Short Communication

Phylogeography and demography of the Blenniid *Parablennius parvicornis* and its sister species *P. sanguinolentus* from the northeastern Atlantic Ocean and the western Mediterranean Sea

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1. Introduction

Studies on the phylogeography and historic demography of the marine fish fauna of the northeastern Atlantic Ocean and the Mediterranean Sea have demonstrated the impact of the Pleistocene glaciations on their populations (Domingues et al., 2006, 2007a,b; Stefanni et al., 2006). Estimates of sea surface temperatures (SST) of the northeastern Atlantic over the last 280 Kyr showed a clear glacial/interglacial evolution and a steep north–south SST gradient between 37 and 45°N during the last glacial period (Calvo et al., 2001). SST at the Azores region were estimated to be 2–3°C lower than present day values (CLIMAP, 1976; Crowley, 1981). The archipelago of Madeira, located further south, experienced negligible variations in SST, while the eastern islands of the Canaries were more affected due to their proximity to the continent (Fig. 1, Calvo et al., 2001). Santos et al. (1995a) mentioned that the drop in SST at the Azores, might have been enough to promote the local disappearance of the warm water marine fish in the region. The same authors propose the Madeira Islands and the western coast of Africa as glacial refugia and source of fish for post-glacial colonization of the Azores. Almada et al. (2001) argued that the warmer water fish of the Atlanto-Mediterranean area survived in two distinct glacial refugia which acted as sources of

post-glacial colonization: one in the west coast of Tropical/Subtropical Africa and Madeira, from which fish reached the Azores, and another inside the Mediterranean, from which the northeastern Atlantic waters adjacent to the Mediterranean entrance were colonized during the interglacials. These ideas have been supported by molecular studies on species like the pomacentrid *Chromis limbata* (Domingues et al., 2006) and the blenniid *Tripterygion delaisi* (Domingues et al., 2007a).

The Mediterranean has also been impacted by the Plesitocene glaciations experiencing considerable reduction in SST (Hayes et al., 2005), except for some preserved warm water pockets in the southern regions of this Sea (Thiede, 1978). Effects of the cooling events of the Mediterranean have also been identified by the molecular analysis of *C. chromis* (Domingues et al., 2005), *T. delaisi* (Domingues et al., 2007a) and *Diplodus sargus* (Bargelloni et al., 2005; Domingues et al., 2007b).

The species pair *Parablennius parvicornis* (Valenciennes, 1836) and *P. sanguinolentus* (Pallas, 1814) (Piscies: Blenniidae) constitutes a promising system to test the biogeographical hypothesis presented above. These blenniids have been described as two distinct species (Almada et al., 2005a) and their sister status has been demonstrated in a molecular phylogeny of the northeastern Atlantic and Mediterranean blenniids (Almada et al., 2005b). Almada et al. (2005a) commented on the pattern of geographic distribution of the species pair, highlighting the interest of a population survey to address biogeographical issues. *Parablennius parvicornis* occurs in the western African coast, south of Cape Blanc to the Congo river, including the

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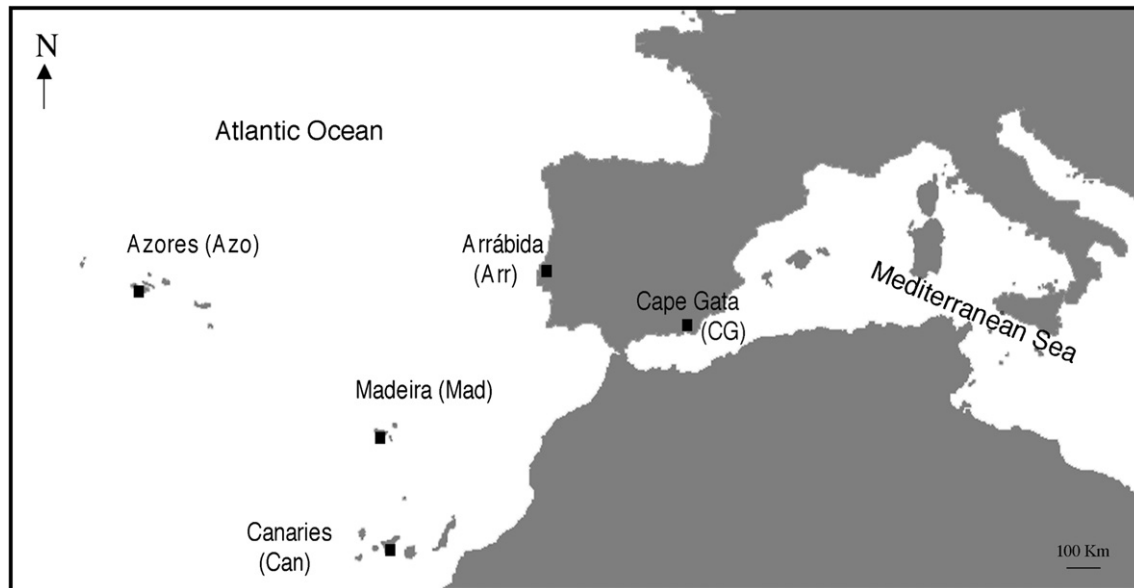*E-mail address:* v_domingues@yahoo.com (V.S. Domingues).

Fig. 1. *Parablennius parvicornis* and *P. sanguinolentus* sampling locations. Individuals of *P. parvicornis* were collected in the islands of Fayal (Azores), Madeira, and Tenerife (Canaries). Samples of *P. sanguinolentus* were collected in Cape of Gata.

archipelagos of Azores, Madeira, Canaries and Cape Verde. The distribution of *P. sanguinolentus* includes the Mediterranean and the Atlantic coast between the Gulf of Biscay and Morocco (north of Casablanca, Almada et al., 2005a and references therein). The two species are allopatric and are separated by a gap of at least 13° of latitude. These fishes are intertidal and are found in well-illuminated sites in sheltered areas with algae-covered rocks (Bath, 1990; Zander, 1986). Like other blenniids, *P. parvicornis* and *P. sanguinolentus* have demersal adhesive eggs guarded by the male (Santos, 1989) and planktonic larvae that remain in the water column for over a month (Santos et al., 1995b; Raventós and Macpherson, 2001).

In this note we analyze mitochondrial control region sequences of populations of *P. parvicornis* from the Atlantic archipelagos of Azores, Madeira and Canaries and *P. sanguinolentus* from a western Mediterranean location. We aim at determining whether the phylogeography and historical demography of these species fit the biogeographical pattern proposed by Almada et al. (2001) and Santos et al. (1995a).

2. Materials and methods

Samples of *P. parvicornis* were collected from the Azores (Fayal), Madeira (Funchal) and Canaries (Tenerife). Individuals of *P. sanguinolentus* were collected from Spain (Cape of Gata). A sequence of *P. sanguinolentus* from Arrábida (western coast of Portugal) available from GenBank database was also included in the analyses. Collection localities and date of collection are shown in Fig. 1 and Table 1. Fin clips were cut immediately after collection of the individuals and stored at ambient temperature in 95% ethanol. Total genomic DNA was extracted by SDS

proteinase K procedure and purified by standard chloroform and isopropanol precipitation (Sambrook et al., 1989). Amplification of the 5′ hypervariable portion of the mitochondrial control region (also called D-loop) was accomplished with primers Lpro1 (Ostellari et al., 1996) and 12S (Nesbö et al., 1998), using an annealing temperature of 50 °C. Direct sequencing was performed with both primers with an ABI 3100 automated sequencer (Applied Biosystems) yielding a final fragment of 556 bp.

Sequences were aligned using CLUSTAL V (Higgins et al., 1991) implemented by Sequence Navigator (Applied Biosystems). Genetic diversity indexes (number of haplotypes, haplotype diversity and nucleotide diversity) were calculated. Population structure was determined by an analysis of molecular variance (AMOVA; Excoffier et al., 1997) and gene flow (Fst) between populations was estimated. These analyses were performed in the program ARLEQUIN (version 2.000; Schneider et al., 2000).

A network of haplotypes was constructed using the statistical parsimony method (Templeton et al., 1992) implemented in TCS (version 1.21, Clement et al., 2000).

The historical demography of each population was examined using mismatch distributions analysis (Rogers and Harpending, 1992; Rogers, 1995) performed in ARLEQUIN (version 2.000; Schneider et al., 2000). The parameters of the expansion θ_0, θ_1 and τ were computed and the time of the expansion (t) was estimated using the formula $\tau = 2t\mu$, where μ is the mutation rate. In the absence of an estimate of μ for the mitochondrial control region of blenniids, we used $\mu = 8.24 \times 10^{-8}$ that was estimated using an internally calibrated molecular clock for two pomacentrid sister species separated by the closure of the isthmus of Panama (Domingues et al., 2005). This value is very similar to the one applied by Bowen et al. (2006) after a revision of

Table 1
Collection localities of *Parablennius parvicornis* and *P. sanguinolentus* used in the present study and diversity indexes for the mitochondrial control region sequences

	N	nH	Hd	π	Date of collection	GenBank Accession Nos.
Parablennius parvicornis						
Azores (Azo)	22	8	0.736	0.002	November 2002/June 2006	EF554601–EF554622
Madeira (Mad)	29	23	0.980	0.005	September 2003	EF554623–EF554651
Canaries (Can)	18	16	0.987	0.005	November 2005	EF554652–EF554669
Parablennius sanguinolentus						
Arrábida (Arr)	1					AY090789
Cape of Gata (CG)	14	6	0.604	0.001	July 2004	EF554670–EF554683

Number of individuals (*N*); number of haplotypes (nH); haplotype diversity (Hd); and nucleotide diversity (π) for each population are shown. Date of collection and GenBank Accession Nos. are shown in the two last columns.

D-loop molecular clock calibrations for several tropical Atlantic fish species. In addition Fu's Fs neutrality test (Fu, 1997) was used to detect possible population expansions.

3. Results

A total of 83 D-loop sequences were obtained and deposited in GenBank database (Table 1). Madeira and Canaries showed similar haplotype and nucleotide diversities, which were higher than the values for Azores and Cape of Gata (Table 1). The AMOVA analysis showed that 24.36% ($P < 0.001$) of the data variance was explained by differences among populations. Gene flow was shown to be higher between Madeira and Canaries (Fst = 0.016, $P = 0.261$) than between Azores and the other archipelagos (Fst = 0.385, $P = 0.000$ for Azores and Madeira and Fst = 0.280, $P = 0.000$ for Azores and Canaries).

The haplotype networks of the two species are not connected at the confidence level of 95% (Fig. 2). Ancestral haplotypes in each network were inferred as the ones that yielded the highest outgroup weights (Castelloe and Templeton, 1994). Both networks showed very simple patterns with few mutational steps separating the most divergent haplotypes from the ancestror (two steps in the case of *P. sanguinolentus* and 6 steps in the case of *P. parvicornis*). *Parablennius parvicornis* show two common haplotypes, differing by four mutations, from which the remaining haplotypes derive by one or two mutations. Haplotypes were shared between Azores, Madeira and Canaries populations and genetic partition between the three populations was not evident. Few network reticulations are observed suggesting the existence of moderate homoplasy.

The model of sudden expansion was not rejected for any of the populations (Table 2) and mismatch distributions were unimodal (figures not shown). While the values of θ (the compound parameter representing the mutation rate and the female effective population size) were similar before the expansion (θ_0) in the four populations, the values corresponding to the same parameter after the expansion (θ_1) were much higher in the Azores and Cape of Gata. Population expansions seemed to have occurred more recently in the Azores and Cape of Gata than in Madeira and Canary islands.

4. Discussion

Our results showed that *P. parvicornis* from the Atlantic archipelagos of Azores, Madeira and Canaries are genetically connected with a particular strong connection of Madeira and Canaries. In this study we wanted to test whether populations of *P. parvicornis* were differentially affected by the Pleistocene glaciations. Evolutionary relationships among *P. parvicornis* haplotypes resulted in a star-like network (Fig. 2), which is consistent with a recent demographic expansion following a considerable reduction in population size. In the parsimony network Azorean haplotypes tend to be closer to the ancestral one while in Madeira and Canaries there are haplotypes linked by more mutational steps to the most common one. This suggests that if bottlenecks took place in these islands the loss of genetic diversity was less accentuated for Madeira and Canaries than for the Azores. The historical demography analysis revealed the existence of a past population expansion for all the populations (Table 2). Comparison of θ_0 and θ_1 values (the compound parameter representing the mutation rate and the female effective population size before and after the expansion, respectively), showed that the demographic expansion of *P. parvicornis* was more accentuated in the Azores than in Madeira and Canary islands. According to CLIMAP (1976) and Crowley (1981) SST during the Pleistocene glaciations decreased about 2–3 °C in the Azores region. Santos et al. (1995a) suggested that such a decrease would have been enough to promote the local disappearance of warm water species such as *P. parvicornis* from the Azores. The less affected tropical western coast of Africa, the archipelago of Madeira, the westernmost Canary islands and some regions of the Mediterranean have been shown to act as refugia for species that were not able to survive the cold phases in the northern Atlantic locations (Domingues et al., 2006, 2007a,b). Our findings support this hypothesis. Indeed, *P. parvicornis* from the Azores show a strong and recent population expansion that
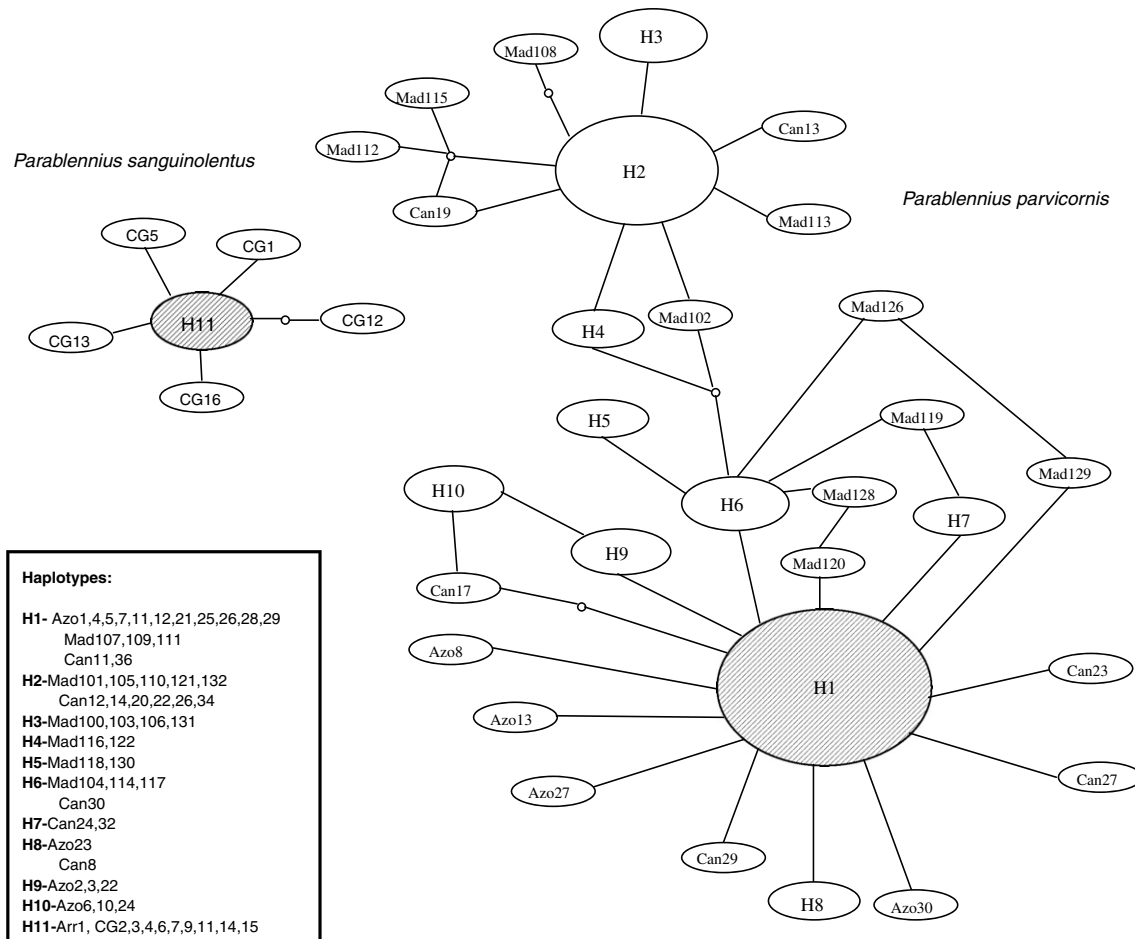
Fig. 2. Statistical parsimony network of *Parablennius parvicornis* D-loop haplotypes. Empty circles represent missing haplotypes. Ancestral haplotypes for each network (Castelloe and Templeton, 1994) are in grey. The size of the circles is proportional to the haplotype frequency. Shared haplotypes are defined in the table. See Fig. 1 for labels.

Table 2
Estimated values for the expansion model for each population of *Parablennius parvicornis* and *P. sanguinolentus* obtained from the D-loop sequences

	SSD	P	θ_0	θ_1	τ	t (Kyr ago)	Fu's Fs	P
Parablennius parvicornis								
Azores	0.006	0.380	0.000	1693	0.228–2.189	1.61–15.5	−4.247	$P < 0.05$
Madeira	0.004	0.460	1.184	40.78	1.132–5.177	8.01–36.1	−23.149	$P < 0.001$
Canaries	0.018	0.360	0.005	8.198	2.051–8.389	14.5–59.3	−13.419	$P < 0.001$
Parablennius sanguinolentus								
Cape of Gata	0.001	0.840	0.000	1252	0.000–1.911	0.00–13.5	−5.997	$P < 0.001$

SSD (sum of square deviations) and its probability P; θ_0 and θ_1 (compound parameter representing the mutation rate and the female effective population size before and after expansion, respectively); and τ (time in generations). The time of the expansion (t) is also presented. Fu's Fs neutrality test and its probability P are shown in the last two columns.

might have occurred after the Younger Dryas at about 12 Kyr (Table 2), when, although already after the Last Glacial Maximum, a large-scale cooling occurred (Lambeck et al., 2002). According to our data, demographic expansions of *P. parvicornis* in Madeira and Canaries were less pronounced and occurred earlier than in the Azores. Interestingly, Azorean fish show lower genetic diversity than Madeira and Canaries populations (Table 1). Lower levels of genetic diversity are typical of recent populations or of populations that have experienced a recent bottleneck, like the one we propose to have occurred in *P. parvicornis* from the Azores. According to this scenario, *P. parvicornis* from the Azores resulted from a post-glacial colonization having its origin in southern, and thus less affected, regions like Madeira. Eddies moving from Madeira towards the Azores, which persist for many weeks and retain the characteristic of the water mass that originated them, have been documented (Santos et al., 1995a and references therein). These eddies can easily transport the pelagic larvae of *P. parvicornis*, which

remain in the water current for over a month (Santos et al., 1995b).

In this study, we were also interested in assessing the effects of this climatic event on one population of *P. sanguinolentus* (the sister species of *P. parvicornis*) from the western Mediterranean. Considerably reductions in SST during the Pleistocene have also been described for the Mediterranean Sea (Hayes et al., 2005), with warm water fish species being confined to southern warmer pockets (Thiede, 1978). The genetic diversity indices and demographic parameters obtained for *P. sanguinolentus* from Cape of Gata yielded a pattern similar to the one obtained for the Azorean *P. parvicornis*. It is thus likely that this population of *P. sanguinolentus* has been drastically reduced during the Pleistocene glaciations having its origin in a post-glacial colonization from the preserved regions of the Mediterranean.

Results on *P. parvicornis* and its sister species *P. sanguinolentus* presented in this study add to the growing evidence of a biogeographical scenario for the Atlanto-Mediterranean warm water benthic species. Previous work on other warm water benthic fish species such as *Tripterygion delaisi* (Domingues et al., 2007a) and *C. limbata/C. chromis* (Domingues et al., 2006) pointed to the existence of two groups of populations: one including the Mediterranean and the Atlantic coast of western Europe and another encompassing the western tropical coast of Africa and the Atlantic archipelagos of the Macaronesia. This pattern may reflect the different effects of the Pleistocene glaciations on warm water fishes that must have become extinct or suffered considerable reductions, in some regions where sea surface temperatures were seriously reduced (western coast of Europe, Azores, eastern Canaries and northern Mediterranean), surviving in less affected regions that acted as refugia. Recolonization of the affected locations may have been possible in the last 10 Kyr, from the western tropical coast of Africa, the western Canaries and Madeira islands, in the case of the Azores, and the southwestern Mediterranean in the case of the Atlantic shores of Iberia (Almada et al., 2001). As suggested by Almada et al. (2001) the operation of this double system of refugia through the entire series of glaciations may have also promoted speciation with the formation of multiple sister species pairs involving one Afro-Macaronesian species and one in the Mediterranean and adjacent Atlantic waters.

Acknowledgments

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