Mar Biol (2008) 154:465–474
DOI 10.1007/s00227-008-0941-z

RESEARCH ARTICLE

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

Vera S. Domingues · Markos Alexandrou · Vitor C. Almada · D. Ross Robertson · Alberto Brito · Ricardo S. Santos · Giacomo Bernardi

Received: 14 March 2007 / Accepted: 19 February 2008 / Published online: 4 March 2008

Abstract The northeastern Atlantic and the Mediterranean Sea share geological histories and display great faunal affinities. The majority of the Mediterranean species have Atlantic origins, with a few species with tropical affinities. These include the parrotfish *Sparisoma cretense* and the wrasse *Thalassoma pavo* that are restricted to the subtropical northeastern Atlantic, the Macaronesian archipelagos (Azores, Madeira, and Canaries) and the southern Mediterranean. The Pleistocene glaciations have been described as having different effects on the fauna of the two regions. During glacial peaks, Mediterranean waters remained warmer than those of the adjacent Atlantic. Within the eastern Atlantic, the effects of Pleistocene glaciations were differentiated. Here, we perform a comparative analysis focusing on *T. pavo* and *S. cretense* populations from the northeastern Atlantic and the Mediterranean to assess the effects of Pleistocene glaciations in these two species. Sequences from the mitochondrial control region were obtained and analyzed combining phylogeographic and demographic approaches. Gene flow between Atlantic and Mediterranean populations was shown to be very high. The Mediterranean populations of *T. pavo* and *S. cretense* showed high levels of genetic diversity, even in the eastern basin, pointing to an ancient colonization event. This suggests that both species must have been able to persist in the Mediterranean during the cold Pleistocene periods. Historical migration estimates revealed a Mediterranean towards Atlantic trend in the case of *T. pavo*, which may reflect the re-colonization of areas in the Atlantic by fish that survived the cold phases in relatively warmer Mediterranean refugia. Our data also showed that within the Macaronesian Archipelagos, migrations occurred from Madeira towards the Azores, for both *T. pavo* and *S. cretense*, thus supporting a post-glacial colonization of the Azores by fish that persisted in the warmer region of Madeira. Similar geographic distributions, thermal affinities, and means of dispersion for *T. pavo* and *S. cretense* resulted in a similar response to the effects of Pleistocene glaciations, as evidenced by identical phylogeographic patterns.

Communicated by J.-M. Gili.

V. S. Domingues · V. C. Almada
Instituto Superior de Psicologia Aplicada,
Unidade de Investigação em Eco-Etologia,
R. Jardim do Tabaco 34, 1149-041 Lisbon, Portugal

V. S. Domingues · R. S. Santos
Departamento de Oceanografia e Pescas,
Universidade dos Açores, 9901-862 Horta, Azores, Portugal

V. S. Domingues (✉) · M. Alexandrou · G. Bernardi
Department of Ecology and Evolutionary Biology,
Long Marine Lab, University of California Santa Cruz,
100 Shaffer Road, Santa Cruz, CA 95060, USA
e-mail: v_domingues@yahoo.com

M. Alexandrou
Department of Biological and Chemical Sciences,
University of London, Queen Mary,
Mile End Road E1 4NS, London, UK

D. R. Robertson
Smithsonian Tropical Research Institute,
Balboa, Republic of Panama

A. Brito
Dpto. Biología Animal (Ciencias Marinas),
Universidad de La Laguna,
Avenida Astrofísico Francisco Sánchez s/n,
38206 La Laguna, Tenerife, Canary Islands, Spain

Introduction

Paleogeography and oceanography have played an important role in shaping the present day phylogeographic

patterns in northeastern Atlantic and Mediterranean marine faunas. Approximately 5–6 million years ago (Mya), the Mediterranean Sea experienced a desiccation event, the Messinian Salitiny Crisis (MSC), that lasted for some hundred thousands years and resulted in a major extinction of its marine fauna (Hsü et al. 1977; Krijgsman et al. 1999; Duggen et al. 2003). This event was followed by replenishment from the adjacent Atlantic Ocean, at the base of the Pliocene, suddenly restoring the open marine conditions. More recently, Pleistocene glaciations (about 3 Mya) were accompanied by lowered seawater temperatures and changes in oceanographic characteristics (Briggs 1996; Adams et al. 1999; Lambeck et al. 2002). During these glacial peaks, Mediterranean waters remained warmer than those of the adjacent Atlantic (Thiede 1978). Within the eastern Atlantic, the effects of Pleistocene glaciations were differentiated. The western coast of Europe and the northwest coast of Africa were particularly affected by a very pronounced southward migration of the polar front, which caused a significant drop in the seawater temperatures of these regions (Crowley 1981; Dias 1997). The Macaronesian archipelagos (Azores, Madeira and Canaries) and Cape Verde islands were also differentially affected. While the Azores region experienced moderate cooling (2–3°C; Crowley 1981), the Madeira archipelago, located further south, was not affected. The Canaries were affected to some extent, and the Cape Verde islands, although remaining considerably warm, were clearly out of the Tropical bio-region (Briggs 1996). These climatic events promoted the extinction of the warm temperate species in the cooler regions of the Atlantic. Although the lowering of sea surface temperatures of the Azores were not very pronounced, they may have still triggered migrations and even local extinction of several littoral fish species, particularly for populations that are not distributed further north than this region. Many species now present in the warm temperate Atlantic are likely to have survived the cold phases of the glacial cycles in less affected regions such as Madeira, the western tropical coast of Africa, and southern Mediterranean, recolonizing the affected regions when more favorable temperatures were reestablished during interglacial phases (Almada et al. 2001). Santos et al. (1995) suggested that a relevant set of the organisms (mainly fish) now present in the Azores would have recolonized the islands from some of the southern regions mentioned above, namely Madeira, after the end of the last glaciation. These geological and climatic events shaped the biota of the Mediterranean, which is currently considered a subtropical temperate sea, with most species being temperate. Most Mediterranean species have Atlantic affinities, which is supported by genetic evidence (Pannacciuli et al. 1997; Pérez Losada et al. 1999; Aurelle et al. 2003; Bargelloni et al. 2003; Costagliola et al. 2004; Duran et al. 2004). A few species show clear tropical affinities. These include the parrotfish *Sparisoma cretense* and the wrasse *Thalassoma pavo*, which belong to the closely related families Scaridae and Labridae, respectively. The similar distributions of both species reflect their preference for warmer water. Indeed, these species are restricted to the subtropical northeastern Atlantic, the Macaronesian archipelagos and the southern Mediterranean. In the Atlantic, *T. pavo* occurs from Portugal (Algarve) southwards to Cape Lopez (Gabon) (Quignard and Pras 1986a), and *S. cretense* has its southern limit in Senegal (Quignard and Pras 1986b; González 1993). In the Mediterranean, the species are slowly migrating north due to recent warming trends (Guidetti et al. 2002). Several studies report stable populations of *T. pavo* in many sections of the northern Mediterranean (Francour et al. 1994; Dulcic 2004; Sara et al. 2005).

Costagliola et al. (2004) studied Atlantic and Mediterranean populations of *T. pavo* and found no genetic discontinuities between the two regions. Within the Mediterranean, populations of *T. pavo* showed a genetic restriction of gene flow between eastern and western regions.

Both species have pelagic eggs and active swimming larvae that remain in the water column for a few weeks (38–49 days in the case of *T. pavo*, Raventós and Macpherson 2001; 50–60 days for *Sparisoma* spp., Robertson et al. 2006). Juvenile fishes then recruit to rocky reefs and seagrass beds.

The evolutionary history of *S. cretense* and *T. pavo* raises a number of interesting questions: (1) how were populations affected when seawater temperatures dropped during the Pleistocene glaciations events? (2) what are the historical dynamics between Atlantic and Mediterranean populations? (3) do *S. cretense* and *T. pavo* populations show similar phylogeographic and demographic patterns and to what extent may this similarity be attributed to the effects of Atlantic and Mediterranean paleoclimatic history?

To address these questions we used a comparative phylogeographic method based on the fast evolving mitochondrial control region gene. We focused on two fish species with tropical affinities, *T. pavo* and *S. cretense*, that are restricted to the Mediterranean and the adjacent Macaronesian and Cape Verde Archipelagos. Sequences from *T. pavo* available from Costagliola et al. (2004) were integrated and reanalyzed in our study and a comparative analysis including *S. cretense* was performed. New data analysis tools based on the coalescent theory, allowed the determination of the direction of migration between populations of *T. pavo* and *S. cretense*. Combined with traditional approaches, these methods shed light on the evolutionary history and population dynamics of the two species in these closely related regions.

Materials and methods

Sampling and laboratory procedures

A total of 84 *S. cretense* and 134 *T. pavo* from the Mediterranean and the islands of the Azores, Madeira, Canaries and Cape Verde were used in this study. We used *S. strigatum,* and *T. ascensionis* and *T. sanctahelenae* from Ascension Island and Saint Helena as outgroups. Sampling localities and number of individuals are given in Table 1 and Fig. 1. Samples of *S. cretense* were collected by spear or hand nets while scuba diving or free diving. 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 (611 bp) was accomplished with universal primers L15725 (revised from Sorenson et al. 1999) 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, CT), 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). Partial mitochondrial control region sequences (321 bp) of *T. pavo* obtained in Costagliola et al. (2004) (GenBank accession numbers AY329698-AY329798) were used in this study. In addition, three different locations in the Mediterranean (Kea, Sifnos and Santorini) were included in the analysis, and sample size of Cape Verde was increased. PCR and sequencing procedures followed Costagliola et al. (2004).

Genetic diversity and phylogenetic relationships

Sequences were aligned using the computer program Clustal V implemented by Sequence Navigator (Applied Biosystems). Diversity indices (number of haplotypes, haplotype diversity and nucleotide diversity) of *S. cretense* and *T. pavo* were calculated using DNAsp (Rozas et al. 2003). Phylogenetic relationships were inferred using Maximum Parsimony (MP) and Neighbor-Joining (NJ) with the substitution model established using Modeltest 3.06 (Posada and Crandall 1998) under AIC (HKY + I + G and TVM + G for *S. cretense* and *T. pavo*, respectively. Topological confidence was evaluated for MP and NJ, with

Table 1 Collection localities and diversity indexes of *Thalassoma pavo* and *Sparisoma cretense* used in the present study

	Thalassoma pavo				*Sparisoma cretense*			
Species locality	***n***	**nH**	**Hd**	**π**	***n***	**nH**	**Hd**	**π**
Atlantic	49	30	0.915	0.011	51	31	0.943	0.005
Azores, Portugal (Azo)	11	7	0.873	0.007	10	9	0.978	0.009
Madeira, Portugal (Mad)	12	6	0.818	0.004	10	9	0.978	0.005
Canaries, Spain (Can)	11	6	0.800	0.005	19	12	0.871	0.003
Cape Verde (CV)	15	15	1.000	0.016	12	9	0.955	0.005
Mediterranean	85	34	0.829	0.006	33	23	0.964	0.007
Western Mediterranean								
Al-Hoceima, Morocco (Mor)	3	3	1.000	0.008	–	–	–	–
Genoa, Italy (Gen)	10	6	0.778	0.007	–	–	–	–
Calvi, Corsica, France (Cal)	3	3	1.000	0.006	–	–	–	–
Olbia, Sardinia, Italy (Olb)	10	4	0.533	0.002	–	–	–	–
Pozzuoli, Italy (Poz)	11	5	0.618	0.004	–	–	–	–
Naples, Italy (Nap)	3	3	1.000	0.008	–	–	–	–
Ustica, Italy (Ust)	2	2	1.000	0.003	–	–	–	–
Lipari, Italy (Lip)	7	3	0.524	0.004	–	–	–	–
Linosa, Italy (Lin)	3	3	1.000	0.004	–	–	–	–
Lampedusa, Italy (Lamp)	–	–	–	–	3	3	1.000	0.004
Eastern Mediterranean								
Otranto, Italy (Otr)	9	6	0.833	0.007	–	–	–	–
Kea, Greece (Kea)	3	2	0.667	0.002	12	11	0.985	0.007
Sifnos, Greece (Sif)	6	5	0.933	0.007	10	9	0.978	0.005
Santorini, Greece (Sant)	–	–	–	–	8	7	0.964	0.011
Rhodes, Greece (Rho)	15	8	0.733	0.005	–	–	–	–

Number of individuals (*n*), number of haplotypes (nH), Haplotype diversity (Hd) and nucleotide diversity (π) for mitochondrial control region were calculated using DNAsp (Rozas et al. 2003). Locality labels are presented between parentheses

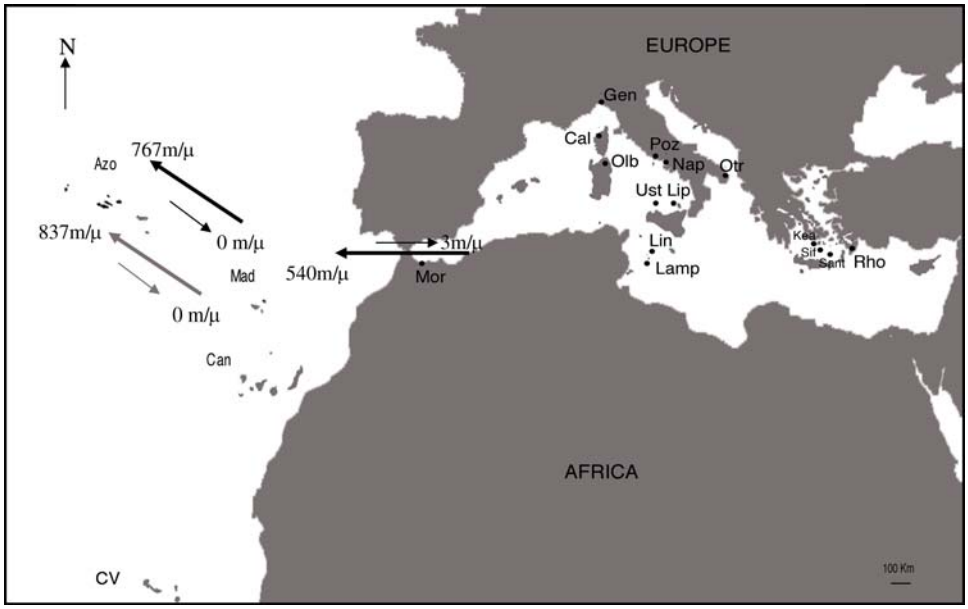

Fig. 1 *Thalassoma pavo* and *Sparisoma cretense* sampling locations. Both species were collected from the Macaronesian and Cape Verde islands, in the Atlantic, and the Mediterranean (sea Table 1 for detailed sampling locations and labels). Extent of migration of *T. pavo* (between Atlantic and Mediterranean populations and between Azores and Madeira) are shown with *black arrows*. Extent of migration of *S. cretense* (between the Azores and Madeira populations) are shown with *grey arrows*. Numbers of migrants (scaled by the mutation rate) in each direction are given close to the *arrows*

1,000 bootstrap replicates (Felsenstein 1985). Phylogenetic analysis was performed using the software package PAUP (version 4.0; Swofford 1998).

Gene flow and historical demography

Gene flow (Fst) and corrected average pairwise differences between populations were estimated for both species using ARLEQUIN (vers. 2.000; Schneider et al. 2000). The *P* distances were estimated for each pair of populations. Population structure was estimated by an analysis of molecular variance (AMOVA, Excoffier et al. 1997) by partitioning the data into two groups (Atlantic and Mediterranean).

Exchanges and range expansions (immigration) between Atlantic and Mediterranean and also between Azores and Madeira were estimated for *S. cretense* and *T. pavo* using MIGRATE 2.0.3 (Beerli and Felsenstein 2001; Beerli 2004). In the case of *T. pavo*, only samples from the western basin of the Mediterranean were included in the analysis generating similar sampling sizes for the two locations. Analyses were repeated ten times, to ensure stability of parameter estimates. Final search strategy varied according to the dataset. For *S. cretense*, final analyses employed ten short Monte Carlo chains with 1,000,000 sampled genealogies and three long chains with 10,000,000 sampled genealogies. We applied an exhaustive search using four heated chains {1, 4, 7, 10} and an interval between swapping trees of 1. For the *T. pavo* dataset, the same heating scheme and number or Monte Carlo chains were used, but 100,000 sampled genealogies for the short chains and 1,000,000 sampled genealogies for the long chains were enough to ensure stability. Finally, two runs were performed using the obtained parameters estimated in the previous runs to ensure stability was reached.

Results

Genetic diversity and phylogenetic relationships

A total of 83 *S. cretense* and 13 *S. strigatum* partial sequences of the mitochondrial control region were obtained (GenBank accession numbers: EF484419–EF484515). New sequences of *T. pavo* were also deposited in GenBank (accession numbers: EF484516–EF484537). Number of haplotypes, haplotype diversity and nucleotide diversity for the mitochondrial control region are shown in Table 1. Both species revealed high diversity values for all locations (Table 1).

Both methods of phylogenetic inference resulted in similar topologies. Figures 2 and 3 show *T. pavo* and *S. cretense* Neighbor-Joining phylogenies based on the mitochondrial control region sequences. Haplotypes were shared between populations and none of the species showed clear signs of geographic partitioning. The majority of *T. pavo* haplotypes from Cape Verde grouped together in a clade containing also 2 haplotypes from Italy. However, this clade was weakly supported (bootstrap below 50%). Similarly, all haplotypes from the island of Rhodes, Greece, grouped together in a clade with a bootstrap below 50%. For *S. cretense* all clades included haplotypes from different locations, thus resulting in a tree with no obvious geographic structure.

Gene flow

The Fst values and *P* distances estimated for *T. pavo* and *S. cretense* populations are shown in Tables 2 and 3, respectively. Gene flow between *S. cretense* and *T. pavo* populations was found to be high. *Sparisoma cretense* in

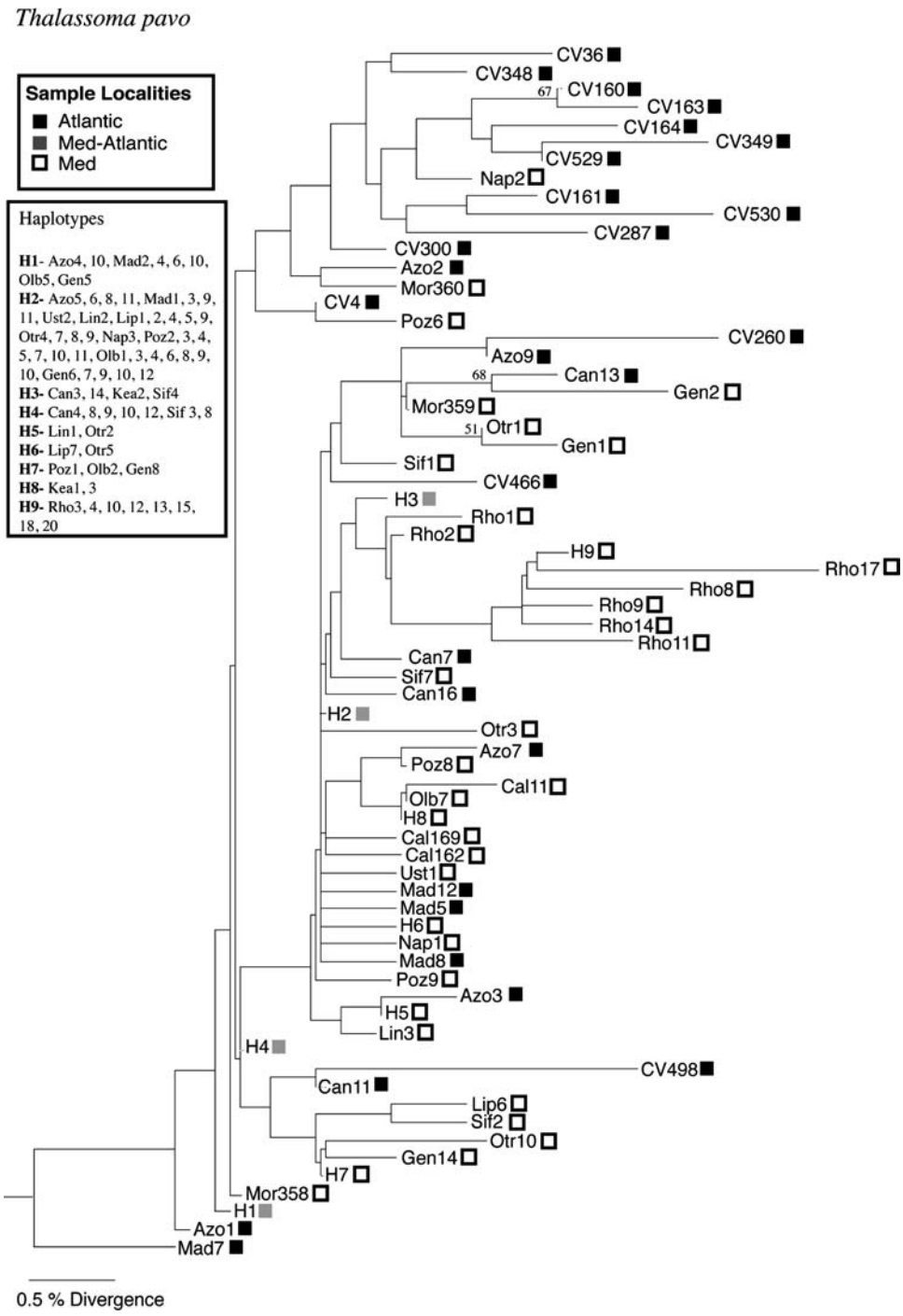

Fig. 2 Phylogenetic relationship within *Thalassoma pavo* for the mitochondrial control region sequences. *T. sanctaehelenae* and *T. ascensionis* were used as outgroup. Neighbor-Joining trees are shown with Neighbor-Joining (above the nodes) and Maximum Parsimony (below the nodes) bootstrap support above 50% indicated at the nodes. Labels are described in Table 1. The length of each branch is proportional to the number of nucleotide substitutions. *Scale bar*: 0.5% divergence

particular, revealed remarkably high levels of gene flow, with all pairwise comparisons showing Fst values below 0.1. Cape Verde appeared as a more isolated population for both species. *Thalassoma pavo* showed gene flow restriction between the Canary islands and the other locations but also between the eastern locations of the Mediterranean (Kea, Sifnos and Rhodes) and the remaining locations.

The AMOVA analyses showed a significant but weak differentiation between Atlantic and Mediterranean populations. Only a very small percentage of the data variance (9.96%, $P = 0.00$ in the case of *T. pavo* and 2.24%, $P = 0.03$ in the case of *S. cretense*) was attributable to the separation between Atlantic and Mediterranean populations.

Historical demography

Migration between Atlantic and Mediterranean was determined for *T. pavo*. Migration between the two basins

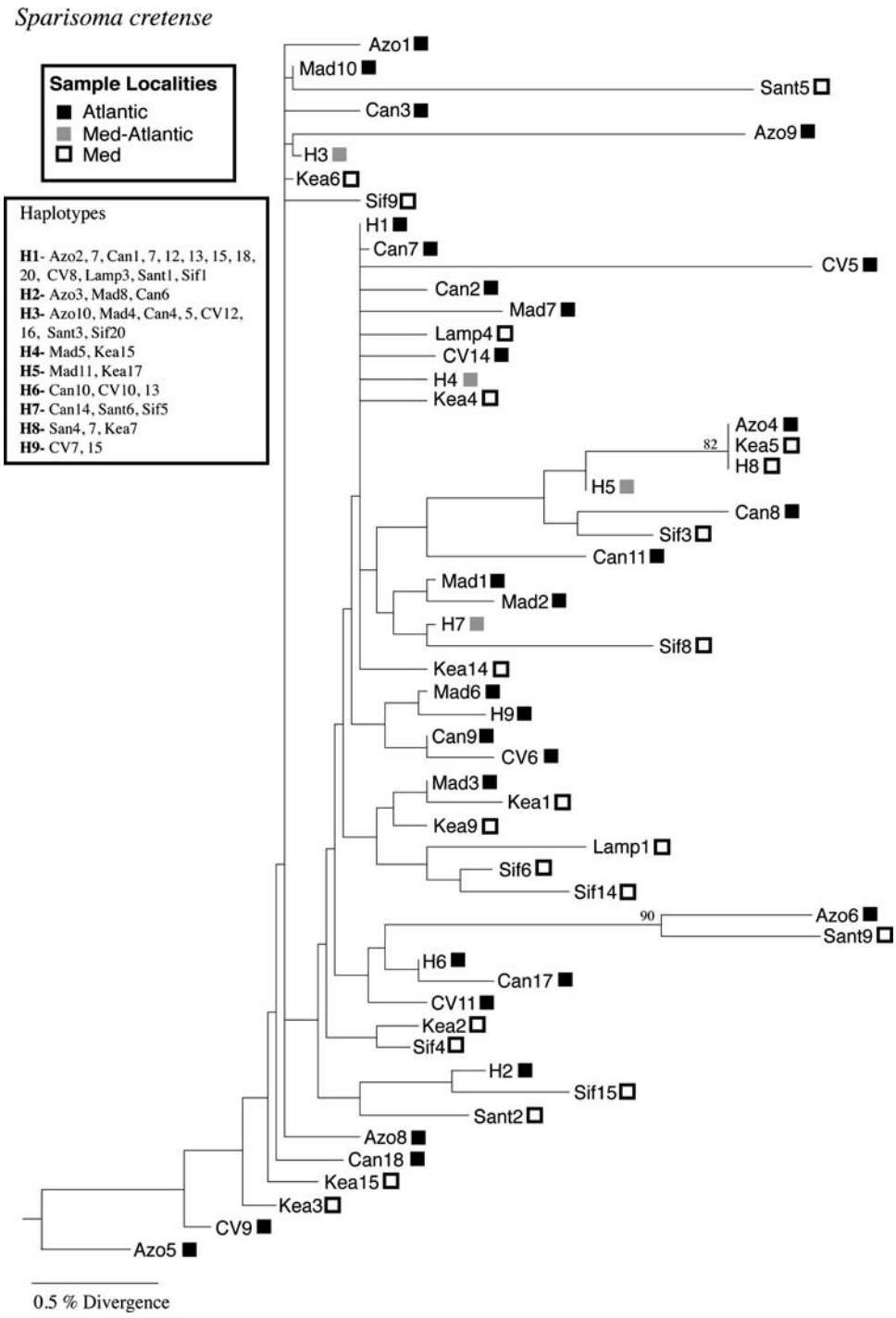

Fig. 3 Phylogenetic relationship within *Sparisoma cretense* for the mitochondrial control region sequences. *S. strigatum* was used as outgroup. Neighbor-Joining trees are shown with Neighbor-Joining (above the nodes) and Maximum Parsimony (below the nodes) bootstrap support above 50% indicated at the nodes. Labels are described in Table 1. The length of each branch is proportional to the number of nucleotide substitutions. *Scale bar*: 0.5% divergence

showed a higher value of Atlantic immigrants revealing a westward trend (Fig. 1). We were not able to determine migration between Atlantic and Mediterranean populations of *S. cretense*. Despite several attempted search strategies, stationarity of parameters was not achieved. Both species showed similar levels of migration from Madeira into the Azores and no migration in the opposite direction.

Discussion

Evolutionary history of the Mediterranean and adjacent Atlantic

The northeastern Atlantic Ocean and the Mediterranean Sea are known for sharing great faunal affinities shaped by an intimately related paleogeography. Pleistocene glaciations

Table 2 Uncorrected *P* distances and Fst values (above and below the diagonal, respectively) among *Thalassoma pavo* populations calculated from mitochondrial control region sequences using ARLEQUIN version 2.000 (Schneider et al. 2000)

	Azo	Mad	Can	CV	Mor	Gen	Cal	Olb	Poz	Nap	Ust	Lip	Lin	Otr	Kea	Sif	Rho
Azo		0.000	0.297	0.541	0.053	0.000	0.222	0.000	0.000	0.044	0.034	0.000	0.000	0.000	0.413	0.284	0.563
Mad	0.000		0.303	0.544	0.066	0.003	0.256	0.002	0.011	0.047	0.047	0.003	0.047	0.013	0.464	0.292	0.575
Can	**0.335**	**0.409**		0.780	0.360	0.308	0.606	0.337	0.347	0.388	0.398	0.341	0.379	0.353	0.189	0.000	0.607
CV	**0.304**	**0.336**	**0.401**		0.473	0.575	0.953	0.643	0.617	0.328	0.744	0.655	0.730	0.649	1.119	0.793	1.225
Mor	0.122	**0.245**	**0.417**	**0.205**		0.076	0.069	0.132	0.144	0.104	0.208	0.149	0.208	0.139	0.625	0.375	0.711
Gen	0.000	0.013	**0.332**	**0.307**	0.125		0.243	0.000	0.000	0.076	0.035	0.000	0.035	0.000	0.451	0.295	0.558
Cal	0.260	0.417	0.534	**0.360**	0.069	0.251		0.194	0.220	0.313	0.208	0.208	0.208	0.208	0.486	0.583	0.753
Olb	0.000	0.003	**0.468**	**0.368**	0.380	0.000	**0.440**		0.000	0.069	0.007	0.000	0.007	0.000	0.382	0.309	0.543
Poz	0.000	0.029	**0.439**	**0.356**	**0.323**	0.000	**0.383**	0.000		0.059	0.011	0.000	0.011	0.000	0.428	0.311	0.545
Nap	0.085	0.197	**0.432**	**0.128**	0.100	0.104	0.273	0.297	0.208		0.104	0.074	0.104	0.081	0.521	0.375	0.621
Ust	0.000	0.083	0.417	0.229	0.137	0.000	0.186	0.089	0.000	0.066		0.000	0.000	0.000	0.417	0.375	0.545
Lip	0.000	0.012	**0.411**	**0.327**	0.263	0.000	**0.315**	0.000	0.000	0.176	0.000		0.000	0.000	0.417	0.301	0.533
Lin	0.000	0.122	0.425	**0.280**	0.222	0.000	0.250	0.100	0.042	0.143	0.000	0.000		0.000	0.417	0.340	0.545
Otr	0.000	0.033	**0.375**	**0.331**	0.192	0.000	0.238	0.000	0.000	0.119	0.000	0.000	0.000		0.417	0.328	0.535
Kea	0.372	**0.559**	0.245	**0.393**	0.500	0.372	0.483	0.621	0.539	0.500	0.638	**0.503**	0.571	**0.374**		0.167	0.649
Sif	**0.296**	**0.391**	0.000	**0.354**	0.336	**0.287**	**0.444**	**0.446**	**0.400**	0.342	0.317	**0.350**	0.333	**0.320**	0.174		0.587
Rho	**0.499**	**0.565**	**0.548**	**0.540**	**0.584**	**0.488**	0.599	**0.578**	**0.500**	**0.555**	**0.510**	**0.525**	**0.524**	**0.488**	**0.570**	**0.520**	

Fst significant *P* values ($P < 0.05$) after Bonferroni correction are bolded

Table 3 Uncorrected *P* distances and Fst values (above and below the diagonal respectively) among *Sparisoma cretense* populations calculated from mitochondrial control region sequences using ARLEQUIN version 2.000 (Schneider et al. 2000)

	Azo	Mad	Can	CV	Lamp	Kea	Sif	Sant
Azo		0.000	0.004	0.023	0.056	0.000	0.011	0.000
Mad	0.000		0.000	0.011	0.018	0.000	0.000	0.000
Can	0.032	0.000		0.016	0.014	0.013	0.010	0.008
CV	0.037	0.021	0.043		0.048	0.060	0.052	0.058
Lamp	0.000	0.024	0.067	0.071		0.053	0.093	0.035
Kea	0.000	0.000	0.037	0.090	0.030		0.005	0.000
Sif	0.015	0.000	0.037	0.084	0.000	0.007		0.000
Sant	0.000	0.000	0.058	**0.089**	0.000	0.000	0.000	

Fst significant *P* values ($P < 0.05$) after Bonferroni correction are bolded

had a differential effect in the two regions, shaping the structure of marine populations, which disappeared as the ice endured and managed to recolonize regions after temperatures stabilized. Distribution ranges of *T. pavo* and *S. cretense* reflect a clear preference for warm waters. Some warm water species such as *Chromis chromis C. limbata* and *Tripterygion delaisi* (Domingues et al. 2006, 2007), have been suggested to have survived the cold periods in warmer regions like Madeira, the tropical African coast and the southern Mediterranean, where the climatic conditions did not change significantly during the Pleistocene (Thiede 1978; Crowley 1981). Indeed, Mediterranean populations of *T. pavo* and *S. cretense* show high levels of genetic diversity even in the eastern basin, pointing to an ancient colonization of this sea. This suggests that both species must have been able to persist in the Mediterranean during the cold periods, possibly in some southern warmer water pockets (Thiede 1978). This is also consistent with the fact that *T. pavo* and *S. cretense* showed no evidence for genetic partition between the Atlantic and the Mediterranean. Gene flow between localities in the two basins was very high for both species. Similarly, AMOVA analyses revealed that only a very small percentage of the data variance was attributable to Atlantic/Mediterranean differentiation, although *T. pavo* showed a slightly higher variance between Atlantic and Mediterranean than *S. cretense*. The Strait of Gibraltar and the Almeria-Oran front have been described as effective barriers to gene flow for some species, while others show no restriction to gene flow between the two basins. Patarnello et al. (2007) suggested that a combination of vicariance phenomena, paleoclimatic fluctuations and life history traits is responsible for the different phylogeographic patterns observed.

Although the direction of historical migration between the Atlantic and the Mediterranean could not be estimated for *S. cretense*, a Mediterranean–Atlantic trend is evident for *T. pavo* (Fig. 1). This trend may reflect the re-colonization of the cooler areas of the Atlantic by fish that survived the cold phases in the Mediterranean. Colonization of the Atlantic from the Mediterranean may have been facilitated, at least for *T. pavo* which is carnivorous, by the numerous submarine banks and seamounts that occur between Europe

and the islands of Madeira and Azores (Kitchingman and Lai 2004; Kitchingman et al. 2007). This colonization route has also been suggested for molluscs (Ávila 2000). *Thalassoma pavo* and *S. cretense* are active demersal (or benthic) fish, with pelagic eggs and larvae, that occur at depths of 50 m or below (Quignard and Pras 1986a, b), thus being able of reaching these seamounts. The interpretation of the Mediterranean–Atlantic migration trend must, however, be viewed with caution, since the Atlantic samples come from island locations, which in the case of Cape Verde and the Azores, are very far away from shore, meaning that many haplotypes may not have been able to reach them. Even though Cape Verde is not genetically isolated from the other locations, Fst values indicate lower levels of gene flow between this archipelago and the other location for both *T. pavo* and *S. cretense*. Sampling on the western African shore is urgently needed to improve our understanding of the phylogeography of these fishes.

According to what has been discussed, persistence of *T. pavo* and *S. cretense* in the Azores archipelago during the Pleistocene glaciations is difficult to admit. Planktonic foraminifera record of the last 150 ky in the region, revealed variations in sea surface temperatures of 2–3°C (Crowley 1981). These small fluctuations might have been enough to promote local disappearance of sub-tropical species like *T. pavo* and *S. cretense*, whose northern limit of distribution is in this archipelago. Indeed, Santos et al. (1995) suggested the recolonization of the Azores by sub-tropical species following the end of the glaciations from some southern regions such as Madeira. Bearing in mind that *T. pavo* and *Sparisoma* spp. have active swimming larvae that remain in the water column for 38–49 and 50–60 days, respectively (Raventós and Macpherson 2001; Robertson et al. 2006), this colonization might have been possible by counter current phenomena that cause sporadic transport of water and plankton from Madeira to Azores (Santos et al. 1995). This colonization route is very well supported by the historical migration trend shown by our data. Migration between these two regions is shown to occur in the Madeira–Azores direction only, for both *T. pavo* and *S. cretense*.

Given the probable post-glacial origin of *T. pavo* and *S. cretense* in the Azores, we would expect low genetic diversities for this population. However, this does not seem to be the case. Our data shows that Madeiran and Azorean populations of both species have extremely high levels of gene flow and exhibit no genetic differentiation (Fst and pairwise *P* distances are zero). Thus, the reason for the high genetic diversity of the Azores could be the present day intense connection of the two populations. For marine organisms with high dispersal potential, colonization of new areas can be achieved in a short period of time, when physical and ecological conditions are suitable. These rapid movements promote genetic homogenization and can easily erase the typical genetic signs of strong population reductions. A strong connection between the Azores and Madeira has been revealed for the marine gastropod *Littorina striatta* (De Wolf et al. 2000) and the barnacle *Chthamalus stellatus* (Pannacciulli et al. 1997).

Evolutionary history within the Mediterranean

In our study, we confirmed the existence of a genetic discontinuity at Peloponnesus for *T. pavo*. This species showed gene flow restriction between Kea, Sifnos and Rhodes and the locations to the west. Thus, even with an increased sample size, our analysis is still consistent with the results presented by Costagliola et al. (2004). Within the Mediterranean, a genetic break has been identified south of the Peloponnesus for two coastal organisms, the pomacentrid *Chromis chromis* (Domingues et al. 2005) and the bivalve *Cerastoderma glaucum* (Nikula and Väinölä 2003). This genetic break is probably linked to sea level changes during the Pleistocene glaciations, which might have promoted the isolation of the two basins. The intense anticyclonic gyre that exists in the region may also act as an effective barrier (Malanotte-Rizzoli and Bergamasco 1989). The genetic discontinuity of *T. pavo* at the Peloponnesus contrasts with the high levels of gene flow between the Atlantic and the Mediterranean. Although the reason for this discrepancy is not evident, one may think that the isolation between western and eastern populations of the Mediterranean during the Pleistocene might have been more effective than the isolation between the Atlantic and Mediterranean basins. It is also possible that the oceanographic circulation pattern south of the Peloponnesus is a more effective barrier to gene flow. Over time, population size differences of the areas in question can also explain this contrasting pattern.

It is very interesting to note that the two species examined in this study share common phylogeographic patterns and a similar historical demography. Several studies have attempted to establish a relation between population structure and species ecological characteristics, especially egg types and pelagic larval duration (e.g. Shulman and Bermingham 1995; Riginos and Victor 2001). Results, however, have not been consistent and a clear relationship between dispersal ability and population structure cannot be generalized. Other factors can influence the phylogeographic pattern of coastal fishes, particularly in regions that have experienced strong climatic and habitat changes such as the ones caused by the Pleistocene glaciations. The two species studied here are active demersal (or benthic) littoral fishes and have similar geographic distributions and thermal affinities. These characteristics seem to have triggered a similar response to the effects of Pleistocene glaciations, resulting in identical phylogeographic patterns.

Acknowledgments We are grateful to R. Araújo from Estação Marítima do Funchal for providing samples from Madeira. We thank M. Ramon for help in the lab and N. Serpa for sampling in the Azores. Vera S. Domingues has a doctoral fellowship from the Portuguese Foundation for Science and Technology (FCT) (SFRH/BD/13069/2003). Research at ISPA-UIE (UI&D #331/94) and IMAR-DOP/UAz (UI&D #531 and LA#9) are funded by FCT through pluri-annual and programmatic funding schemes (FEDER). IMAR-DOP/UAz research is also granted by DRCT. This paper is also a contribution to MarBEF (Network of Excellence: "Marine Biodiversity and Ecosystem Functioning"—contract number GOCE-CT-2003–505446).

References

Adams J, Maslin M, Thomas E (1999) Sudden climate transitions during the Quaternary. Prog Phys Geogr 23(1):1–36

Almada VC, Oliveira RF, Gonçalves EJ, Almeida AJ, Santos RS, Wirtz P (2001) Patterns of diversity of the north-eastern Atlantic blennid fish fauna (Pisces:Blenniidae). Glob Ecol Biogeogr 10:411–422

Aurelle D, Guillemaud T, Afonso P, Morato T, Wirtz P, Santos RS, Cancela ML (2003) Genetic study of *Coris julis* (Osteichtyes, Perciformes, Labridae) evolutionary history and dispersal abilities. C R Biol 326:771–785

Ávila SP (2000) Shallow-water marine mollusc of the Azores: biogeographical relationships. Arquipélago Life Mar Sci Suppl 2:99–131

Bargelloni L, Alarcorn JA, Alvarez MC, Penzo E, Magoulas A, Reis C, Patarnello T (2003) Discord in the family Sparidae (Teleostei): divergent phylogeographical patterns across the Atlantic-Mediterranean divide. J Evol Biol 16:1149–1158

Beerli P (2004) MIGRATE: documentation and program, part of LAMARK. Version 2.0. http://evolution.gs.washington.edu/lamarc.html

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc Natl Acad Sci USA 98:4563–4568

Briggs JC (1996) Global biogeography vol. 14. Developments in paleontology and stratigraphy. Elsevier, Amsterdam

Costagliola D, Robertson DR, Guidetti P, Stefanni S, Wirtz P, Heiser JB, Bernardi G (2004) Evolution of coral reef fish *Thalassoma pavo* spp. (Labridae). 2. Evolution of the eastern Atlantic species. Mar Biol 144:377–383

Crowley TJ (1981) Temperature and circulation changes in the eastern north Atlantic during the last 150000 years: evidence from the planktonic foraminiferal record. Mar Micropaleontol 6:97–129

De Wolf H, Verhagen R, Backeljau T (2000) Large scale population structure and gene flow in the planktonic developing periwinkle, *Littorina striata*, in Macaronesia (Mollusca: Gastropoda). J Exp Mar Biol Ecol 246:69–83

Dias JA, 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

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, Almada VC, Santos RS, Brito A, Bernardi G (2007) Phylogeography and evolution of the triplefin *Tripterygion delaisi* (Pisces, Blennioidei). Mar Biol 150:509–519

Domingues VS, Santos RS, Brito A, Almada VC (2006) Historical population dynamics and demography of the eastern Atlantic pomacentrid *Chromis limbata* (Valenciennes, 1833). Mol Phylogenet Evol 40:139–147

Duggen S, Hoernie K, vanden Bogaard P, Rupke L, Morgan JP (2003) Deep roots of the Messinian salinity crisis. Nature 422:602–606

Dulcic J (2004) Record of ornate wrasse *Thalassoma pavo* (Labridae) in the Northern Adriatic Sea. Cybium 28:75–76

Duran S, Pascual M, Turon X (2004) Low levels of genetic variation in mtDNA sequences over the western Mediterranean and Atlantic range of the sponge *Crambe crambe* (Poecilosclerida). Mar Biol 144:31–35

Excoffier L, Smouse PE, Quattro JM (1997) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Francour P, Boudouresque CF, Harmelin JG, Harmelin-Vivien ML, Quignard JP (1994) Are the Mediterranean waters becoming warmer? Information from biological indicators. Mar Pollut Bull 28:523–526

González JA (1993) *Sparisoma (Euscarus) cretense* (L.) (Pisces, Scaridae): Zoogeografía y ciclo reproductor en las Islas Canarias. Courier Forsch.-Inst Senckenberg 159:429–435

Guidetti P, Bianchi CN, La Mesa G, Modena M, Morri C, Sara G, Vacchi M (2002) Abundance and size structure of *Thalassoma pavo* (Pisces: Labridae) in the western Mediterranean Sea: variability at different spatial scales. J Mar Biol Assoc UK 82:495–500

Hsü KJ, Montadert L, Bernoulli D, Cita MB, Erickson A, Garrison RE, Kidd RB, Mèlierés F, Müller C, Wright R (1977) History of the Mediterranean salinity crisis. Nature 267:399–403

Kitchingman A, Lai S (2004) Inferences on potential seamount locations from mid-resolution bathymetric data. In: Morato T, Pauly D (eds) Seamounts: biodiversity and fisheries, Fisheries Centre Research Report 12 (5), pp 7–12

Kitchingman A, Lai S, Morato T, Pauly D (2007) How many seamounts are there and where are they located? In: Pitcher TJ, Morato T, Hart PJB, Clark MR, Haggan N, Santos RS (eds) Seamounts: ecology, conservation and management. Fish and aquatic resources series. Blackwell, Oxford

Krijgsman W, Hilgen FJ, Raffi I, Sierros FJ, Wilson DS (1999) Chronology, causes and progression of the Messinian salinity crisis. Nature 400:652–655

Lambeck K, Esat TM, Potter E-K (2002) Links between climate and sea levels for the past three million years. Nature 419:199–206

Lee WJ, Conroy J, Howell WH, Kocher TD (1995) Structure and evolution of teleost mitochondrial control regions. J Mol Evol 41:54–66

Malanotte-Rizzoli P, Bergamasco A (1989) The circulation of the eastern Mediterranean. Part I. Oceanol Acta 12:335–351

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

Pannacciulli FG, Bishop JDD, Hawkins SJ (1997) Genetic structure of populations of two species of *Chthamalus* (Crustacea: Cirripedia) in the north-east Atlantic and Mediterranean. Mar Biol 128:73–82

Patarnello T, Volckaert AMJ, Castilho R (2007) Pillars of Hercules: is the Atlantic-Mediterranean transition a phylogeographic break? Mol Ecol 16:4426–4444

Pérez-Losada M, Guerra A, Sanjuan A (1999) Allozyme differentiation in the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) from the NE Atlantic and Mediterranean. Heredity 83:280–289

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818

Quignard J-P, Pras A (1986a) Labridae. 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 919–942

Quignard J-P, Pras A (1986b) Scaridae. 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 943–944

Raventós N, Macpherson E (2001) Planktonic larval duration and settlement marks on the otoliths of Mediterranean littoral fishes. Mar Biol 138:1115–1120

Riginos C, Victor BC (2001) Larval spatial distributions and other early life-history characteristics predict genetic differentiation in eastern Pacific blennioid fishes. Proc R Soc Lond B 268:1931–1936

Robertson DR, Karg F, de Moura RL, Victor BC, Bernardi G (2006) Mechanisms of speciation and faunal enrichment in Atlantic parrotfishes. Mol Phylogenet Evol 40:795–807

Rozas J, Sánchez-Del Barrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497

Sara G, Bianchi CN, Morri C (2005) Mating behaviour of the newly-established ornate wrasse *Thalassoma pavo* (Osteichthyes: Labridae) in the Ligurian Sea (north-western Mediterranean). J Mar Biol Ass UK 85:191–196

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York

Santos RS, Hawkins S, Monteiro LR, Alves M, Isidro EJ (1995) Marine research, resources and conservation in the Azores. Aquat Conserv Mar Freshw Ecosyst 5:311–354

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN, Version 2.000: a software for population genetics data analysis. University of Geneva, Switzerland

Shulman MJ, Bermingham E (1995) Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution 49:897–910

Sorenson MD, Ast JC, Dimcheff DE, Yuri T, Mindell DP (1999) Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Mol Phyl Evol 12(2):105–114

Swofford DL (1998) PAUP: phylogenetic analysis using parsimony and other methods. Sinauer Associates, Sunderland, MA

Thiede J (1978) A glacial Mediterranean. Nature 276:680–683