

Phylogeographic pattern and glacial refugia of a rocky shore species with limited dispersal capability: the case of Montagu's blenny (*Coryphoblennius galerita*, Blenniidae)

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Abstract Phylogeographic patterns among coastal fishes are expected to be influenced by distinct ecological, biological and life history traits, along with historical events and oceanography (past and present). This study focuses on the broad range phylogeography of the Montagu's blenny *Coryphoblennius galerita*, a species with well-known ecological features, strictly tied to rocky environments and with limited dispersal capability. Eleven locations from the western Mediterranean to the Bay of Biscay (including the Macaronesian archipelagos) were sampled. Mitochondrial DNA control region (CR) and the first intron of the S7 ribosomal protein gene were used to address the population structure, the signatures of expansion/contraction events retained in the genealogies and potential glacial refugia. The genetic diversity of the Montagu's blenny was high throughout the sampled area, reaching maximum values in the Mediterranean and western Iberian Peninsula. The results confirmed a marked structure of *C. galerita*

along the sampled area, with a major separation found between the Mediterranean and the Atlantic populations, and suggesting also a separation between the Azores and the remaining Atlantic locations. This study revealed complex and deep genealogies for this species, with Montagu's blenny populations presenting signatures of events clearly older than the Last Glacial Maximum, with lineages coalescing in early Pleistocene and Pliocene. Three potential glacial refugia where this species might have survived Pleistocene glaciations and from where the recolonization process might have taken place are suggested: South of Iberian Peninsula/North Africa, Mediterranean and Azores.

Introduction

Many biological factors have been proposed to shape genetic structure in marine organisms, namely ecological and environmental preferences, life history characteristics and duration of planktonic larval stage (Wares and Cunningham 2001). Abiotic factors such as dynamic circulation of regional ocean currents and past historical events also interact with the previously mentioned factors influencing the genetic structure of these organisms (e.g. Muss et al. 2001; Sotka et al. 2004).

Climatic changes have caused marine species to shift their ranges, expanding, retracting or getting in contact with each other along latitudinal gradients associated with Pleistocene glacial cycles (Hewitt 2000). The northeastern Atlantic is a transitional region between the tropics and boreal regions, and its climate has been very dynamic since the Pleistocene, with drastic changes in sea surface temperature (SST) (Lambeck et al. 2002) and in the location of the polar front at the western Portuguese coast during glaciations (Climap 1984; Alveirinho-Dias et al. 1997). Species'

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ranges became fragmented, with the populations of some species driven south and being restricted to isolated refugia during glacial maxima (e.g. Beebee and Rowe 2004). Although these refugia were predominantly located in the south, where the climate was relatively buffered against glacial cycles, some populations are thought to have persisted in northern refugia (for a review see Maggs et al. 2008). Populations of different species vary in their phylogeographic patterns, and a comprehensive picture of how European marine ichthyofauna evolved during the Pleistocene is still far (see Francisco et al. 2014 for a review). Cases of panmixia with no genetic structure throughout the European shore were reported in the literature (e.g. *Petromyzon marinus* in Almada et al. 2008; *Sprattus sprattus* in Debes et al. 2008; *Lipophrys pholis* in Francisco et al. 2011). Other species display clear population differentiation (e.g. *Pomatoschistus microps* in Gysels et al. 2004; *Pomatoschistus minutus* in Larmuseau et al. 2009). Still, in other cases, the present-day structure suggests the existence of small refugial pockets in the north and, potentially, two sources of recolonization after the glaciations (e.g. *Taurulus bubalis* in Almada et al. 2012). The obtained genealogies also vary greatly, from species with populations presenting expansion after the last glaciation (e.g. *Symphodus melops* in Robalo et al. 2012) to deep genealogies dated from early Pleistocene (e.g. *Pholis gunnellus* in Hickerson and Cunningham 2006; *Gasterosteus aculeatus* in Mäkinen and Merilä 2008).

This study focuses on the broad range phylogeography of the Montagu's blenny *Coryphoblennius galerita* (Linnaeus 1758), a species with well-known ecological features, strictly tied to rocky environments and with limited dispersal capability. It is a rocky intertidal fish inhabiting exposed shores of the northeastern Atlantic (Zander 1986). Its distribution ranges from the southwestern coast of England to Morocco, including the Macaronesian archipelagos (Azores, Madeira and Canaries), the Mediterranean and the Black Sea (Zander 1986; Quéro et al. 1990). Breeding takes place in distinct periods of the year, depending on the geographical region: spring/summer in the Atlantic (Almada et al. 1996) or winter in the Mediterranean (Rich-tarski and Patzner 2000). The demersal eggs are guarded by males, and the larvae spend 26–27 days in the plankton (Raventós and Macpherson 2001). The poor dispersal ability of the Montagu's blenny could be indicative of some degree of population structure. Indeed, several studies reported differences between fishes from the Mediterranean and the Atlantic, based on morphological (Bath 1978; Domingues et al. 2007) and molecular data (Almada et al. 2005; Domingues et al. 2007). Using a dataset combining mitochondrial, nuclear and morphological measurements, Domingues et al. (2007) found the existence of two groups in *C. galerita*: one in the Mediterranean and another in the northeastern Atlantic, with a subdivision of the latter with

Azores versus the remaining locations. However, some of the sample sizes in this study were very small, limiting the conclusions in terms of phylogeographic pattern for this species.

This paper represents the more complete phylogeographic study of the Montagu's blenny done so far. We extended the sampling coverage already reported by our research team in the abovementioned work, obtaining a total of eleven locations from the western Mediterranean to the Bay of Biscay (including the Macaronesian archipelagos) and performed the same and new analyses (including coalescent approaches) in an enlarged data set. Mitochondrial DNA CR and the first intron of the S7 ribosomal protein gene were used to address the following questions: (1) Is there any phylogeographic structure in *C. galerita* along its distributional range? (2) How old are the signatures of expansion/contraction events retained in the genealogies? and (3) Where did the Montagu's blenny survived the Pleistocene glaciations?

Materials and methods

Sampling

Specimens of *C. galerita* were obtained from eleven locations along its distributional range in the Northeastern Atlantic and the Mediterranean (Fig. 1, Table 1). These sites included the Macaronesian Islands (Azores—Az, Madeira—Mad and Canary—Can), Gijon—Gij (Asturias, Spain), Camariñas—Cam (Galicia, Spain), Cabo-do-Mundo—CM (Northern Portugal), Estoril—Es (Central Portugal), Praia-da-Luz—Luz (Southern Portugal),

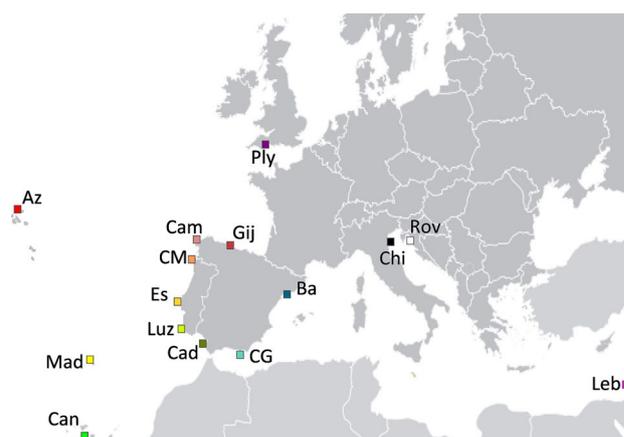


Fig. 1 Map of sampling locations for *Coryphoblennius galerita*. Ply Plymouth, Gij Gijon, Cam Camariñas, CM Cabo-do-Mundo, Es Estoril, Luz Praia-da-Luz, Cad Cádiz, Az -Azores, Mad Madeira, Can Canary, CG Cabo-de-Gata, Ba Barcelona, Chi Chioggia, Rov Rovinj and Leb Lebanon

Table 1 Diversity measures for the collecting sites of *Coryphoblennius galerita* for CR and S7: number of sequences (N), number of haplotypes (N_h), haplotype diversity (h), nucleotide diversity (π) and mean number of pairwise differences (PD)

	Location	Country		N	N_h	h	π	PD
CR								
Atlantic	Gijon	Spain	43°32'N–5°42'W	16	9	0.700	0.007	2.309
	Camariñas	Spain	43°07'N–9°11'W	19	16	0.977	0.006	1.779
	Cabo-Mundo	Portugal	41°13'N–8°42'W	22	15	0.905	0.008	2.352
	Estoril	Portugal	38°42'N–9°23'W	37	11	0.581	0.004	1.256
	Praia-Luz	Portugal	37°05'N–8°44'W	30	11	0.641	0.004	1.322
	Cadiz	Spain	36°31'N–6°16'W	11	9	0.946	0.014	4.535
	Canarias	Spain	28°10'N–15°40'W	21	7	0.557	0.001	0.358
	Madeira	Portugal	32°39'N–16°54'W	21	11	0.724	0.002	0.4869
Mediterranean	Cabo-Gata	Spain	36°47'N–2°06'W	25	23	0.970	0.017	5.671
	Barcelona	Spain	41°23'N–2°11'E	18	18	0.989	0.024	8.176
S7								
Atlantic	Gijon	Spain	43°32'N–5°42'W	30	20	0.977	0.017	10.503
	Camariñas	Spain	43°07'N–9°11'W	42	18	0.921	0.012	7.347
	Cabo-Mundo	Portugal	41°13'N–8°42'W	46	38	0.992	0.027	17.812
	Estoril	Portugal	38°42'N–9°23'W	42	34	0.988	0.033	21.405
	Praia-Luz	Portugal	37°05'N–8°44'W	46	31	0.980	0.033	21.786
	Cadiz	Spain	36°31'N–6°16'W	38	23	0.979	0.012	7.785
	Azores	Portugal	41°44'N–25°40'W	42	26	0.959	0.006	3.429
	Canarias	Spain	28°10'N–15°40'W	46	28	0.936	0.005	3.303
	Madeira	Portugal	32°39'N–16°54'W	42	21	0.928	0.003	1.828
Mediterranean	Cabo-Gata	Spain	36°47'N–2°06'W	40	32	0.990	0.018	11.411
	Barcelona	Spain	41°23'N–2°11'E	40	22	0.977	0.035	21.846

Cádiz—Cad (Andalusia, Spain), Cabo-de-Gata—CG (Andalusia, Spain) and Barcelona—Ba (Catalonia, Spain). Fish were collected in rocky tide pools, and a small piece of dorsal fin was clipped and preserved in 96 % ethanol. The majority of the samples were newly collected for this study; however, sequences obtained for a previous work published by members of our research team (Domingues et al. 2007) were also retrieved from GenBank (Table S-1 in supplementary materials).

DNA extraction, amplification and sequencing

Total genomic DNA extraction was performed with the REDEExtract-N-mp kit (Sigma–Aldrich) following the manufacturer's instructions. PCR amplification of mitochondrial CR and the first intron of the nuclear S7 ribosomal protein gene (S7) were performed with the following pairs of primers: CR—LPro1 (5'-ACTCT CACCC CTAGC TCCCA AAG-3') and HDL1 (5'-CCTGA AGTAG GAACC AGATG CCAG-3') (Ostellari et al. 1996), and S7—S7RPEX1F (5'-TGG CCT CTT CCT TGG CCG TC-3') and S7RPEX2R (5'-AAC TCG TCT GGC TTT TCG CC-3') (Chow and Hazama 1998). Polymerase chain reactions (PCR) were performed in a 20 μ l total reaction volume with 10 μ l of REDEExtract-N-ampl PCR mix (Sigma–Aldrich), 0.8 μ l of each primer (10 μ M), 4.4 μ l of Sigma

water and 4 μ l of template DNA. An initial denaturation at 94 °C for 3 min was followed by 35 cycles (denaturation at 94 °C for 30/45 s, annealing at 55/58 °C for 30/45 s, and extension at 72 °C for 1 min; values CR/S7, respectively) and a final extension at 72 °C for 10 min on a Bio-Rad MyCycler thermal cycler. The same primers were used for the sequencing reaction, and the PCR products were purified and sequenced in STABVIDA (<http://www.stabvida.net/>).

Sequences were edited with Codon Code Aligner (<http://www.codoncode.com/index.htm>) and aligned with Clustal X2 (Larkin et al. 2007). Whenever possible, both strands of the same specimen were recovered for S7 following the approach of Sousa-Santos et al. (2005). This approach takes advantage of the presence of indels in a given nuclear marker and uses them to accurately reconstruct the individual haplotypes without the need of probabilistic estimation. Sequences obtained were deposited in GenBank (Accession numbers KJ440525–KJ440838). Sequences EF521666–EF521790 and EF527585–EF527802 were retrieved from GenBank (Table S1 in supplementary materials).

DNA analyses

The appropriate model of sequence evolution for each fragment was determined using the jModeltest program

(Guindon and Gascuel 2003; Posada 2008), under the Akaike information criterion (AIC) (Nei and Kumar 2000). For both fragments, haplotype networks were constructed with Network v.4.6 (Bandelt et al. 1999) using the median-joining algorithm. For these analyses, some additional sequences from Plymouth—Ply (UK), Chioggia—Chi (Italy), Rovinj—Rov (Croatia) and Lebannon—Leb were added in order to include also haplotypes present in the British Islands and in the eastern Mediterranean.

ARLEQUIN software package v.3.5 (Excoffier and Lischer 2010) was used to estimate the genetic diversity within each sample, to access potential population differentiation and to perform neutrality tests. It was also used to perform analyses of molecular variance (AMOVA; Excoffier et al. 1992) and to compute pairwise FSTs. The correlation between geographic distance and FST was computed with the Mantel test (Mantel 1967; Smouse et al. 1986) (also implemented in Arlequin; 10,000 permutations; geographic distances measured along the shore line). For the CR data set, the Azores sampling location (Domingues et al. 2007) was not included in the population study due to its low size ($N = 9$).

The spatial analysis of molecular variance (SAMOVA 1.0) (Dupanloup et al. 2002) was used to identify groups of sampling locations that are geographically and genetically homogeneous and maximally differentiated from each other. The most likely number of groups was identified by running SAMOVA with two to ten groups and choosing the partition scheme with the highest FCT value. The sequences of the locations included in each of the groups that maximized FCT (see the Results section) were pooled and mismatch analysis (Rogers and Harpending 1992; Rogers 1995), and Fu's F_s (Fu 1997) and Tajima's D (Tajima 1983) tests were performed to test for possible bottlenecks and population expansion in each group.

A MCMC approach taking into account phylogenetic relationships among haplotypes as implemented in LAMARC 2.1.9 (Kuhner 2006) was used to estimate effective population size (N_{ef}), the exponential growth parameter (g) and the migration rates among adjacent groups of populations. In order to compute estimates of effective population size, their changes with time and the age of populations, we used the following mutation rates: 5 % for CR (Bowen et al. 2001) and 0.23 % for S7 (Bernardi and Lape 2005).

Past population demography of *C. galerita* was inferred using the linear Bayesian skyline plot (BSP) (Drummond et al. 2005) model as implemented in BEAST v.1.7 (Drummond and Rambaut 2007), employing the Bayesian MCMC coalescent method and a strict clock. The Bayesian distribution was generated using results from five independent runs of 150 million MCMC steps obtaining effective samples sizes (ESS) of parameter estimates of over 200, with a

burn-in of 10 %. The time to most recent common ancestor (tMRCA) and the median and corresponding credibility intervals of the BSP were depicted using Tracer v.1.5 (Rambaut and Drummond 2007).

Results

For CR, after alignment, a 342-bp fragment was analysed. We obtained 220 sequences defining 120 haplotypes, with a total of 237 polymorphic sites. Differences among haplotypes corresponded to 84 transitions, 57 transversions and 173 indels. The CR sequences revealed a complex pattern, previously referred by Domingues et al. (2007). Motifs of variable length were repeated several times and found to be characteristic of each geographical area. Individuals from the Mediterranean presented a variable number of the motif TATATGTACTAGG. For the Northeastern Atlantic fish, the motif TATATGTACTATACAC was only found in the Azores, while the motif TATATGTACTATACAGTATATGTATGGGTACA was present in fish from other locations. Following the procedure of Domingues et al. (2007), we decided to exclude the central region of the sequences, focusing our analyses on the remaining 342 bp. This decision was based on the alignment and in difficulties in the analyses of sequences caused by the variable number of these repeats (0–12) that would likely cause bias. For the S7 gene, we obtained 454 sequences (corresponding to 227 diploid individuals) corresponding to 252 distinct haplotypes. The fragment obtained was 662 bp long, with 197 polymorphic sites. Differences between haplotypes included 47 transitions, 54 transversions and 115 indels.

The CR haplotype network revealed three subnetworks (Fig. 2). The first includes all the samples from the Mediterranean (group 1; Fig. 2). The second group includes the northeastern Atlantic fish (with the exception of the Azorean samples) and shows a star-like pattern, centred in a dominant Atlantic haplotype (group 2; Fig. 2). The third subnetwork groups together all sequences from the Azores (group 3; Fig. 2). Overall, the global network represents a deep genealogy with multiple levels of diversification.

The haplotype network obtained for the S7 of *C. galerita* is equally deep and diversified (Fig. 3). This haplotype network reveals a more complex pattern, partially due to the twofold number of sequences, with a less obvious geographical structure than the one detected for the CR. Instead of grouping together as in the CR network, the Mediterranean samples are divided in two subgroups, very distant from each other and connected to samples from the adjacent Atlantic. Also, the Azorean specimens do not form an individualized group, sharing haplotypes with the other Atlantic locations.

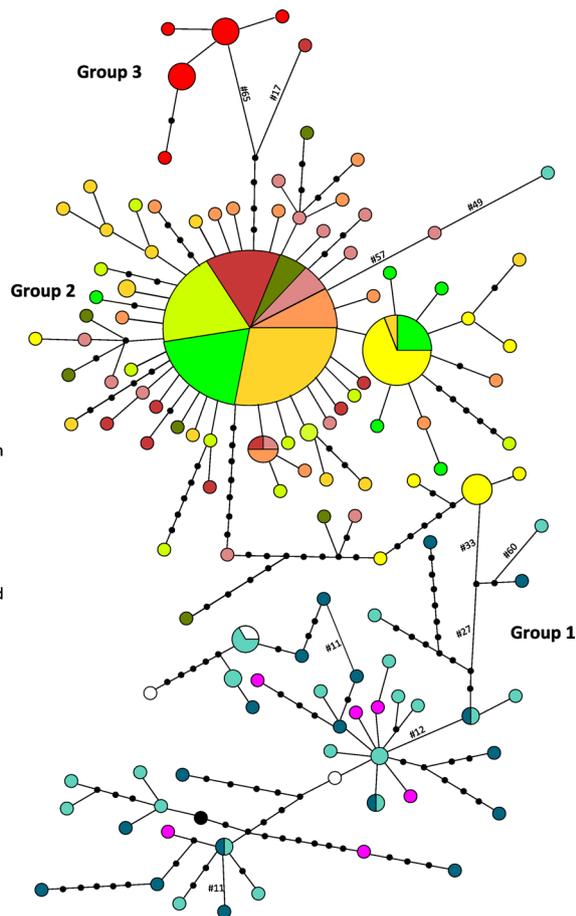


Fig. 2 Median-joining network for the CR of *Coryphoblennius galerita*. The area of the circles is proportional to each haplotype frequency. The colours refer to the region in which haplotypes were found. In the case where haplotypes are shared among regions, shading is proportional to the frequency of the haplotype in each region. Ply Plymouth, Gij Gijon, Cam Camariñas, CM Cabo-do-Mundo, Es Estoril, Luz Praia-da-Luz, Cad Cádiz, Az -Azores, Mad Madeira, Can - Canary, CG Cabo-de-Gata, Ba Barcelona, Chi Chioggia, Rov Rovinj and Leb Lebanon

Genetic diversity indices for each location are summarized in Table 1. CR showed more variability in the magnitude of these indices, with higher values in the Mediterranean and in the west coast of the Iberian Peninsula. Haplotype diversity also yielded high values for S7. For both fragments, Macaronesian locations presented lower nucleotide diversity.

The AMOVA analyses computed for both markers revealed genetic structure along the distribution area of *C. galerita* ($F_{ST} = 0.819$, $P < 0.001$ for CR; and $F_{ST} = 0.342$, $P < 0.001$ for S7). Table 2 shows genetic differentiation and levels of gene flow among collecting sites for the Montagu's blenny. For the CR, significant F_{ST} and corrected average pairwise differences were found between locations in the Mediterranean and Atlantic coasts. The

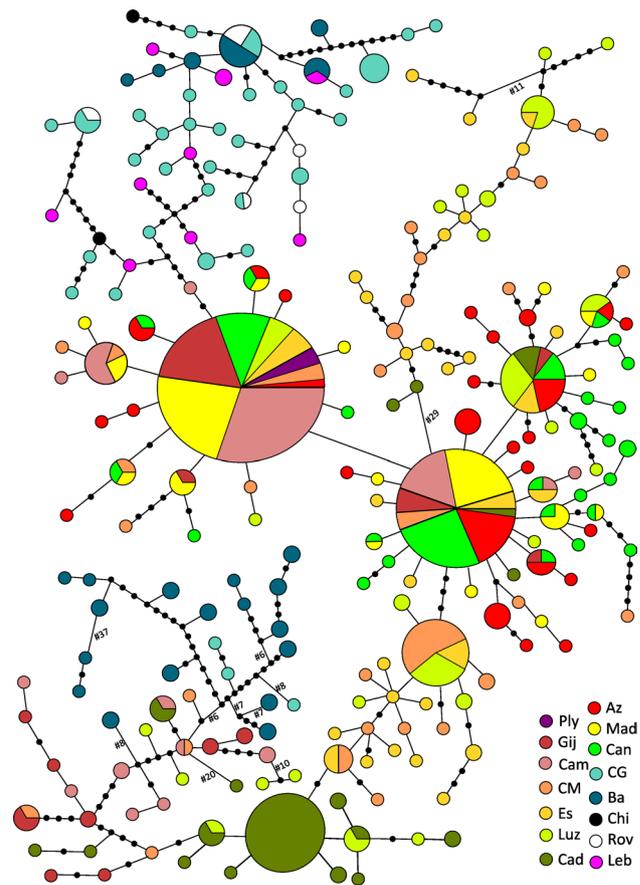


Fig. 3 Median-joining network for the S7 of *Coryphoblennius galerita*. The area of the circles is proportional to each haplotype frequency. Colours refer to the region in which haplotypes were found. In the case where haplotypes are shared among regions, shading is proportional to the frequency of the haplotype in each region. Ply Plymouth, Gij Gijon, Cam Camariñas, CM Cabo-do-Mundo, Es Estoril, Luz Praia-da-Luz, Cad Cádiz, Az Azores, Mad Madeira, Can - Canary, CG Cabo-de-Gata, Ba Barcelona, Chi Chioggia, Rov - Rovinj and Leb Lebanon

S7 data set revealed a more structured distribution of *C. galerita* with almost every pair of collecting sites presenting significant differences (Table 2). For both fragments, the number of migrants was consistently low for pairs of Atlantic and Mediterranean locations ($N_m < 1$, i.e. inexistent), reaching higher values between locations within the two basins (Table S-2).

The SAMOVA of the CR data set yielded a maximized FCT (0.849, $P < 0.001$) for a two-groups set: the two Mediterranean locations (Ba, CG) versus the eight Atlantic locations (Gij, Cam, CM, Es, Luz, Cad, Mad, Can). For the S7, the SAMOVA resulted in a maximized FCT (0.379, $P < 0.001$) for a six-groups set: Barcelona, Cabo-de-Gata in the Mediterranean, Cadiz, Portuguese coast (Luz, Es, CM), Gijon and Macaronesia (Az, Mad, Can) + Camariñas. Given the concordance between pairwise F_{ST} s and

Table 2 Gene flow among collecting sites of *Coryphoblennius galerita* represented by FST (above diagonal) and corrected average pairwise distances (below diagonal) among collecting sites of *Coryphoblennius galerita* for CR and S7

CR	Gij	Cam	CM	Es	Luz	Cad	Az	Mad	Can	CG	Ba
Gij		-0.001	0.009	0.039	0.023	0.086	-	0.267	0.028	0.729	0.816
Cam	0.024		0.004	0.047	0.028	0.001	-	0.160	0.023	0.706	0.778
CM	0.015	-0.003		0.033	0.003	0.123	-	0.287	0.013	0.765	0.848
Es	0.035	0.103	0.053		0.032	0.218	-	0.394	0.036	0.816	0.888
Luz	0.027	0.071	0.001	0.052		0.169	-	0.339	0.040	0.794	0.871
Cad	0.570	0.022	0.554	0.638	0.592		-	0.105	0.177	0.681	0.767
Mad	2.879	2.010	2.553	2.852	2.665	1.197			0.323	0.712	0.784
Can	0.039	0.094	0.024	0.050	0.071	0.633	-	2.587		0.776	0.863
CG	44.882	44.586	45.773	45.687	45.773	43.468	-	45.836	43.958		-0.026
Ba	46.721	46.700	47.679	47.554	47.683	45.423	-	47.693	46.152	-0.463	
S7											
Gij		0.086	0.132	0.204	0.129	0.163	0.301	0.344	0.309	0.450	0.374
Cam	0.798		0.161	0.217	0.180	0.368	0.109	0.107	0.113	0.460	0.481
CM	2.320	2.471		0.007	-0.008	0.214	0.241	0.257	0.253	0.406	0.426
Es	4.393	3.981	0.130		0.002	0.298	0.281	0.293	0.291	0.418	0.443
Luz	2.634	3.289	-0.166	0.046		0.179	0.270	0.283	0.280	0.394	0.393
Cad	1.738	4.401	3.646	6.367	3.414		0.570	0.611	0.578	0.552	0.426
Az	2.707	0.659	3.500	4.846	4.846	7.267		0.008	0.025	0.542	0.590
Mad	2.807	0.547	3.535	4.821	4.870	7.301	0.021		0.008	0.551	0.604
Can	2.701	0.664	3.572	4.871	4.887	7.282	0.084	0.023		0.567	0.600
CG	9.035	7.941	10.159	11.891	11.062	11.898	8.651	8.515	8.665		0.284
Ba	10.230	13.371	14.601	17.206	14.115	11.130	17.833	17.697	17.813	6.589	

Gij Gijon, Cam Camariñas, CM Cabo-do-Mundo, Es Estoril, Luz Praia-da-Luz, Cad Cádiz, Az Azores, Mad Madeira, Can Canary, CG Cabo-de-Gata, Ba Barcelona

Significant values of probability $P < 0.05$ are shown in bold

SAMOVA, we decided to use the mentioned groups, pooling together the sequences for further analyses. The Mantel test did not support a significant correlation between the geographical distance and FST for both CR ($r = 0.397$, $P = 0.289$) and S7 ($r = 0.000$, $P = 1.000$), thus ruling out the hypothesis of isolation by distance for *C. galerita*.

The results of neutral test analyses of the CR indicated negative and highly significant values for Tajima's D and Fu's F_s for both Atlantic and Mediterranean groups, suggesting population expansion in the Montagu's blenny (Table 3). The pattern was not so clear for S7, with F_s for Barcelona being positive and the values for Gijon (F_s and D), Cabo-de-Gata (D) and Barcelona (D) being nonsignificant, although negative (Table 3).

Mismatch distribution analyses suggested demographic and spatial expansion for most locations' groups (Table 3). The estimated time for the demographic expansion yielded 200 thousand years (ky) for the CR in the Mediterranean, while in the Atlantic group a sudden expansion model was not confirmed. For the S7, the estimated time for demographic expansions yielded a complex pattern. Within the Mediterranean basin, a much younger date was estimated for Cabo-de-Gata (98 Ky) than for Barcelona (3,696 Ky). Concerning the Atlantic populations, a time of 4,302 Ky was estimated for the spatial expansion of Gijon, and a much younger value was yielded for Cádiz (197 Ky). Concerning the spatial expansion, the estimated times were

older for the Mediterranean (150 Ky) than for the Atlantic (17 Ky), when considering the CR dataset. For the S7, the estimated times of spatial expansion yielded older values for the Iberian Peninsula shores (10,435 Ky for Cadiz and 10,367 Ky for Portugal) and the youngest value for the group Macaronesia + Camariñas (892 Ky) (Table 3). For both markers, Mediterranean locations presented larger female effective population size (before and after the expansion) when compared to Atlantic locations (Table 3). Lamarc results were generally consistent with the ones obtained from the mismatch distribution analyses (Table 3).

BSP runs failed to converge for the CR of the Atlantic group and for the S7 groups. For the CR of the Mediterranean group, the BSP showed a rapid population growth in the last 4 Ky reaching an effective population size (N_{ef}) of 100,000 fish in the present day. The t_{MRCA} yielded a value of 14 Ky for this group (Table 3).

For the CR, it was not possible to estimate the migration rate, as the connectivity assumption of Lamarc was not met. Migration rates for the S7 of *C. galerita* (Table S-3 in supplementary materials) did not present an obvious pattern. Indeed, within the Mediterranean, the migration rate was higher from Cabo-de-Gata towards Barcelona (average 736.652) than in the opposite direction (average 68.004). In the Atlantic, the migration rate was higher southwards along the Iberian Peninsula coast (average 2,080.386 from Gijon to Portugal and 5,576.298 from Portugal to Cadiz,

Table 3 Demographic parameters of *Coryphoblennius galerita* based CR and S7

	S7								
	CR	Atlantic	Mediterranean	Mac + Cam	Gij	Portugal	Cad	CG	Ba
Neutrality tests									
F_S		-25.200*	-20.312*	-25.592*	-3.688	-23.862*	-6.580*	-14.291*	0.870
D		-2.679*	-1.573*	-2.000*	-1.472	-1.755*	-1.868*	-0.482	-0.063
Mismatch distribution									
<i>Demographic expansion</i>									
τ (95 % CI)		0.555 (0.430 -0.734)	6.771 (1.691 -27.553)	3.300 (1.279 -5.793)	20.100 (7.771 -91.762)	0.600 (0.371 -0.836)	13.100 (0.738 -75.471)	7.200 (4.154 -14.773)	13.900 (7.311 -43.684)
t (95 % CI) (Ky)		na	200 (50-813)	1,313 (594-1,950)	4,302 (242 -24,783)	na	197 (0-5,494)	98 (0-977)	2,400 (4,564 -14,345)
θ_0		0	7	0.000	0.371	0.000	0.002	4.359	15.216
N_0		na	214,049	1,668	577	na	502,199	574,353	4,996,595
θ_1		99,999	21,914	6,953	13,416	99,999	4,099	44,238	55,309
N_1		na	646,432	4,149,717	1,346,187	na	40,686,982	32,838,237,225	18,162,807
SSD		0.183 *	0.008	0.003	0.035	0.401 *	0.024	0.004	0.008
Hri		0.019	0.008	0.011	0.035	0.002	0.025	0.007	0.013
<i>Spatial expansion</i>									
τ (95 % CI)		0.558 (0.491 -5.446)	5.082 (1.050 -39.914)	1.351 (0.525 -4.182)	12.848 (3.814 -18.336)	31.689 (3.967 -61.389)	9.352 (0.896 -15.847)	5.700 (3.497 -11.415)	12.085 (8.804 -37.378)
t (95 % CI) (Ky)		17 (15-192)	150 (31-1,089)	892 (439 -1,493)	3,071 (294 -5,204)	10,367 (815 -18,052)	10,435 (861 -15,922)	6,001 (272 -8,237)	3,969 (2,891 -12,274)
θ		1.830	9.608	1.711	2.840	13.978	2.575	5.951	16.254
N		54,633	283,418	328,376	845,744	4,601,182	4,374,093	744,037	5,337,564
M		99,999	4,990	25,341	1,749	0.740	0.662	47,258	6,419
N_m		50,000	2	9,735	0.331	0.441	0.286	0.199	3.21
SSD		0.005	0.009	0.002	0.015	0.009	0.009	0.005	0.007
Hri		0.019	0.009	0.011	0.035	0.002	0.025	0.007	0.013
<i>Lamarck</i>									
θ (95 % CI)		nc	0.081 (0.064 -0.275)	0.095 (0.024 -1.147)	0.008 (0.002 -0.047)	0.032 (0.009 -0.370)	0.004 (0.002- 0.009)	0.083 (0.033 -0.311)	0.036 (0.017 -0.079)
N_T (95 % CI)		nc	810,000 (640,000 -2,750,000)	20,656,630 (5,262, 935 -249,308,609)	1,742,752 (485,174 -10,112,109)	6,963,478 (1,916,717 -80,384,826)	862,391 (345,391 -2,031,304)	17,952,826 (714,109 -67,628,826)	7,758,870 (3,794,804 -7,176,239)

Table 3 continued

	S7							
	CR	Atlantic	Mediterranean	Mac + Cam	Gijón	Portugal	Cad	CG
G (95 % CI)	<i>nc</i>	193,127 (24,851–544,478)	1,983,713 (919,681–4,261,039)	1,873,091 (–269,144–8,059,959)	1,814,158 (557,035–5,324,932)	–129,484 (–460,493–70,886)	597,248 (218,576–1,377,907)	133,379 (–56,587–369,708)
$N_{1\%}$ (95 % CI) (Ky)	<i>nc</i>	477 (170–3,706)	1,009 (470–2,177)	1,069 (248– <i>na</i>)	1,086 (376–3,594)	<i>na</i> (<i>na</i> –28,246)	3,352 (1,453–9,160)	15,012 (5,416– <i>na</i>)
BEAST								
t_{MRCA} (95 % CI) (Ky)	<i>nc</i>	14 (10–19)	<i>nc</i>	<i>nc</i>	<i>nc</i>	<i>nc</i>	<i>nc</i>	<i>nc</i>

Neutrality tests: F_s (Fu's), D (Tajima's). Mismatch distributions: τ time in generations; upper and lower bounds of 95 % CI in parenthesis, t time in years, θ_0 theta0, N_0 female effective population size before the expansion, θ_1 theta1, N_1 female effective population size after the expansion, SSD sum of square deviation and Hri Harpending's Raggedness index. Estimates of population parameters with LAMARC: θ theta, N_f female effective population size, g growth rate and N_i age of population, accessed as the age at which N_f drops below 1 %. Estimates of t_{MRCA} time to most recent common ancestor with BEAST

Mac + Cam Macaronesia and Camariñas, *Gij* Gijón, *Cad* Cádiz, *CG* Cabo-de-Gata, *Ba* Barcelona, *na* nonapplicable, *nc* no convergence

Significant values of probability P are shown with an *

versus 605.506 and 1,488.349, respectively, in the opposite direction). When we consider the migration rate between mainland shores and the Macaronesia + Camariñas, the signal is not so clear: the migration is higher from Gijón towards Islands + Camariñas (1,037.794) than in the opposite direction (173.342), but is higher from Islands + Camariñas to mainland Portugal (1,417.166) than in the opposite direction (465.749).

Discussion

Phylogeographic patterns among coastal fishes are expected to be influenced by distinct ecological, biological and life history traits, along with historical events and oceanography (past and present). Several marine species present phylogeographic patterns that challenge the idea of extensive connectivity within the marine environment and the corresponding panmixia (see Francisco et al. 2014 for a review). Thus, previously unrecognized levels of diversity among marine fishes and even extensive population subdivision have both been recorded in the literature (e.g. *P. microps* in Gysels et al. 2004; *Taurulus bubalus* in Almada et al. 2012).

Population structure

The genetic diversity of the Montagu's blenny is high throughout the sampled area, with the highest values recorded in the Mediterranean and western Iberian Peninsula, and the lowest in Macaronesia locations (Table 1). Several other studies found similarly high diversity levels (e.g. *S. melops* in Robalo et al. 2012; *T. bubalis* in Almada et al. 2012). The results presented in our study reveal a marked structure of *C. galerita* along the sampled area. A major separation is found, for both molecular markers, between the Mediterranean and the Atlantic populations. Domingues et al. (2007) suggested the existence of a phylogeographic barrier preventing gene flow between Atlantic and Mediterranean populations of the Montagu's blenny. Our results, using a much larger dataset (more samples and more sampling locations) corroborate this hypothesis. In fact, the number of migrants between locations from the two basins is below the threshold of 1, strongly suggesting the existence of an effective isolation between them. This pattern is common to other organisms inhabiting the Mediterranean and the adjacent waters of the Atlantic, being frequently associated with the Strait of Gibraltar or the Almeria-Oran Front (see Patarnello et al. 2007 for a review). In more detail, for the CR, the SAMOVA grouped together all the Atlantic locations, including the ones from Macaronesia (Canaries and Madeira) and Continental Europe. Domingues et al. (2007) found some degree of

separation between the Azores and the group formed by mainland Portugal, Madeira and Canaries. As we decided not to include the Azores in the CR dataset used for the population study due to its low sample size, the comparison with the study by Domingues et al. (2007) is not straightforward. Nevertheless, both the pattern of repeated motifs and the CR network (Fig. 2) suggest the separation between the Azores and other Atlantic locations. In fact, this separation within the Atlantic basin also seems to be corroborated by morphological characters (meristic characters and colour patterns in Bath 1978; crest height and width in Domingues et al. 2007). The genetic isolation of Azores has been reported for other fishes (e.g. *L. pholis* in Stefanni et al. 2006; *Aphanopus carbo* in Stefanni and Knutsen 2007). S7 results evidenced a more complex genetic structuring of *C. galerita*: Cádiz (southwest Spain), Portugal, Gijon (northern Spain), a group comprising the Macaronesian locations (Azores, Madeira and Canaries) + Camariñas (northwestern Spain), Barcelona and Cabo-de-Gata. Interestingly, the two latter groups are located in the western Mediterranean, and structuring within this basin is a relatively uncommon pattern in the literature, as a pattern of panmixia within western Mediterranean is generally reported (e.g. *Dicentrarchus labrax* in Bahri-Sfar et al. 2000). More often, population structure is found between west and east Mediterranean (e.g. *Cerastoderma glaucum* in Nikula and Väinölä 2003; *Thalassoma pavo* in Domingues et al. 2008), and between adjacent Atlantic and west versus east Mediterranean (e.g. *Scomber scombrus* in Zardoya et al. 2004; *Chromis chromis* in Domingues et al. 2005).

For species whose dispersal entirely depends on the planktonic phase, the hydrographic patterns, with their inter-annual variability, are expected to influence the populations supply (e.g. Galarza et al. 2009) and, thus, deeply affect the genetic structure along the species distributional range. The rich current system of the temperate Northeastern Atlantic is dominated by the Gulf Stream, a subtropical gyre, which detaches from the eastern American coast and heads eastwards as the North Atlantic Current (Willebrand et al. 2001). Off the coast of West Ireland, it splits in two: one part recirculates eastwards in the subtropical gyre as the Azores Current, and the other part heads northwards in the subpolar gyre. Near the Azores archipelago, the Azores Current divides into two branches both flowing southwards and feeding the Madeira and the Canary Currents (Stramma 1984; Santos et al. 1995). Despite the fact that dominant average circulation reaches the Macaronesian archipelagos from the west, their marine littoral fauna share affinities with the Mediterranean and western European and African coasts (e.g. Briggs 1974). This is probably related to the fact that this multi-branch oceanographic system also contains coastal upwelling, filaments and eddies and seasonal variations in the mean directions adding even more

complexity to this system (Santos et al. 1995). This circulation pattern simultaneously accounts for the Azorean differentiation of *C. galerita* (as most peripheral population) (CR, Fig. 2) and the lack of structure between the Macaronesian archipelagos and the western Iberian Peninsula (S7, Table 2). When one considers the migration directions between the group Macaronesia + Camariñas and the other Iberian locations, the pattern is not straightforward (Table S-3). Along the Atlantic Iberian shore, the net migration found for the S7 of *C. galerita* (Table S-3) is in accordance with the southwards average flow of the Portugal Current during Spring–Summer (Martins et al. 2002) when this species is described to breed in the Atlantic (Almada et al. 1996). The predominant northeastward gene flow depicted by the S7 of *C. galerita* (from Cabo-de-Gata towards Barcelona, Table S-3) apparently contradicts the circulation pattern around the east coast of the Iberian Peninsula (southwestwards, along the Catalanian and Andalusian shores) (Millot 1999). These results are probably related to the considerable amount of mesoscale activity (eddies, filaments and meanders) and heavy influence of wind patterns that characterize the complex current system of this coast during winter (Millot 1999), when the Montagu's blenny is known to breed in the Mediterranean Sea (Richtarski and Patzner 2000).

Another factor reported in the literature as being in close relationship with a species population structure is the planktonic larval duration (PLD) (reviewed by Weersing and Toonen 2009). The Montagu's blenny presents a PLD of 26–27 days (Raventós and Macpherson 2001). Taken together, the hydrographic patterns of the northeastern Atlantic (past and present) and the relatively long PLD of *C. galerita* could account for the generally high levels of gene flow found, for both CR and S7, between the archipelagos of Macaronesia and western European shores (Tables 2, S-2 and S-3).

Complexity and depth of the genealogies

A new and striking result of this study is the complexity and depth of the genealogies found (Figs. 2 and 3; Table 3). The genealogies of the Montagu's blenny populations showed signatures of events clearly older than the Last Glacial Maximum (LGM, 21–18kya) (Shakun and Carlson 2010), with lineages coalescing in early Pleistocene and Pliocene. Similar results have been found for other fish species in northern Europe (e.g. *G. aculeatus* in Mäkinen and Merilä 2008; *L. pholis* in Francisco et al. 2011, *S. melops* in Robalo et al. 2012).

At the LGM, the European ice sheet extended southwards with the polar front being at the Bay of Biscay (Zaragosi et al. 2001) or even far south at the latitude of Lisbon (38°42'N) according to more conservative works

(Alveirinho-Dias et al. 1997). This implies that the current northern European biota was established, at best, after the LGM. Species with high dispersal capabilities and large effective population size may disperse in large numbers, exporting much of the genetic diversity across large geographical scales and eroding previous phylogeographic signals (Francisco et al. 2014). This hypothesis may explain the deep genealogies found for the populations of *C. galerita*.

Glacial refugia

Judging from the present distribution of *C. galerita*, it is unlikely that this species kept viable populations north of Iberian Peninsula shores. Taking into account the SST during the LGM (Climap 1984), the present-day distribution of the Montagu's blenny and the results obtained in the present work, we suggest three potential glacial refugia where this species might have survived Pleistocene glaciations and from where the recolonization process might have taken place: (1) south of the Iberian Peninsula/North Africa, (2) Mediterranean and (3) Azores. This hypothesis is in agreement with the refugial areas identified for several other species and compiled in Maggs et al. (2008).

At the LGM, the west Iberian coast yielded water temperatures of 7.2 °C (according to Climap 1984), a little lower comparing to the present-day SST in the southwestern England coast where this species inhabits. Thus, at glacial peaks, waters around the Iberian Peninsula and/or northwest Africa were suited for populations of the Montagu's blenny. As the SST rose along the European coastline, these locations must have played an important role for the recolonization of northern areas. Evidences of glacial refugia in the southern European Atlantic shore were also suggested for other coastal fish species (e.g. *Atherina presbyter* in Francisco et al. 2009; *S. melops* in Robalo et al. 2012).

During the LGM (and other Pleistocene glacial stadial), the Mediterranean presented a milder environment with colder (but ice-free) conditions and even warm-water pockets, especially in the south (Thiede 1978). Hence, the role of the Mediterranean as a potential glacial refugium has been profusely suggested, and several cases have been reported (e.g. *Zostera marina* in Olsen et al. 2004; *S. sprattus* in Debes et al. 2008; *Salaria pavo* in Almada et al. 2009). Our results of prolonged isolation between the Mediterranean and the adjacent Atlantic (for both CR and S7 markers) support the existence of a Mediterranean refugium for *C. galerita*, as suggested by Domingues et al. (2007), although with fewer sample locations. However, the role of the Mediterranean as a glacial refugium for Atlantic taxa is yet controversial and far from consensual, as reviewed by Patarnello et al. (2007).

According to several authors, the drop in SST was only moderate at the Azorean archipelago during the LGM (e.g. Crowley 1981; Morton and Britton 2000), and a temperate fish like *C. galerita* might have survived there during Pleistocene glaciations. This would explain our results [and those of Domingues et al. (2007)] on the differentiation of the Azorean Montagu's blenny population. Similar results were already described in the literature (e.g. *Raja clavata* in Chevolut et al. 2006).

Caution should be taken while interpreting the present results. First, and although the present work is the more complete phylogeographic study of the Montagu's blenny so far, it is important that future studies try to cover the entire species range, from the extreme south (Morocco) to the north (Bay of Biscay and southern UK), including eastern Mediterranean and Black Sea. Second, it is important that sample sizes are consistent along the sampled area, allowing more accurate population genetic analyses. Only then, it will be possible to have a more complete picture of the historical demography and phylogeography of this species. Third, we are aware that distinct divergence rates might influence the magnitude of the genealogies depth. In the absence of a clock calibration for the CR of *C. galerita*, we decided to address the uncertainty by tentatively assuming a within-lineage mutation rate of 5 %/MY, which is within the range of values found by Bowen et al. (2001) after a review of CR molecular clock calibrations for several tropical Atlantic fish species. Despite these caveats, ours is the most complete population genetic study of the Montagu's blenny, adding, unquestionably, important knowledge to the study of phylogeographic patterns in the northeastern Atlantic.

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References

- Almada VC, Carreiro H, Faria C, Gonçalves EJ (1996) The breeding season of *Coryphoblennius galerita* in Portuguese waters. *J Fish Biol* 48:295–297
- Almada F, Almada VC, Guillemaud T, Wirtz P (2005) Phylogenetic relationships of the north-eastern Atlantic and Mediterranean blenniids. *Biol J Linn Soc* 86:283–295
- Almada VC, Pereira AM, Robalo JI, Fonseca JP, Levy A, Maia C, Valente A (2008) Mitochondrial DNA fails to reveal genetic structure in sea-lampreys along European shores. *Mol Phylogenet Evol* 46:391–396

- Almada VC, Robalo JI, Levy A, Freyhof J, Bernardi G, Doadrio I (2009) Phylogenetic analysis of Peri-Mediterranean blennies of the genus *Salaria*: molecular insights on the colonization of freshwaters. *Mol Phylogenet Evol* 52:424–431
- Almada V, Almada F, Francisco S, Castilho R, Robalo JI (2012) Unexpected high genetic diversity at the extreme northern geographic limit of *Taurulus bubalis* (Euphrasen, 1786). *PLoS One* 7:e44404
- Alveirinho-Dias J, Rodrigues A, Magalhães F (1997) Evolução da linha de costa em Portugal, desde o último máximo glaciário até à actualidade: síntese dos conhecimentos. *Estudos Do Quaternário* 1:53–66
- Bahri-Sfar L, Lemaire C, Hassine OKB, Bonhomme F (2000) Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. *Proc R Soc Lond B* 267:929–935
- Bandelt H, Forster P, Rohlf A (1999) Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 16:37–48
- Bath H (1978) Geographische variation der körperfärbung und flossenformel von *Coryphoblennius galerita* (Linnaeus 1758) (Pisces: blenniidae). *Senckenb Biol* 59:317–324
- Beebee T, Rowe G (2004) Phylogeography. An introduction to molecular ecology. Oxford University Press, Oxford, pp 165–197
- Bernardi G, Lape J (2005) Tempo and mode of speciation in the Baja California disjunct fish species *Anisotremus davidsonii*. *Mol Ecol* 14:4085–4096
- Bowen BW, Bass AL, Rocha LA, Grant WS, Robertson DR (2001) Phylogeography of the trumpetfishes (*Aulostomus*): ring species complex on a global scale. *Evolution* 55:1029–1039
- Briggs JC (1974) Marine zoogeography. McGraw-Hill Book Co., New York
- Chevolot M, Hoarau G, Rinjndorp AD, Stam WT, Olsen JL (2006) Phylogeography and population structure of thornback rays (*Raja clavata* L., Rajidae). *Mol Ecol* 15:3693–3705
- Chow S, Hazama K (1998) Universal PCR primers for S7 ribosomal protein gene introns in fish. *Mol Ecol* 7:1247–1263
- Climap Project Members (1984) The last interglacial ocean. *Quat Res* 21:123–224
- Crowley TJ (1981) Temperature and circulation changes in the eastern north Atlantic during the last 150,000 years: evidence from the planktonic foraminiferal record. *Mar Micropaleontol* 6:97–129
- Debes PV, Zachos FE, Hanel R (2008) Mitochondrial phylogeography of the European sprat (*Sprattus sprattus* L., Clupeidae) reveals isolated climatically vulnerable populations in the Mediterranean Sea and range expansion in the northeast Atlantic. *Mol Ecol* 17:3873–3888
- Domingues VS, Bucciarelli G, Almada VC, Bernardi G (2005) Historical colonization and demography of the Mediterranean damselfish, *Chromis chromis*. *Mol Ecol* 14:4051–4063
- Domingues VS, Faria C, Stefanni S, Santos RS, Brito A, Almada VC (2007) Genetic divergence in the Atlantic-Mediterranean Montagu's blenny, *Coryphoblennius galerita* (Linnaeus 1758) revealed by molecular and morphological characters. *Mol Ecol* 16:3592–3605
- Domingues VS, Alexandrou M, Almada VC, Robertson DR, Brito A, Santos RS, Bernardi G (2008) Tropical fishes in a temperate sea: evolution of the wrasse *Thalassoma pavo* and the parrotfish *Sparisoma cretense* in the Mediterranean and the adjacent Macaronesian and Cape Verde Archipelagos. *Mar Biol* 154:465–474
- Drummond AJ, Rambaut A (2007) BEAST: bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7:214
- Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol Biol Evol* 22:1185–1192
- Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Mol Ecol* 11:2571–2581
- Excoffier L, Lischer H (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 10:564–567
- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479–491
- Francisco SM, Castilho R, Soares M, Congiu L, Brito A, Vieira MN, Almada VC (2009) Phylogeography and demographic history of *Atherina presbyter* (Pisces: Atherinidae) in the North-eastern Atlantic based on mitochondrial DNA. *Mar Biol* 156:1421–1432
- Francisco SM, Faria C, Lengkeek W, Vieira MN, Velasco EM, Almada VC (2011) Phylogeography of the shanny *Lipophrys pholis* (Pisces: Blenniidae) in the NE Atlantic records signs of major expansion event older than the last glaciation. *J Exp Mar Bio Ecol* 403:14–20
- Francisco SM, Robalo JI, Levy A, Almada VC (2014) In search of phylogeographic patterns in the northeastern Atlantic and adjacent seas. In: Pontarotti P (ed) *Evolutionary biology, Genome, Speciation, Coevolution and Origin of life*. Springer, pp 323–338. doi:10.1007/978-3-319-07623-2_15
- Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147:437–460
- Galarza JA, Carreras-Carbonell J, Macpherson E, Pascual M, Roques S, Turner GF, Rico C (2009) The influence of oceanographic fronts and early-life-history traits on connectivity among littoral fish species. *PNAS* 106:1473–1478
- Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum likelihood. *Syst Biol* 52:696–704
- Gysels ES, Hellemans B, Pampoulie C, Volckaert FAM (2004) Phylogeography of the common goby, *Pomatoschistus microps*, with particular emphasis on the colonization of the Mediterranean and the North Sea. *Mol Ecol* 13:403–417
- Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature* 405:907–9013
- Hickerson MJ, Cunningham CW (2006) Nearshore fish (*Pholis gunnellus*) persists across the North Atlantic through multiple glacial episodes. *Mol Ecol* 15:4095–4107
- Kuhner MK (2006) LAMARC 2.0: maximum likelihood and Bayesian estimation of population parameters. *Bioinformatics* 22:768–770
- Lambeck K, Esat TM, Potter E-K (2002) Links between climate and sea levels for the past three million years. *Nature* 419:199–206
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA et al (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948
- Larmuseau MHD, Van Houdt JKJ, Guelinckx J et al (2009) Distributional and demographic consequences of Pleistocene climate fluctuations for a marine demersal fish in the north-eastern Atlantic. *J Biogeogr* 36:1138–1151
- Maggs CA, Castilho R, Foltz D et al (2008) Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. *Ecology* 89:S108–S122
- Mäkinen HS, Merilä J (2008) Mitochondrial DNA phylogeography of the three-spined stickleback (*Gasterosteus aculeatus*) in Europe: evidence for multiple glacial refugia. *Mol Phylogenet Evol* 46:167–182
- Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res* 27:209–220
- Martins CS, Hamann M, Fuiza AFG (2002) Surface circulation in the eastern North Atlantic from drifters and altimetry. *J Geophys Res* 107:3217–3240
- Millot C (1999) Circulation in the Western Mediterranean Sea. *J Mar Sys* 20:423–442

- Morton B, Britton JC (2000) The origins of the coastal and marine flora and fauna of the Azores. *Oceanogr Mar Biol* 38:13–84
- Muss A, Robertson DR, Stepien CA, Wirtz P, Bowen BW (2001) Phylogeography of *Ophioblennius*: the role of ocean currents and geography in reef fish evolution. *Evolution* 55:561–572
- Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press, Oxford
- Nikula R, Väinölä R (2003) Phylogeography of *Cerastoderma glaucum* (Bivalvia: Cardiidae) across Europe: a major break in the Eastern Mediterranean. *Mar Biol* 143:339–350
- Olsen JL, Stam WT, Coyer JA, Reusch TBH, Billingham M, Boström C, Calvert E, Christie H, Granger S, La Lumière R, Milchakova N, Oudot-Le Secq MP, Procaccini G, Sanjabi B, Serrão E, Veldsink J, Widdicombe S, Wyllie-Echeverria S (2004) North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. *Mol Ecol* 13:1923–1941
- Ostellari L, Bargelloni L, Penzo E, Patarnello P, Patarnello T (1996) Optimization of single-strand conformation polymorphism and sequence analysis of the mitochondrial control region in *Pagellus bogaraveo* (Sparidae, Teleostei): rationalized tools in fish population biology. *Anim Genet* 27:423–427
- Patarnello T, Volckaert FMJ, Castilho R (2007) Pillars of Hercules: is the Atlantic-Mediterranean transition a phylogeographical break? *Mol Ecol* 16:4426–4444. doi:10.1111/j.1365-294X.2007.03477.x
- Posada D (2008) jModelTest: phylogenetic model averaging. *Mol Biol Evol* 25:1253–1256
- Quéro JC, Hureau JC, Karrer C, Post A, Saldanha L (1990) Check-list of the fishes of the eastern Tropical Atlantic. JNICT, Lisbon
- Rambaut A, Drummond AJ (2007) Tracer v1.4, Available from <http://beast.bio.ed.ac.uk/Tracer>
- Raventós N, Macpherson E (2001) Planktonic larval duration and settlement marks on the otoliths of Mediterranean littoral fishes. *Mar Biol* 138:1115–1120
- Richtarski U, Patzner RA (2000) Comparative morphology of male reproductive systems in Mediterranean blennies (Blenniidae). *J Fish Biol* 56:22–36
- Robalo JJ, Castilho R, Francisco SM et al (2012) Northern refugia and recent expansion in the North Sea: the case of the wrasse *Symphodus melops* (Linnaeus, 1758). *Ecol Evol* 2:153–164
- Rogers AR (1995) Genetic evidence for a Pleistocene population explosion. *Evolution* 49:608–615
- Rogers AR, Harpending HC (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Mol Biol Evol* 9:552–569
- Santos RS, Hawkins S, Monteiro LR, Alves M, Isidro EJ (1995) Marine research, resources and conservation in the Azores. *Aquat Conserv* 5:311–354
- Shakun JD, Carlson AE (2010) A global perspective on Last Glacial Maximum to Holocene climate change. *Quat Sci Rev* 29:1801–1816
- Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Syst Zool* 35:627–632
- Sotka EE, Wares JP, Barth JA, Grosberg RK, Palumbi SR (2004) Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. *Mol Ecol* 13:2143–2156
- Sousa-Santos C, Robalo JJ, Collares-Pereira MJ, Almada VC (2005) Heterozygous indels as useful tools in the reconstruction of DNA sequences and in the assessment of ploidy level and genomic constitution of hybrid organisms. *DNA Seq* 16:462–467
- Stefanni S, Knutsen H (2007) Phylogeography and demographic history of the deep-sea fish *Aphanopus carbo* (Lowe, 1839) in the NE Atlantic: vicariance followed by secondary contact or speciation? *Mol Phylogenet Evol* 42:38–46
- Stefanni S, Domingues V, Bouton N, Santos RS, Almada F, Almada V (2006) Phylogeny of the shanny, *Lipophrys pholis*, from the NE Atlantic using mitochondrial DNA markers. *Mol Phylogenet Evol* 39:282–287
- Stramma L (1984) Geostrophic transport in the warm water sphere of the eastern subtropical North Atlantic. *J Mar Res* 42:537–558
- Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. *Genetics* 105:437–446
- Thiede J (1978) A glacial Mediterranean. *Nature* 276:680–683
- Wares JP, Cunningham CW (2001) Phylogeography and historical ecology of the North Atlantic intertidal. *Evolution* 55:2455–2469
- Weersing K, Toonen RJ (2009) Population genetics, larval dispersal, and connectivity in marine systems. *Mar Ecol Prog Ser* 393:1–12
- Willebrand J, Barnier B, Böning C, Dieterich PD, Le Provost C, Jia Y, Molines JM, New AL (2001) Circulation characteristics in three eddy-permitting models of the North Atlantic. *Prog Oceanogr* 48:123–161
- Zander CD (1986) Blenniidae. In: Whitehead PJP, Bauchot M-L, Hureau J-C, Nielsen J, Tortonese E (eds) Fishes of the North-Eastern Atlantic and the Mediterranean. UNESCO, Paris, pp 1096–1112
- Zaragosi S, Eynaud F, Pujol C, Auffret GA, Turon JL, Garlan T (2001) Initiation of the European deglaciation as recorded in the northwestern Bay of Biscay slope environments (Meriadzek Terrace and Trévelyan Escarpment): a multi-proxy approach. *Earth Planet Sci Lett* 188:493–507
- Zardoya R, Castilho R, Grande C, Favre-Krey L, Caetano S, Marcato S, Krey G, Patarnello T (2004) Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the Mediterranean Sea. *Mol Ecol* 13:1785–1798