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Population structure and identification of two matrilinear and one patrilinear mitochondrial lineages in the mussel *Mytella charruana*

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ABSTRACT

The mitochondrial gene cytochrome *c* oxidase subunit I (*COI*) was sequenced from *Mytella charruana* ($N = 243$) at 10 Brazilian coastal localities to search for cryptic species, doubly uniparental inheritance and investigate genetic population structure and demography. Three haplogroups were found: two matrilinear (A and B) in males and females, and one patrilinear (C) found only in males. The p-distances were 0.0624 (A and B), 0.2097 (A and C) and 0.2081 (B and C). Coalescence of *M. charruana* occurred around 12.5 Mya, and the origins of the lineages were 3.4 and 4 Mya (matrilinear A and B) and 51.2 Mya (patrilinear), which split before the separation of the genera *Perna* and *Mytella*. All individuals from the northern coast of Brazil belonged to haplogroup A, whereas haplogroup B predominated among individuals from the eastern and northeastern coasts, with one exception, Goiana. Haplogroup C was found in males from the northern to the eastern coast. GenBank sequences of *M. charruana* from Colombia, Ecuador and four populations introduced to the USA joined Brazilian haplogroup B. Nuclear gene 18S-ITS1 sequences confirmed that all specimens belong to the same species. Four populations from the northern coast of Brazil were homogenous with evidence of recent population expansion. All populations from the northeastern and eastern coasts of Brazil were significantly structured (pairwise F_{ST} and AMOVA). The heterogeneity among Brazilian populations requires that relocation for aquaculture be preceded by genetic identification of the haplogroups. Differences in salinity and temperature may have selected for distinct lineages of mussels and changing conditions in coasts and estuaries may allow only resistant lineages of mussel to persist with the loss of others. In the light of global climate change, more detailed data on temperature, pH, salinity and local currents could help explain the genetic structuring observed among populations of Brazilian *M. charruana*.

1. Introduction

Mytella charruana (d'Orbigny, 1842) (Mytilidae, Bivalvia), the charru mussel, is native to Central and South America where it ranges, along the Pacific coast, from Guayamas, Mexico to southern Ecuador and the Galapagos Islands (Rios, 1994; Cardenas and Aranda, 2000) and, along the Atlantic coast, from Colombia to Argentina (Keen, 1971). Introíni et al. (2010) listed the diverse synonyms attributed to *M. charruana,* including *Mytella falcata* d'Orbigny, 1846, *Modiola falcata* Von Ihering, 1897, *Mytilus strigatus* Von Ihering, 1900, *Mytilus arciformis* Dall, 1909, *Mytilus mundahuensis* Duarte, 1926 and *Modiolus falcatus* Morretes, 1949.

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Laboratory experiments show that *Mytella charruana* (synonym of *Mytella falcata*) planktonic larvae begin to settle on the substrate only after at least seven days (Paranaguá and Carvalheira, 1972), thus allowing a high level of dispersion. The latter is common in species with a long planktonic larval stage and results in greater gene flow and low levels of population genetic structure (Hoskin, 1997; Murray-Jones and Ayre, 1997; Collin, 2001), in contrast to species with direct development (Collin, 2001). Environmental factors, such as temperature, salinity, physical barriers, long geographic distances, and oceanic current patterns, may, however, limit gene flow in species with planktonic development (Launey et al., 2002).

The charru mussel is an euryhaline species (Leonel and Silva, 1988; Gillis et al., 2009) that tolerates salinities varying between 2 and 40 (Yuan et al., 2010). These bivalves may be found in shallow coastal lagoons and in muddy areas of bays and estuaries (Sibaja, 1988) in water temperatures of between 6 °C and 31 °C (Brodsky et al., 2009). Along the eastern coast of the United States, the charru mussel is considered an invasive species and has been found in Florida since 1986, most likely being introduced via ballast water or encrusted on ship hulls (Gillis et al., 2009).

Molecular studies carried out on marine and freshwater bivalves show evidence for the presence of two lineages of mitochondrial DNA (mtDNA), one maternal (mtDNA F) and another paternal (mtDNA M) in origin due to doubly uniparental inheritance (DUI) of mtDNA (Skibinski et al., 1994; Hoeh et al., 1996; Theologidis et al., 2008). In this pattern of inheritance, females are typically homoplasmic for mtDNA F and transmit it to both sons and daughters, whereas males are heteroplasmic, carrying both mitochondrial genomes, whereas mtDNA F is predominant in somatic cells and M in gonadal cells, but transmitting mtDNA M only to their sons (Hoeh et al., 1996, 2002; Zouros, 2013). Although both lineages evolve independently (Hoeh et al., 1996; Breton et al., 2007), mtDNA M evolution is more rapid (Hoeh et al., 1997; Passamonti and Ghiselli, 2009). DUI has been widely studied in bivalves and has been recorded in the Mytilidae (Breton et al., 2007; Passamonti and Ghiselli, 2009; Zouros, 2013). Alves et al. (2012) detected mitochondrial heteroplasmy due to DUI in *Mytella charruana* from the Brazilian coast, showing evidence for the presence of this type of inheritance in yet another species of mytilid. According to the latter authors, intraspecific divergence between mtDNA F and M in *M. charruana* cytochrome *c* oxidase subunit I (*COI*) varied between 20.5 and 20.8%. However, the DUI pattern of inheritance was not found in three species of the mussel *Perna* (Wood et al., 2007) and, according to Gillis et al. (2009), DUI appeared to be absent in *M. guyanensis* and *M. charruana*. The discrepancy between the results of the latter two studies regarding *M. charruana* is explained by Alves et al. (2012) as a consequence of the choice of primers used by Gillis et al. (2009), that were only able to amplify *COI* fragments of matrilinear lineages.

An ancient polymorphism (L and M groups) with a 7.3% COI sequence divergence was found between populations of the mussel *Brachidontes pharaonis* in the Mediterranean Sea and in the Red Sea between which, despite differences in haplotype frequencies, gene flow appears to be extensive (Sirna-Terranova et al., 2006). However, the population from Salina di Marsala had private haplotypes from L and M groups in high frequencies and the researchers hypothesize that these haplotypes are selectively advantageous in waters with high temperature and salinity. A more widespread analysis of the genus *Brachidontes* by the same authors, using COI and 16S rDNA mitochondrial sequences, revealed three geographically distinct monophyletic clades: *B. pharonis* from the Mediterranean and Red Seas, *B. variabilis* from the Indian Ocean that also has DUI, and *B. variabilis* from the western Pacific Ocean, which have divergence values corresponding to interspecific values in other bivalves and thus belong to three cryptic species (Sirna-Terranova et al., 2007).

Oliveira et al. (2005), using allozymes, found intraspecific structure among populations of *Mytella charruana* and *M. guyanensis* from the Brazilian coast, and, although analyzes indicated limited gene flow, the observed structure was not according to the isolation by geographic distance model. These authors were unable to give reasons for the retention of gene flow due to the lack of detailed studies of larval dispersion and settlement. On the other hand, Gillis et al. (2009) analyzing molecular data (*COI*) of two invasive populations of *M. charruana* in Florida (USA) and two native populations from South America (Cartagena in Colombia and Guayaquil in Ecuador), found distinct haplotypes among the South American populations. Zardi et al. (2007), sequencing *COI*, found two genetic lineages (a western and an eastern one) of the brown mussel *Perna perna* on the South-East coast of South Africa that could be explained by the pattern of ocean currents in that region. Populations of bivalves from other families have also been studied from the Brazilian coast using *COI* gene sequences. Arruda et al. (2009) found heterogeneity among four populations of *Anomalocardia brasiliana*, whereby one population presented isolation by distance (Ilha Canela, Bragança) and another isolation due to physical barriers (Camurupim). Population studies carried out by Lazoski et al. (2011) with allozymes and *COI* sequences, show intraspecific genetic structure in the oysters *Crassostrea brasiliana* and *Crassostrea rhizophorae*, which, according to the authors, follows the isolation by distance model.

There are only a few genetic studies of mussels of the genus *Mytella* (Oliveira et al., 2005; Gillis et al., 2009; Alves et al., 2012). The present study is the first based on molecular data (*COI* and 18S-ITS1) from a wide range of populations along the Brazilian coast and the objective of this paper is to investigate the genetic diversity, population structure, and demographic history of the native mussel species *Mytella charruana*. Moreover, our study aims to identify useful DNA markers for genetically characterizing the species *M. charruana*. As three very distinct groups of *COI* sequences were found, this led us to investigate the possibility of the presence of cryptic species, an ancient polymorphism and/or doubly uniparental inheritance, all of which have previously been described in the Mytilidae (Lee and Ó Foighil, 2004; Sirne Terranova et al., 2006, 2007; Alves et al., 2012).

2. Material and methods

A total of 243 individual mussels were sampled at 10 localities along the Brazilian coast, from Oiapoque on the northern coast, to Antonina, on the eastern coast. The sample size ranged from 15 to 30 at each locality (Table 1). Whole specimens were conserved in 92% ethanol and frozen at −20 °C pending laboratory analyzes. In order to determine which COI haplotypes are patrilinear and which are matrilinear, the sex of each individual was determined in six specimens of *Mytella charruana* from Bragança and ten from Maceió using hematoxilin-eosin dye and the methods described by Alves et al. (2012). The sex of charru mussels from Antonina ($N = 10$) was determined by using fresh gonad smears by adding one drop of distilled water to the gonad material, which was examined under the light microscope (400×). All mussels were morphologically identified to species and the shells were deposited in the Laboratório de Conservação e Biologia Evolutiva of the Universidade Federal do Pará (LCBE/UFPA) or in the Museu de Zoologia da Universidade de São Paulo (MZSP).

DNA was extracted from adductor muscles using the phenol-chloroform protocol of Sambrook and Russell (2001). DNA from gonads was extracted when the muscle sequences (mitochondrial