began about 50,000 YBP with sequences coalescing around 78,000 YBP. In the Northern group haplotype network, a star-like topology is observed, with many of the haplotypes diverging between 2 and 3 bp from two abundant and widely distributed central haplotypes. The typical star-like topology of a demographic expansion is not so evident in the Southern group haplotype network (Figure 2).

The southern kingcroaker is considered an estuarine dependent species, so this characteristic could explain the restriction of the Southern group to the Rio de la Plata. This is a particularly big estuary that receives freshwater from the Uruguay and Paraná Rivers. Probably the great size of the Rio de la Plata, could allowed the development of a very differentiated population of individuals better adapted to live in low salinities. This might have favored the differentiation with those individuals who frequently use more typically oceanic waters such as those grouped in the Northern clade. Between Chuy and Rio de Janeiro there were no other genetically divergent sequences that might suggest that the process that led to the diversification of the individuals of the Rio de la Plata could have been repeated in other regions. In the white croaker, *Micropogonias furnieri* (Perciformes Sciaenidae), two genetically distinct populations, one in the Rio de la Plata and the other in oceanic waters of Uruguay have been documented (D'ANATRO et al., 2011; D'ANATRO; LESSA, 2011; PEREIRA et al., 2009). For this species, it was suggested that the genetic drift, together with the adaptation to local environmental conditions could explain the observed genetic differences, but they do not discard that selective pressures imposed by fisheries are promoting differentiation (D'ANATRO et al., 2011) and similar explanations were given to explain genetic differences in estuarine dependent kingfishes (BEHEREGARAY; SUNNUCKS, 2001). Also it was detected genetic differentiation in its southernmost distribution in the sciaenid fish *Macrondon atricauda* (RODRIGUES et al., 2014), and in the marine mammal *Pontoporia blainvillei* (COSTA-URRUTIA et al., 2012;

GARIBOLDI et al., 2016). However, it should be noted that in all of these cases, the differentiation was never as deep as that observed in *M. americanus*, with two mutually monophyletic groups.

Both monophyletic groups went through processes of population expansion that differed in time. Demographic expansion events would have occurred 40,000-50,000 YBP for *M. americanus* in the Río de la Plata and about 25,000 YBP in the Northern group. The absolute times for the demographic events depend on the chosen rate of mutation, but it should be noted that whatever the chosen rate the demographic phenomena in the Rio de la Plata are older than those in the Northern Group. The differences could be explained by differential responses to changes in the sea level of the Pleistocene in the Río de la Plata and in other coastal regions of South America (VIOLANTE; PARKER, 2004). The wider edge of the platform of the Argentinian Shelf could have allowed a greater environmental availability for coastal fishes, compared to northern regions in South America. Additionally, the different historical distribution of suitable environments could cause a limitation to the genetic flux in coastal fish species like *M. americanus*, between the Rio de la Plata and the rest of the South American coast, with the consequent long-term genetic differences.

The identification of one genetically divergent group of the southern kingcroaker in the Rio de la Plata suggest its importance for estuary dependent fish species. The Rio de la Plata seems to be central in providing conditions for the development of genetic differences as was observed in many fish species. Conducting studies of genetic divergence in aquatic species with similar environmental requirements could test the hypothesis that the Rio de la Plata promotes a particularly biodiversity. In the case of the southern kingcroaker, morphological studies could reveal if the genetic differences have a morphological correlation as observed in the case of the whitemouth croaker (GALLI BEDUCHAUD, 2001). The use of other nuclear molecular markers could also uncover the extent of the genetic difference detected with mitochondrial DNA. In order to clarify fine-scale population structure and evaluate connectivity among areas, we also suggest the use of non-maternally inherited hypervariable genetic markers, like microsatellites.

Coasts are highly diverse compared to open waters, but in turn are more vulnerable to disturbance by the presence of cities and, more generally, the concentration of human activities, such as marine traffic and fishing (GRAY, 1997). This is the case of the Rio de la Plata and the recognition

of geographically concordant genetic variation in various species in these waters indicates the need to take particular care for the protection of their diversity.

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