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Historical population dynamics and demography of the eastern Atlantic pomacentrid *Chromis limbata* (Valenciennes, 1833)

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Abstract

Recent studies have focused on the relationship between the marine fauna of the eastern Atlantic and the Mediterranean Sea, but within the Atlantic, little is known about genetic relationships between populations of the Macaronesian islands. In this study, we tested whether the paleo-climatology and paleo-oceanography of the region could predict the genetic relationships among three eastern Atlantic populations (Azores, Madeira, and Canaries) of a damselfish, *Chromis limbata*, and compared our results with its Mediterranean and adjacent Atlantic sister species, *Chromis chromis*. We combined phylogeographic and coalescent approaches using the fast evolving mitochondrial control region gene. No population structure was found for the three archipelagos. The coalescence time estimated for *C. limbata* (0.857–1.17 Mya) was much greater than that estimated for *C. chromis*. We propose that this difference reflects differences in glaciating extents in the Northeastern Atlantic and the Mediterranean. Diversity indexes (Hd and genetic distances) together with historical demographic parameters of *C. limbata* (Θ and g) revealed a more stable population history when compared to *C. chromis*. Our results suggest that the Macaronesian populations of *C. limbata* have probably been less affected by the last glaciation than the Mediterranean populations of *C. chromis*. Migration across the three archipelagos was estimated and a prevailing northwest trend was detected. This result supports the idea of a colonization of the Azores by warm water fish from Madeira or the westernmost Canary islands which acted as major glacial refugia for the tropical and subtropical marine fauna during the glaciations.

Keywords: *Chromis limbata*; Northeastern Atlantic islands; Phylogeography; Glaciations; D-loop

1. Introduction

To understand the relationships between speciation and dispersal/population dynamics in marine species, genetic connectivity between populations are usually studied across geographic boundaries. Recently, studies have focused on the relationship between the eastern Atlantic and Mediterranean (Alvarado Bremer et al., 2005; Bargelloni et al., 2003, 2005; Costagliola et al., 2004; Domingues et al., 2005; Lemaire et al., 2005; Naciri et al., 1999; Stamatis et al., 2004; Zardoya et al., 2004). In contrast, little is known about genetic relationships between populations of the Macaronesian islands (Azores, Madeira, Canaries, and Cape Verde; Almada et al., 2005; Guillemaud et al., 2000). These isolated oceanic islands constitute interesting model systems for the study of colonization processes, as several climatic and oceanographic phenomena have played a major role in the history of the ichthyofauna of these islands (Almada et al., 2001; Miller, 1984; Santos et al., 1995; Zander, 1980).

During the Pliocene, the eastern Atlantic experienced a progressive cooling that reached its extreme with the Pleistocene glaciations (Adams et al., 1999; Briggs, 1996). The western coast of Portugal was particularly affected by a

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very pronounced southward migration of the polar front, which caused a significant dropping of sea water temperatures in this region (Crowley, 1981; Dias et al., 1997). The Madeira islands, located further south, were less affected, while the Canaries were severely affected due to its proximity to the continent, although probably less in the western islands, which nowadays present the higher temperatures (Barton et al., 1998) and warmer fauna of all the temperate Macaronesia (Brito et al., 2001). The Cape Verde islands, although remaining considerably warm, were clearly out of the Tropical bio-region (Briggs, 1996). The sea surface temperatures in the Azores region experienced a small cooling (2–3 °C) (Crowley, 1981). Several authors (Briggs, 1974; Miller, 1984; Santos et al., 1995) suggested that this drop in sea surface temperatures has probably resulted in mass extinctions of littoral fish at the Azores, and that most of the organisms now present would have recolonized the islands after this glaciating event from some southern regions such as Madeira.

In marine organisms, oceanographic conditions play an important role in colonization processes, particularly in those whose dispersal is restricted to their planktonic larval phase. The Northeastern Atlantic current system is dominated by the Gulf Stream, which splits into two main branches, the North Atlantic Current (flowing north) and the Azores Current (flowing east). Close to the Azores islands, each of these currents divides into two branches, one of which flows south, feeding the Madeira and Canaries currents (Santos et al., 1995; Stramma, 1984). This multibranch system is even more complex due to seasonal variations of the mean current directions, and as it is a source of meanders and eddies (Santos et al., 1995; Stramma, 1984). Although dominant average ocean current circulation reaches the Northeastern Atlantic islands from the west, the marine littoral fauna and flora of the temperate Macaronesia (Azores, Madeira, and Canaries) share affinities with the eastern coasts of the Atlantic and the Mediterranean (Boury-Esnault and Lopes, 1985; Brito et al., 2001; Brito and Ocaña, 2004; Gofas, 1990; Lloris et al., 1991; Prud'homme van Reine, 1988; Weerdt, 1989; Wirtz and Martins, 1993). This is probably the result of episodic anomalies of the water movements described above (Santos et al., 1995), and of the temperate conditions that the northwest African upwelling confers to these islands.

Chromis limbata (Valenciennes, 1833) is a species restricted to the Macaronesian islands (Azores, Madeira, and Canaries) and the western coast of Africa (between Senegal and Congo, Edwards, 1986; Wood, 1977; L. Rocha et al., unpublished). The Cape Verde islands bear an endemic and a tropical amphiatlantic *Chromis* species (Edwards, 1986). *Chromis chromis* (Linnaeus, 1758), the most likely sister species of *C. limbata* (Edwards, 1986; Wood, 1977; L. Rocha et al., unpublished) is found in the Mediterranean and adjacent Atlantic. *Chromis limbata* inhabits rocky areas from 3 to 50 m, where it forms aggregations in midwater (Brito et al., 2002). During the summer, nesting males defend territories and take care of the eggs that are attached to the substratum (Mapstone and Wood, 1975). In the case of *C. chromis*, after a pelagic larval phase of 18–19 days (Raventós and Macpherson, 2001) fish settle to adult grounds. It is likely that *C. limbata* have similar life history parameters.

The goal of this study was to test whether the paleo-climatology and paleo-oceanography of the region could predict the genetic relationships among the three eastern Atlantic populations (Azores, Madeira, and Canaries) of *Chromis limbata*. Our working hypothesis was that relict populations of *C. limbata* from the Madeira refugium would have been the source of re-colonization of the other two populations. To address this question, we combined a phylogeographic and coalescent approach using the fast evolving mitochondrial control region gene.

2. Materials and methods

2.1. Sampling and DNA extraction

Samples of *C. limbata* were obtained from one island of the archipelagos of the Azores, Madeira, and the Canaries (Fig. 1). *C. chromis* (*C. limbata* sister species) was used as outgroup. Samples were collected by spear fishing or hand nets while scuba diving. Fin clips were cut immediately after collection of the individuals and stored at ambient temperature in 95% ethanol. Tissues were digested overnight at 55 °C in 700 μl of extraction buffer (400 mM NaCl, 10 mM Tris, 2 mM EDTA, 1% SDS). We purified the DNA by standard chloroform extraction and isopropanol precipitation (Sambrook et al., 1989).

2.2. DNA amplification and sequencing

Amplification of the 5′ hypervariable portion of the mitochondrial control region (also called D-loop) was accomplished with universal primers CR-A and CR-E (Lee et al., 1995), and used a cycling profile of 45 s at 94 °C, 45 s at 52 °C, 1 min at 72 °C, for 35 cycles. Each 13 μl reaction contained 5–50 ng of DNA, 10 mM Tris–HCL (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1.25 u of *Taq* DNA polymerase (Perkin-Elmer, Norwalk, Conn.), 150 mM of each dNTP, and 0.3 mM of each primer. After purification following the manufacturer's protocol (Applied Biosystems, Forter City, CA), direct sequencing was performed with an ABI 3100 automated sequencer (Applied Biosystems).

2.3. Data analysis

We used the computer program Clustal V implemented by Sequence Navigator (Applied Biosystems) to align the sequences. Number of haplotypes and haplotype diversity were calculated using the software package DNAsp (Rozas et al., 2003).

Phylogenetic relationships of *C. limbata* individuals were assessed using the neighbor-joining and maximum parsimony methods implemented by the software package PAUP