Molecular Ecology (2007) **16**, 4426–4444 doi: 10.1111/j.1365-294X.2007.03477.x

INVITED REVIEW

Pillars of Hercules: is the Atlantic–Mediterranean transition a phylogeographical break?

TOMASO PATARNELLO,* FILIP A. M. J. VOLCKAERT† and RITA CASTILHO‡
**Dipartimento di Sanità Pubblica Patologia Comparata e Igiene Veterinaria, Agripolis, Università di Padova, Viale dell'Università, 16, I-35020 Legnaro (Pd), Italy, †Laboratory of Animal Diversity and Systematics, Katholieke Universiteit Leuven, Ch. Deberiotstraat 32 B-3000 Leuven, Belgium, ‡Center for Marine Sciences (CCMAR), Campus de Gambelas – University of Algarve, P-8005-139 Faro, Portugal*

Abstract

The geological history of the Mediterranean Sea, its hydrography and connection with the Atlantic Ocean have been well documented. Despite a wealth of historical and oceanographic data, the Atlantic–Mediterranean transition remains controversial at the biological level as there are discordant results regarding the biogeographical separation between the Atlantic and Mediterranean biota. The opening of the Strait of Gibraltar at the end of the Messinian Salinity Crisis (some 5.33 million years ago), removed the land barrier that impeded the marine biota allowing it to disperse freely into the Mediterranean Sea. However, present day genetic patterns suggest a limitation to gene flow for some marine species, preventing population admixture. In the last few years, a large number of studies have challenged the hypothesis of the Strait of Gibraltar representing a phylogeographical break. A review of more than 70 papers reveals no obvious relationship between either dispersal ability or life history, and observed patterns of partial or complete genetic isolation between Atlantic and Mediterranean populations. We re-analysed a selection of this large body of data (20 studies in total) in order to provide a homogeneous and coherent view on the generality of the phylogeographical patterns and the presence of a phylogeographical barrier. This offered the opportunity to summarize the state of the art on this matter and reach some general conclusions on the evolutionary history across the Atlantic–Mediterranean range. Geographically, some species in the transition zone showed step changes of allele frequencies associated with the Almeria-Oran Front rather than with the Strait of Gibraltar itself. A major part of the data describe evolutionary events well within the time frame of the Quaternary age as very few taxa pre-date closure of the Tethys Sea. Results point to a combined signature of vicariance, palaeoclimate fluctuation and life-history traits on the Atlantic–Mediterranean phylogeographical patterns. Principal component analysis failed to show any particular association between biological traits and genetic variables. It would argue that organismal determinism may play a far less significant role than marine biogeographers have generally believed.

Keywords: Almeria-Oran front, Atlantic Ocean, evolution, Mediterranean Sea, phylogeography, population genetics, Strait of Gibraltar

Received 1 March 2007; revision accepted 26 June 2007

Phylogeography in the marine realm

Origin and maintenance of genetic diversity is a central issue in evolutionary biology. Understanding these processes in the ocean realm is particularly difficult because barriers to gene flow are far less obvious in marine compared to continental species. Vicariance is usually invoked as the most likely model of speciation promoting genetic discontinuities across geographical ranges (Cunningham & Collins 1998). However, historical environmental factors related

Correspondence: Tomaso Patarnello, Fax: +39–0498276209, E-mail: tomaso.patarnello@unipd.it

to habitat, currents and glaciations (Roy *et al*. 1996; Wares 2002) combined with species-specific traits play a pivotal role in shaping the pattern of inter- and intraspecific differentiation. Their relative contribution remains hard to disentangle. These ancestral interactions combined with present-day environmental patterns are the focus of marine phylogeographical investigations.

Taxa with congruent geographical patterns of intraspecific structure have provided evidence as well as documented cases of phylogeographical breaks in the marine environment. Both ancestral and contemporary discontinuities in the marine environment are, for example, reflected in the biogeographical realm (Longhurst 1998). Well known cases are ancient breaks between the Southern Ocean and the surrounding oceanic waters associated with the formation of the Antarctic Polar Front some 20–30 million years ago (Ma) (Bargelloni *et al*. 2000), as well as the Equatorial Pacific and Atlantic Ocean at the Isthmus of Panama some 3.1–3.5 Ma (Avise 2000). Breaks of a more recent date are located between the Baltic Sea and the North Sea some 7500 years ago (Olsen *et al*. 2004), the northern and southern Atlantic Shelf (Cape Hatteras) where the Labrador Current and Gulf Stream have met on and off during the Quaternary (Weinberg *et al*. 2003). Sharp genetic breaks were also documented in the Indo-Pacific region north and south of the Flores and Java Seas (Barber *et al*. 2000), and at Cape Canaveral in the Florida Keys (reviewed in Avise 1992). Many of these cases turned out to be influenced by the large climatic fluctuations during the Quaternary. These fluctuations left a detectable trace in the genomic architecture of the contemporary terrestrial (Hewitt 2000; Soltis *et al*. 2006) and freshwater fauna (e.g. Bernatchez 2001), and less so in the oceans because of the ability of offshore organisms to move latitudinally (Cunningham & Collins 1998).

Biogeographical analysis focused on the Gulf of Mexico-Florida range revealed that several species of invertebrates and vertebrates have concordant genetic separation with reciprocal monophyly, indicative of a deep historical partition. However, not all taxa showed the same population subdivision. In fact, some species occupying the same area revealed very shallow to no phylogeographical pattern. Interestingly, neither the present-day distribution pattern (continuous or discontinuous) across the geographical boundary of Southern Florida, nor the dispersal abilities were good indicators of the genetic structure displayed by each taxon (Cunningham & Collins 1998). This suggests that shared biological features between species do not necessarily imply similar population structure. The lack of life-history characteristics associated with patterns of population subdivision at Cape Canaveral is consistent with a hypothesis that the vicariant events affected many species without regard to their life history. The reciprocal monophyly between populations of the southeast Atlantic coast and the Gulf of Mexico reported for a number of unrelated species can be associated with the same historical process (Avise 2000). However, as this is not always the case, the question arises why in some species such a historical event has left clearly detectable and concordant signs, whereas in other species it has not? Why did vicariance, separating populations across a given boundary, not equally affect all species over the same geographical range? Two possible explanations were given; the simplest and more intuitive is that taxa with higher dispersal ability are expected to have less pronounced population structure but, this does not hold true for the species studied north and south of Cape Canaveral (see above) since almost all of them have planktonic larvae that potentially contribute to high dispersal (Cunningham & Collins 1998). For instance, fish with equally high dispersal ability show either cases of reciprocal monophyly (e.g. black sea bass and toadfish) or shallow population structure (e.g. sturgeon and menhaden) with evidence of a recent genetic connection between the Atlantic Ocean and the Gulf of Mexico (Avise 1992). Extinction followed by recolonization is an alternative explanation to justify the absence of genetic subdivision among populations. Even assuming that cladogenetic processes have determined genetic discontinuity across a geographical boundary, extinction of one of the two lineages, followed by recolonization by members of the other lineage results in a genetic homogenization over the entire distribution range of the species (Wares & Cunningham 2001). The genetic signature of the recent extinction/recolonization is detectable as (i) reduced genetic diversity in the colonized area compared to the source population, and (ii) similar allele phylogenies on both sides of the geographical boundary.

Biogeographical origins of the Mediterranean biota

The term 'biodiversity' usually refers to species richness but it encompasses several interrelated aspects of biological diversity ranging from population to ecological communities. Although in this review we will develop those aspects of biodiversity related to population genetics, thus concentrating on intraspecific diversity, it is worth providing an overview of the species richness and diversity as well as a historical context of the Mediterranean Sea.

More than 8500 species of macroscopic organisms have been reported in this semiclosed sea (Longhurst 1998; Bianchi & Morri 2000), representing between 4% and 18% of the world's marine biodiversity. Interestingly, this value of species diversity seems rather high for a basin representing only 0.82% of the surface area and 0.32% of the volume of the world's oceans. In addition, more than one-quarter of the species are endemic to the Mediterranean (Tortonese 1985). This high biodiversity could be attributed to the historical interest in the Mediterranean Sea, but it may also have its roots in the troubled geological history of the Mediterranean region (Fig. 1). The Mediterranean Sea

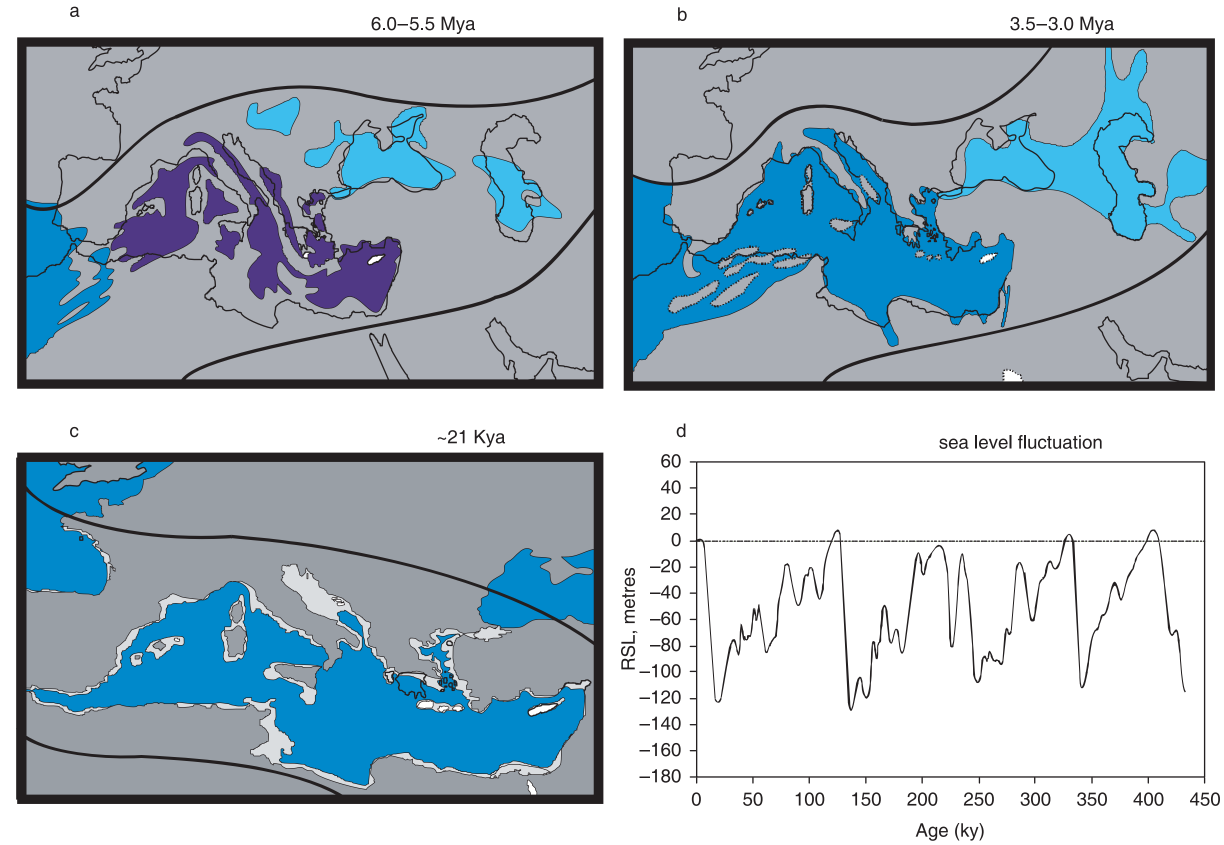

Fig. 1 Panels a, b and c report palaeogeographical maps of the Mediterranean Sea and adjacent regions over time; some present-day coastlines have been drawn for reference. Grey indicates land extension; light blue, low salinity waters; dark blue, hypersaline regions (from: Rögl & Steininger 1983; Dawson 1992; Rögl 1999). Black lines indicate the geographical limits within which the palaeogeographical events were considered. Panel d depicts sea-level fluctuation during the Pleistocene (from: Waelbroeck *et al*. 2002).

ranged, in the last few million years, from almost complete desiccation during the 'Messinian salinity crisis' (MSC) (up to 5.33 Ma) to cycles of cold glacial periods followed by warm interglacial periods during the Quaternary (Box 1). The present-day biota are largely the result of colonization, mostly from the Atlantic Ocean (Almada *et al*. 2001; Domingues *et al*. 2005) and to a minor extent from the Red Sea. In fact, after an isolation and desiccation period that lasted about 0.5 million years, re-flooding of the Mediterranean basin was possible because of the inflow of Atlantic waters through the newly opened Straits of Gibraltar. The MSC drove the pre-existing Indo-Pacific biota to extinction, with the few exceptions of taxa of Miocene origin that have survived in shallow-water refuges, for example killifishes (Hrbek & Meyer 2003).

This geographical history suggests that species communities contemporarily inhabiting the Mediterranean waters can be divided into the following biogeographical categories: (i) temperate Atlantic-Mediterranean species; (ii) cosmopolitan/panoceanic species; (iii) endemic species, including palaeo-endemic (Miocenic) and neo-endemic (Pliocenic) species; (iv) subtropical Atlantic species (interglacial remnants); (v) boreal Atlantic species (glacial remnants); (vi) Red Sea invasive (Lessepsian) species entering through the Suez Canal; and (vii) eastern Atlantic invasive species. Bianchi & Morri (2000) identified 10 biogeographical regions in the Mediterranean according to the relative abundance of each of the aforementioned categories. Transition from one biogeographical zone to another results from a combination of geological (orogenesis and hydrogeology), physical (present-day hydrography, coastal and seabed profile) and biological factors (species biology and evolutionary history), all contributing to shape the intra- and interspecific diversity.

Population structure across the Atlantic–Mediterranean transition

The information reported above, although pertaining to interspecific diversity, is useful to set a historical and biogeographical context for the Mediterranean Sea and adjacent regions. However, the goal of this work is not to investigate differences between species but rather to concentrate on the geographical partitioning of genetic

Box 1 Mediterranean palaeoceanography

Four features dominate the palaeoceanography of the Mediterranean Sea: (i) it is enclosed geographically with the presence of sills separating individual basins; (ii) its connection to the Atlantic Ocean — throughout history the main source of biota for colonization; (iii) the climate regime where temperature determines the natural range of living organisms, and where the temperature and evaporation balance determine seawater density and hence thermohaline circulation; and (iv) the sea level, which determines hydraulic connectivity between basins.

Enclosed geography

The Mediterranean Sea is a fully enclosed sea, except for a 12.9-km wide and 286-m deep connection with the Atlantic Ocean between Cape Trafalgar and Cape Spartel (Straits of Gibraltar) (Figs 1 and 2). The Mediterranean Sea has a depth of approximately 3400 m in the western basin and 4200 m in the eastern basin; therefore, it can be named a proper ocean basin. It is divided into two units by a shallow sill (350 m deep) in the Strait of Sicily, namely the Eastern and the Western Mediterranean. The Bosporus–Dardanelles sill (40–70 m) separates the Black Sea from the Mediterranean Sea. The Pelagosa sill (160 m) separates the central Adriatic Sea from the Eastern Mediterranean Sea. The man-made Suez Canal (opened in 1869) in the southeastern Mediterranean Sea has led to a steady influx of saline water (and biota) from the subtropical Red Sea.

Connection to the Atlantic Ocean

Apart from the Suez Canal, the only source of oceanic water to the Mediterranean Sea originates from the North Atlantic Ocean, entering through the Straits of Gibraltar. The Mediterranean Sea became stably separated from the Atlantic Ocean during the Messinian salinity crisis (MSC), which occurred in the late Miocene (5.59–5.33 Ma) (Hsü et al. 1973; Krijgsman et al. 1999). The sea level of the Mediterranean Sea dropped dramatically. Once the obstruction to this gateway was removed because of tectonic uplifting, faulting and sea level changes, the Mediterranean Sea was flooded catastrophically and synchronously with oceanic water, one of the most dramatic events of the Cenozoic (Fig. 1a–c). Recurrent, short periods of separation between the Atlantic and Mediterranean waters occurred during the Quaternary in correspondence to cyclic ice ages and the associated sea level changes (Fig. 1d).

Climate regime

The relatively small and marginal basin of the Mediterranean Sea has tracked global climate oscillations faithfully. The Lago-Mare facies during the MSC represented evidence of extensive desiccation and the formation of hypersaline and freshwater sub-basins (Krijgsman *et al*. 1999). Although large areas of low salinity were present in the proximity of river outflows, few coastal marine taxa survived this period (Penzo *et al*. 1998; Hrbek & Meyer 2003; Huyse *et al*. 2004). Termination of the MSC coincided with the catastrophic flooding of the Mediterranean Sea. Following the restoration of the marine ecosystem, the Late-Pliocene epoch saw several climate-induced increases in marine production associated with processional minima and coincidental regional warming (Passier *et al*. 1999; Haywood *et al*. 2000; Meyers & Arnaboldi 2005). About 3 Ma, the Mediterranean climate was warmer and wetter than today (Haywood *et al*. 2000). During the Quaternary, the Mediterranean Sea continues to register and amplify the smallest climatic variations occurring at mid-latitude regions (Sbaffi *et al*. 2001). Hence, the glacial–interglacial cycling so typical during the Pleistocene is well reflected in the palaeontological record. Relatively warm periods (interglacials) have been dated at ~630 000, ~330 000, ~200 000, ~130 000 (the last interglacial) and 11 500 years ago (the current interglacial, named Holocene). Glacial stadia, including the last glacial maximum (LGM) — 24 000–20 700 years ago (Lambeck *et al*. 2002) have led to colder (but ice-free) conditions. Local analysis of dated deposits by means of proxies of sea surface temperature, point to a close tracking of the climate by the biota (Zonneveld 1996; Paterne *et al*. 1999; Sbaffi *et al*. 2001; Rohling *et al*. 2002).

During the Pleistocene period, circulation in the Mediterranean Sea became so restricted at times that the deeper waters became anoxic as in the Black Sea today. Primary productivity of the basin increased and a series of organic rich sediments, called sapropels, were deposited (e.g. at 10 500 and 6100 years ago) (Krom *et al*. 2005). The climate around the basin was less arid; there was increased rainfall in central and eastern Africa and a higher river flow in the Nile. It is not clear whether the circulation remained anti-estuarine or became estuarine. Such climate changes often translated to an impoverishment in taxa as there was no latitudinal range to track the isothermals. Hence, extinction and recolonization have played a major role in the Mediterranean Sea (Cunningham & Collins 1998).

Currently a Mediterranean climate dominates the region, that is wet cool winters and dry warm summers, with a total precipitation of 420 mm. a^{-1}. River discharge into the Mediterranean Sea is dominated by rivers from the northern temperate zone, such as the Ebro, Rhône, Po and Seyhan, and especially from the East and Central European rivers flowing into the Black Sea (Danube, Dnjestr, Dnjepr and Don). Runoff from the arid southern (sub)tropical zone is very reduced (e.g. Nile), such that there is a 1:5 shortfall in precipitation and runoff over evaporation.

Sea level

Sea level changes are related to tectonics, solar modulation of climate (and ice sheets), geoid and deformation of ocean basins (Lambeck *et al*. 2002). They have globally and locally had a major effect on the oceans and especially the continental shelves. After the dramatic events of the MSC, sea levels have changed regularly throughout the Pliocene and Pleistocene. The lowest sea levels were observed 140 000 years ago (–130 m) and 30 000 years ago (–120 m) (Fig. 1d). The Mediterranean Sea with its sill determined topography has directly felt the impact of changing sea levels through changing flow regimes between the Black Sea, the Eastern Mediterranean, the Western Mediterranean and the Atlantic Ocean. The Black Sea was separated on and off from the Mediterranean Sea through exposure of the shallow sills at the Straits of Dardanelles and Bosporus (Aksu *et al*. 2002).

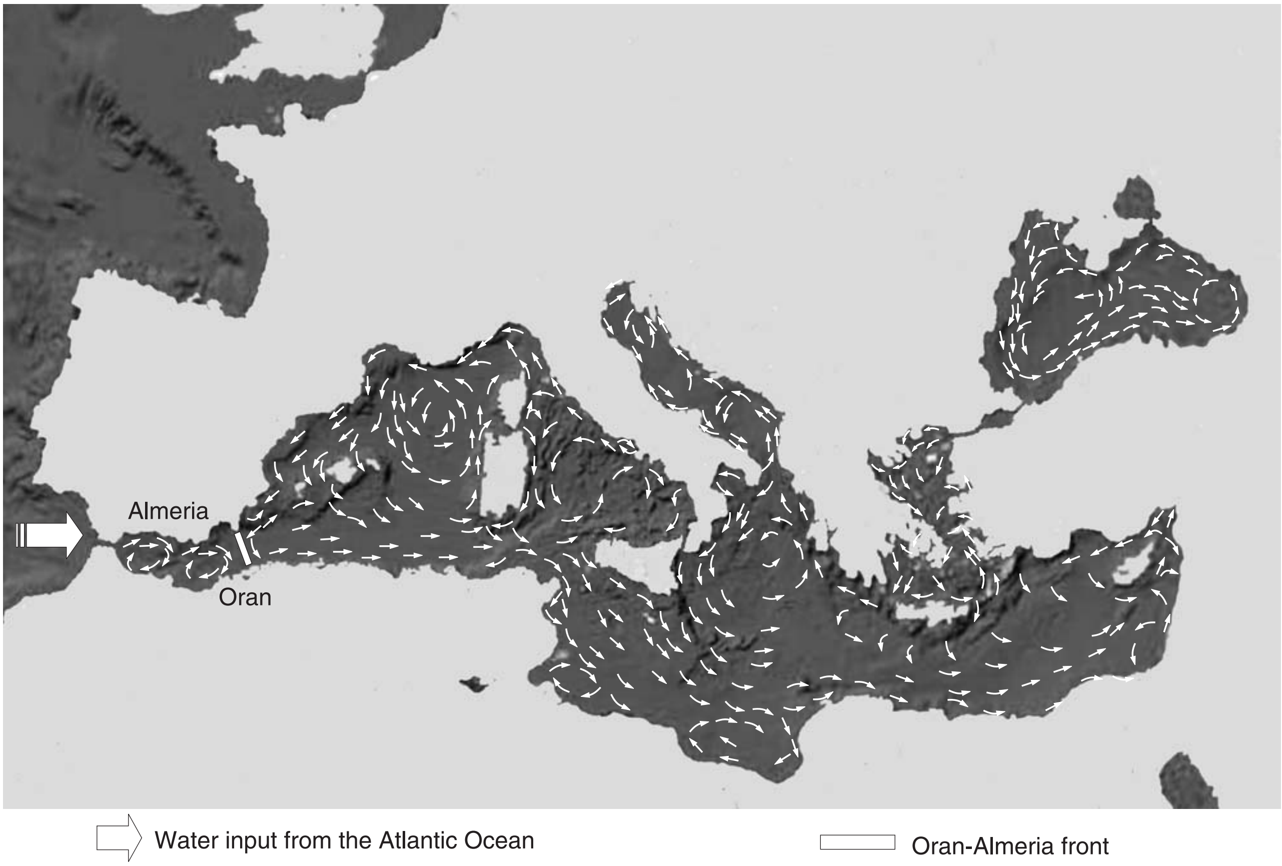

Fig. 2 Schematic representation of the main currents characterizing water circulation in the Mediterranean and Black Sea.

diversity within species across Atlantic–Mediterranean transition zones. For this purpose, we consider three major geographical provinces: the eastern Atlantic, the western Mediterranean and the eastern Mediterranean, that largely follow the bathygraphy (Fig. 2). Intraspecific phylogenetic breaks and/or genetic transitions will be considered in association with two main geographical boundaries separating the aforementioned provinces: the Straits of Gibraltar (dividing the Atlantic Ocean and western Mediterranean Sea) and the Strait of Sicily (separating the eastern and western Mediterranean). The geological history of the Mediterranean Sea has been well documented (Fig. 1) as has its present-day hydrography (Fig. 2) within the Mediterranean and between the Mediterranean and the Atlantic Ocean (see Box 1 and Box 2). Despite the wealth of geological and oceanographic data, the Atlantic–

Box 2 Physical oceanography of the Mediterranean Sea

Mediterranean circulation is forced by topography (water exchange through the various straits), wind stress, and buoyancy flux at the surface due to fresh water and heat fluxes (Robinson *et al*. 2001). It is highly variable at the basin, sub-basin and mesoscale spatiotemporal scales. As evaporation (most intense in the Eastern Basin) dominates over precipitation, an anti-estuarine (reverse thermohaline) circulation and locally deep convection characterize the Mediterranean Sea (Malanotte-Rizzoli *et al*. 1996; Malanotte-Rizzoli *et al*. 1997) (Fig. 2). Less dense Atlantic water enters at the surface through the Straits of Gibraltar with an average salinity of 36.15 ppt (Millot 1999). Salinity tends to increase eastward generating a west-east gradient with a maximum in the eastern Mediterranean Sea (38.0–38.5 ppt) because of the intense water evaporation. The inflowing Atlantic water describes in the Alboran Sea, a quasi-permanent anticyclonic gyre in the west and a more variable one in the east. The particular water circulation in the Alboran Sea generates an oceanographic front located from Almeria (Spain) to Oran (Morocco), called the Almeria-Oran Front (AOF). This is an oceanographic front exhibiting a pronounced step temperature (1.4 °C) and salinity (2 ppt) gradient over a distance of 2 km with average water current speed of 40 cm/s flowing southeastward from the Spanish coast to the coast of North Africa (Tintore *et al*. 1988). The flow of surface water to the east follows the North African coast (Algerian Current) with a strong jet near the Libyan coast (Alhammoud *et al*. 2005). Surface circulation in the Eastern Mediterranean is counterclockwise (Hamad *et al*. 2005). It is complicated by the many mesoscale eddies and jets that are characteristic of the Mediterranean in general. The Eastern Mediterranean is starved of phosphorus and hence ultra-oligotrophic. It is reflected in the high water transparency, the low depth-integrated chlorophyll values, the high contribution of pico- and nanophytoplankton and the deeper position of the deep chlorophyll maximum (DCM) (Krom *et al*. 2005). Deep convection is observed in the South Adriatic Sea with cascading through the Strait of Otranto, the South Aegean Sea and between Rhodes and Cyprus.

Mediterranean transition at the biological level is controversial, as there are discordant results regarding the biogeographical separation between the Atlantic and Mediterranean biota. The opening of the Straits of Gibraltar at the end of the MSC allowed free dispersal of the marine biota between the Atlantic Ocean and Mediterranean Sea. Since then, several geological events influenced the history of the Mediterranean region, most importantly recurrent glaciations during the Pleistocene.

In the past few years, the hypothesis that the Straits of Gibraltar acts as a barrier to gene flow has been tested in many organisms including plants and marine mammals. However, the rapidly growing literature on the Atlantic–Mediterranean divide is largely based on a 'single marker–single species' scheme. Differences in the biology of species combined with differences in the data analysis make comparison between studies complex. To compensate for this discrepancy, we performed a re-analysis of 20 selected species (3 invertebrates and 17 vertebrates) over the target geographical range (see Table 1 and 'Data re-analysis' section below). In particular, we consider case reports on those situations that fit three basic scenarios: (i) no or weak population structure; (ii) population structure but no sign of Mediterranean–Atlantic separation; (iii) population structure with significant differentiation between Mediterranean and Atlantic populations. In the latter case, further substructuring at a smaller geographical scale within each basin may also be observed.

Before concentrating on the 20 selected cases, we summarize hereafter the extensive recent literature with particular attention to (i) species showing clear Atlantic–Mediterranean differentiation, (ii) contrasting results reported in closely related species.

Atlantic–Mediterranean differentiation

Previously, population differentiation across the Atlantic–Mediterranean transition was described in a number of marine species (Borsa *et al*. 1997), with extreme cases of reciprocal monophyly (see below and Table 1). Investigations were carried out both at the large and fine geographical scale providing phylogeographical scenarios of various degrees of detail. Population surveys over a wide geographical range spanning the northeast Atlantic Ocean, the Mediterranean sea (west and east) and the Black Seas were reported for two sea grass species (*Zostera marina* and *Z. noltii*, Coyer *et al*. 2004; Olsen *et al*. 2004), for the blue mussel (*Mytilus galloprovincialis*, Ladoukakis *et al*. 2002), for the chaetognath (*Sagitta setosa*, Peijnenburg *et al*. 2004, 2006) and for the anchovy (*Engraulis encrasicolus*, Magoulas *et al*. 2006). Mitochondrial DNA analysis showed a very similar pattern in all species with distinct gene pools associated with each of the three basins. The strongest genetic separation was observed in the two invertebrate species which showed complete lineage sorting between Atlantic vs. Mediterranean and Black Sea samples.

Table 1 List of candidate papers considered for the present review and summary of the relevant information reported in each work

Species	Type of organism	Genetic information	*N*	Structure; Atl/ medical	Monophyly (MY of Atl-medical separation)	References	Selected
Anguilla anguilla	Fish	Cytochrome *b*	107	Weak; no	—	Daemen *et al*. 2001¶	Yes (1)
Anguilla anguilla	Fish	Microsatellites	2626	No	—	Dannewitz *et al*. 2005†	No
Anguilla anguilla	Fish	Allozymes	304	Yes; yes	No	Maes *et al*. 2002†	No
Asparagopsis armata	Red algae	LSU Rdna COX 2–3 spacer RuBisCo spacer	32	No	—	Andreakis *et al*. 2004†	No
Asparagopsis taxiformis	Red algae	LSU rDNA COX 2–3 spacer RuBisCo spacer	21	Yes; yes	Yes	Andreakis *et al*. 2004†	No
Asterina gibbosa	Echinoderm	AFLP	159	Yes; yes	NA	Baus *et al*. 2005*	No
Balaenoptera physalus	Mammal	D-loop	139	Yes; yes	No	Bérubé *et al*. 1998**	Yes (2)
Calanus helgolandicus	Crustacean	COX1, Cytochrome *b*	72	Yes, yes	No	Papadopoulos *et al*. 2005	No
Carcinus maenas	Crustacean	COX1	230	Yes, yes	Yes††	Roman & Palumbi 2004	No
Caretta caretta	Turtle	D-loop	152	Yes; no	—	Bolten *et al*. 1998*	No
Cerastoderma glaucum	Mollusk	COX1/Allozymes	129	Yes; no	—	Nikula *et al*. 2003†	No
Chthamalus montagui	Crustacean	Allozymes	807	Yes; yes	No	Pannacciulli *et al*. 1997†	No
Coris julis	Fish	Microsatellites	249	No	—	Aurelle *et al*. 2003†	No
Crambe crambe	Sponge	Microsatellites	286	Yes; yes	No	Duran *et al*. 2004c†	No
Crambe crambe	Sponge	COX1	86	No	—	Duran *et al*. 2004d*	No
Crambe crambe	Sponge	ITS-1, 2	111	Yes; yes	No	Duran *et al*. 2004a†	No
Dentex dentex	Fish	D-loop	126	Yes; yes	No (1.2–1.8)	Bargelloni *et al*. 2003¶	Yes (3)
Dicentrarchus labrax	Fish	Allozymes	388	Yes; yes	No	Allegrucci *et al*. 1997†	No
Dicentrarchus labrax	Fish	Microsatellites	226	No	—	Bonhomme *et al*. 2002†	No
Dicentrarchus labrax	Fish	Microsatellites	630	Yes; yes	Yes	Naciri *et al*. 1999†	No
Dicentrarchus puntactus	Fish	Microsatellites	92	No	—	Bonhomme *et al*. 2002†	No
Dicentrarcus labrax	Fish	Cytochrome *b*	580	Yes; yes	Yes (0.27–1.1)	Lemaire *et al*. 2005¶	Yes (4)
Diplodus puntazzo	Fish	D-loop	147	Yes; yes	No	Bargelloni *et al*. 2005¶	Yes (5)
Diplodus sargus	Fish	D-loop	97	No	—	Bargelloni *et al*. 2005¶	Yes (6)
Engraulis encrasicolus	Fish	mtDNA	673	Yes; yes	No	Magoulas *et al*. 1996†	No
Engraulis encrasicolus	Fish	mtDNA	1238	Yes; yes	No	Magoulas *et al*. 2006†	No
Homarus gammarus	Crustacean	mtDNA	3283	Yes; yes	No	Triantafyllidis *et al*. 2005	No
Hydrobia acuta	Mollusk	COX1	76	Yes; yes	NA	Wilke & Pfenninger 2002‡	Yes (7)
Lophius budegassa	Fish	D-loop	134	Yes; yes	No	Charrier *et al*. 2006	No
Lophius piscatorius	Fish	D-loop	382	No; no	—	Charrier *et al*. 2006	No
Lithognathus mormyrus	Fish	D-loop	97	Yes; yes	Yes (1.2–1.8)	Bargelloni *et al*. 2003¶	Yes (8)
Meganyctiphanes norvegica	Crustacean	NADH1	980	Yes; yes	No	Papetti *et al*. 2005¶	Yes (9)
Merluccius merluccius	Fish	Allozymes	1306	Yes; yes	No	Cimmaruta *et al*. 2005†	No
Merluccius merluccius	Fish	Allozymes	420	Yes; yes	No	Lo Brutto *et al*. 2004†	No
Merluccius merluccius	Fish	Microsatellites	483	Yes; yes	No	Lundy *et al*. 1999†	No
Mesopodopsis slabber	Crustacean	COX1, 16S rRNA	103	Yes; yes		Remerie *et al*. 2006	No
Mytilus galloprovincialis	Mollusk	COX3, 16S rRNA	124	Yes; yes	No	Ladoukakis *et al*. 2002†	No
Nephrops norvegicus	Crustacean	mtDNA	379	Weak; no	—	Stamatis *et al*. 2004†	No
Ostrea edulis	Mollusk	Microsatellites	587	No	—	Launey *et al*. 2002†	No
Pagellus bogaraveo	Fish	D-loop	111	No	—	Bargelloni *et al*. 2003¶	Yes (10)
Pagrus pagrus	Fish	D-loop	160	No	—	Bargelloni *et al*. 2003¶	Yes (11)
Paracentrotus lividus	Sea Urchin	COX1	127	Yes; yes		Duran *et al*. 2004d	No
Pecten maximus	Mollusk	Allozymes	168	No	—	Rios *et al*. 2002*†	No
Platichthys flesus	Fish	Allozymes	796	Yes; yes	No	Borsa *et al*. 1997a†	No
Pomatoschistus microps	Fish	Cytochrome *b*	272	Yes; yes	No	Gysels *et al*. 2004a**	Yes (12)
Pomatoschistus minutus	Fish	Cytochrome *b*	228	Yes; yes	No	Gysels *et al*. 2004b**	Yes (13)
Pomatoschistus minutus	Fish	D-loop	51	Yes; no	—	Stefanni & Thorley 2003¶	Yes (14)
Raja clavata	Fish	Cytochrome *b*, microsatellites	385	Yes; yes	No	Chevolot *et al*. 2006	No

Table 1 *Continued*

Species	Type of organism	Genetic information	*N*	Structure; Atl/medical	Monophyly (MY of Atl-medical separation)	References	Selected
Sagitta setosa	Chaetognath	COX2	82	Yes; yes	Yes (1.7)	Peijnenburg *et al*. 2004¶	Yes (15)
Sarda sarda	Fish	D-loop	195	Yes; no	—	Viñas *et al*. 2004*	No
Sardina pilchardus	Fish	D-loop	261	Yes; no	—	Atarhouch *et al*. 2006¶	Yes (16)
Sardinella aurita	Fish	mtDNA	20	Yes; yes	NA	Chikhi *et al*. 1998†	No
Scomber japonicus	Fish	D-loop	263	No	—	Zardoya *et al*. 2004¶§	Yes (18)
Scomber scombrus	Fish	D-loop	50	Yes; yes	No	Nesbø *et al*. 2000¶	Yes (17)
Scomber scombrus	Fish	D-loop	246	Yes; yes	No	Zardoya *et al*. 2004¶	Yes (17)
Sepia officinalis	Mollusk	Microsatellites	807	Yes; yes	No	Perez-Losada *et al*. 2002†	No
Sparus aurata	Fish	D-loop, microsatellite, allozymes	259	Yes, weak	—	Alarcon *et al*. 2004†	No
Sparus aurata	Fish	Microsatellites	361	Yes, weak	—	De Innocentiis *et al*. 2004†	No
Spondyliosoma cantharus	Fish	D-loop	52	Yes; yes	Yes (1.2–1.8)	Bargelloni *et al*. 2003¶	Yes (19)
Stenella coeruleoalba	Mammal	mtDNA	127	Yes; yes	No	Garcia-Martinez *et al*. 1999†	No
Symbium pandora	Cycliophoran	COX1	143	Yes; no	—	Obst *et al*. 2005*	No
Thalassoma pavo	Fish	D-loop	101	No	—	Costagliola *et al*. 2004*	No
Thunnus alalunga	Fish	Allozymes	188	No	—	Pujolar *et al*. 2003*†	No
Thunnus thynnus	Fish	D-loop	179	No	—	Ely *et al*. 2002¶	No
Thunnus thynnus thynnus	Fish	Allozymes	783	No	—	Pujolar *et al*. 2003*†	No
Trachurus mediterraneus	Fish	mtDNA	182	No	—	Karaiskou *et al*. 2004†	No
Trachurus picturatus	Fish	mtDNA	140	No	—	Karaiskou *et al*. 2004†	No
Trachurus trachurus	Fish	mtDNA	302	No	—	Karaiskou *et al*. 2004†	No
Tursiops truncatus	Mammal	D-loop, microsatellites	145	Yes; yes	No	Natoli *et al*. 2005**	Yes (20)
Xiphias gladius	Fish	D-loop	120	Yes; yes	No	Chow *et al*. 2000†	No
Xiphias gladius	Fish	Allozymes	401	No	—	Pujolar *et al*. 2002†	No
Ziphius cavirostris	Mammal	D-loop	17	Yes; yes	No	Dalebout *et al*. 2005*	No
Zostera marina	Seagrass	Microsatellites	1706	Yes; no	—	Coyer *et al*. 2004	No
Zostera noltii	Seagrass	Microsatellites	1756	No	—	Coyer *et al*. 2004†	No
Zostera noltii	Seagrass	ITS-1, 2	383	Yes; no	—	Olsen *et al*. 2004†	No

*Data not used because of incomplete geographical sampling coverage, insufficient number of individuals or extremely low polymorphism; †data type other than mitochondrial DNA sequences [allozymes, single-strand conformation polymorphism (SSCP), restriction fragment length polymorphism (RFLP), microsatellites etc.]; ‡two subspecies with disjunct geographical distribution, therefore configuring a case of incomplete geographical sampling coverage; §samples from Nesbø *et al*. 2000 included; ¶datafile provided by authors; **datafile reconstructed from sequences in GenBank; ††samples from the Alboran Sea group with the Atlantic samples; N.A., information not available. In the last column, numbers in parenthesis refer to Fig. 4 and Tables S2 and S3.

Mediterranean and Black Sea populations appeared more closely related in both *M. galloprovincialis* and *S. setosa*, suggesting that the Black Sea populations originated from ancestral Mediterranean populations. In *S. setosa*, the separation between Atlantic and Mediterranean lineages was estimated to have occurred about 1.7 Ma, whereas divergence between Mediterranean Sea and Black Sea lineages were as recent as 400 000 years ago. These time estimates may be imprecise considering that they were based on a 2% mutation rate for the entire mtDNA. However, they are in agreement with similar observations reported for two copepod species *Calanus helgolandicus* and *C. euxinus*, inhabiting the Mediterranean Sea and the Black Sea, respectively, which diverged during the Pleistocene (Papadopoulos *et al*. 2005). Three well-resolved groups were also identified in the two species of *Zostera*, matching three geographical areas: Northern Europe, Mauritania and the Black/Azov Sea. The Mediterranean samples were separated from all the others and placed intermediate between the northern Atlantic and the Black/Azov Sea populations accounting for a general scheme of ‘isolation by distance’. Anchovy populations appeared more structured with some puzzling haplotype distribution. However, four main geographical groups were clearly identified: Atlantic, central Mediterranean, Aegean Sea, and Black Sea. Interesting is the evidence produced to support the affinity of the alboran sea samples to the atlantic samples. Large-scale population surveys were also carried out in the

European hake, *Merluccius merluccius* over the entire distribution range of the species, which extends form the northeast Atlantic to eastern Mediterranean (Lundy *et al*. 1999; Cimmaruta *et al*. 2005). This commercially valuable species also showed clear separation between Atlantic and Mediterranean stocks detectable by both microsatellites and allozymes. Interestingly, allozyme pattern showed a strong correlation with salinity and temperature suggesting a role for these environmental factors in maintaining the genetic differentiation among the two population groups through selective processes (Cimmaruta *et al*. 2005). Genetic discontinuity between Atlantic and Mediterranean hake populations was observed to correspond with the Almeria-Oran front.

At the fine geographical scale, the Atlantic–Mediterranean transition area was investigated in the cuttlefish (*Sepia officinalis,* Perez-Losada *et al*. 2002), the blue mussel (*M. galloprovincialis,* Quesada *et al*. 1995), the sea urchin (*Paracentrotus lividus,* Duran *et al*. 2004b) and the European sea bass (*Dicentrarchus labrax,* Lemaire *et al*. 2005). In all cases, sampling was aimed at providing a detailed population analysis of the region. With the exception of the sea urchin, which showed a slight (although significant) differentiation between basins, the three other species revealed significant clinal changes in the transition from Atlantic to Mediterranean populations. More importantly, all species showed an abrupt 'step' change in allele frequencies associated with the Almeria-Oran Front (AOF) (Box 2). Such genetic discontinuity was clearly detectable irrespective of the molecular marker used (mtDNA for *M. galloprovincialis* and *D. labrax*, microsatellites for *Se. officinalis*). A number of other species showed significant differences between Atlantic and Mediterranean populations as summarized in Table 1. In the next section, we will focus on cases where closely related species show opposite situations.

Contrasting results in closely related species

A useful approach for identifying common phylogeographical signals may come from the comparison between closely related species with a comparable biology. One may assume that with biological traits (i.e. life history and dispersal ability) being similar as much as possible, it should be more straightforward to identify a shared history of habitat fragmentation. From this perspective, the most extensively investigated group is the family Sparidae, a highly diversified group of demersal perciform fish (Bargelloni *et al*. 2003; Alarcon *et al*. 2004; De Innocentiis *et al*. 2004; Bargelloni *et al*. 2005). Bargelloni *et al*. (2003) surveyed population structure in *Dentex dentex*, *Lithognathus mormyrus*, *Pagellus bogaraveo*, *Pagrus pagrus* and *Spondyliosoma cantharus* with the same mitochondrial fragment (D-loop) and the same set of nuclear (allozymes) markers. The sampling scheme largely overlapped in order to maximize comparability. The results showed stark discordance among species. Three of the five species (*De. dentex*, *L. mormyrus* and *Sp. cantharus*) revealed a sharp Atlantic–Mediterranean separation, whereas the other two (*P. bogaraveo* and *Pa. pagrus*) showed very little or no population structure and no signs of Atlantic–Mediterranean division. Concordance of *De. dentex*, *L. mormyrus* and *Sp. cantharus* in reciprocal monophyly between Atlantic and Mediterranean populations suggested that the same historical biogeographical factors might have influenced intraspecific patterns of genetic differentiation. Further support for this view was given by the estimates of divergence time between Atlantic and Mediterranean clades. In fact, both *De. dentex* and *L. mormyrus* showed a gene flow extremely limited or absent between the two basins over the past 1.2–1.8 Myr. Such a temporal (early Pleistocene) and spatial (Strait of Gibraltar-AOF) placement of a phylogeographical boundary concurs with geological data (Nilsson 1982). The data confirm that climate fluctuations during the entire Quaternary period have produced episodes of habitat fragmentation between the Atlantic and the Mediterranean (Box 1). On a shorter timescale, some hydrological features such as the AOF might have reduced gene flow between both sides of the Strait. An analogous incongruent phylogeographical pattern was described for two sparids belonging to the same genus, *Diplodus puntazzo* and *Dip. sargus*. They share a similar ecological behaviour and have no or little differences in biological traits (Bargelloni *et al*. 2005). However, the pronounced population structure of *Dip. puntazzo* contrasts sharply with the little intraspecific differentiation in *Dip. sargus.* The opposite situation for the two congeneric species was interpreted as a consequence of differences in the population dynamics, such as fluctuation in the effective population size due to bottlenecks and expansions. Sparids are not the only example of differences in population structure between closely related species. Discordance was recently reported for two species of anglerfish (*Lophius budegassa* and *Lo. pescatorius*) (Charrier *et al*. 2006) and two species of mackerel (*Scomber japonicus* and *Sc. scombrus*) (Zardoya *et al*. 2004). Also, the comparative phylogeography of two large pelagic fish, the Atlantic bluefin tuna (*Thunnus thynnus*) and the swordfish (*Xiphias gladius*), reveals contrasting results (Alvarado-Bremer *et al*. 2005). Both species were characterized by a complex haplotype structure with two distinct clades within each species. However, no obvious geographical partition of D-loop haplotypes was observed for the bluefin tuna samples. Deep clades in the swordfish reflect a history of vicariant separation between the Atlantic Ocean and Mediterranean Sea. The proposed explanation for the observed difference between the two species relates to differences in their demographic history. Temperature fluctuation that characterized the Mediterranean Sea during the Pleistocene could have prevented bluefin tuna from reproducing and occupying the areas that this species only recently re-colonized. On the contrary, swordfish adapted to Mediterranean Pleistocene conditions, possibly occupying a refuge area in the eastern Mediterranean where it remained isolated from the Atlantic population for the last 0.7 million years.

The literature on invertebrates largely consists of 'single marker–single species' studies. The only case that allows comparison between closely related species are two lobster species, the Norway lobster (*Nephrops norvegicus*) and the European lobster (*Homarus gammarus*). They belong to the same family and exhibit a very similar biology. They were investigated in two papers, both using a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) approach, applied to a mtDNA fragment (Stamatis *et al*. 2004; Triantafyllidis *et al*. 2005). Population structure of the Norway lobster was analysed across a wide geographical range encompassing the North Sea, Irish Sea, Portuguese coast and Aegean Sea (Stamatis *et al*. 2004). No signs were detected of Atlantic–Mediterranean differentiation or isolation by distance. The absence of a clear pattern of geographical structure for this burrowing crustacean with pelagic larvae might be only partly justified by gene flow over distances as far apart as the Aegean Sea and North Sea. In fact, a more likely explanation is a recent expansion. This possibility is supported by the relatively shallow haplotype network and by the mismatch analysis, which calculates the period of expansion to be as old as 280 000–440 000 years. In contrast, pronounced geographical structuring was reported for the European lobster (Triantafyllidis *et al*. 2005) whose range of distribution considerably overlaps with *N. norvegicus*. At least three well-differentiated groups were identified in *H. gammarus*, with the Mediterranean being well differentiated from all Atlantic samples. This species also showed significant substructuring within the Mediterranean. Notably, the Adriatic population appeared genetically divergent from all the others as reported for other invertebrate (Peijnenburg *et al*. 2006) and vertebrate species (Stefanni & Thorley 2003; Gysels *et al*. 2004b). Distinctiveness of populations inhabiting this semiclosed sea suggests that the Adriatic Sea might represent a region of further phylogeographical discontinuity deserving an in-depth analysis.

Data re-analysis

We prepared a meta-analysis of the most complete and accessible data sets to provide an integrated picture of Atlantic–Mediterranean phylogeography. Representative, balanced data were selected both in terms of species, sampling coverage and molecular markers that would enable the search for common historical patterns. We have thus selected all species for which mitochondrial DNA sequences were available (mtDNA offers the opportunity to compare the evolutionary history, giving a fair degree of population-level resolution), with samples from the northeast Atlantic Ocean and from the Mediterranean Sea, and with at least 10 individuals in each main basin (Atlantic and Mediterranean). These criteria proved to be quite stringent; from the initial 68 candidate data sets (Table 1), 20 were selected for the meta-analysis. They were kindly provided by the authors of the original publications (see Acknowledgements). A standardized data re-analysis was required so that all data matrices were scrutinized in a compatible and directly comparable way. The papers considered did not present all the same data analysis, particularly with regards to the inferences of historical demography. Moreover, geographical partitions of samples were not always the same as intended for the analysis of the Atlantic–Mediterranean divide. We determined evolutionary models for each data set, so that AMOVA analysis could be performed under the most optimal assumptions.

Genetic diversity and population divergence

Evolution models for each species were determined by the software package MODELTEST 3.06 (Posada & Crandall 1998). However, as the most inclusive evolution model implemented in the software package ARLEQUIN 3.01 (Excoffier *et al*. 2005) is the Tamura–Nei model with the inclusion of gamma value (Tamura & Nei 1993), more complex models than this were downgraded to the Tamura–Nei model. To perform a hierarchical analysis of molecular variance (AMOVA, Excoffier *et al*. 1992), populations were grouped in a hierarchical decreasing spatial scale when significant genetic differences of the established partitions were detected. Groups were organized as follows: (i) group one, all populations as one single panmictic population; (ii) group two, Atlantic Ocean vs. Mediterranean Sea; and (iii) group three, Atlantic vs. Western Mediterranean vs. Eastern Mediterranean. Permutation procedures were used to construct null distributions and test the significance of variance components for each hierarchical comparison (Guo & Thompson 1992).

Demographic history

Mismatch analysis of sequences (frequency of pairwise differences between haplotypes) was carried out to explore the demographic history of the populations studied. The method is based on the assumption that population growth or decline leaves distinctive signatures in the DNA sequences compared with a constant population size. Recent growth is expected to generate a unimodal distribution of pairwise differences between sequences (Rogers & Harpending 1992). The distribution is compared with that expected under a model of population expansion (Rogers 1995), calculating the estimator expansion time, tau (τ) and the mutation parameter (θ) according to Schneider & Excoffier

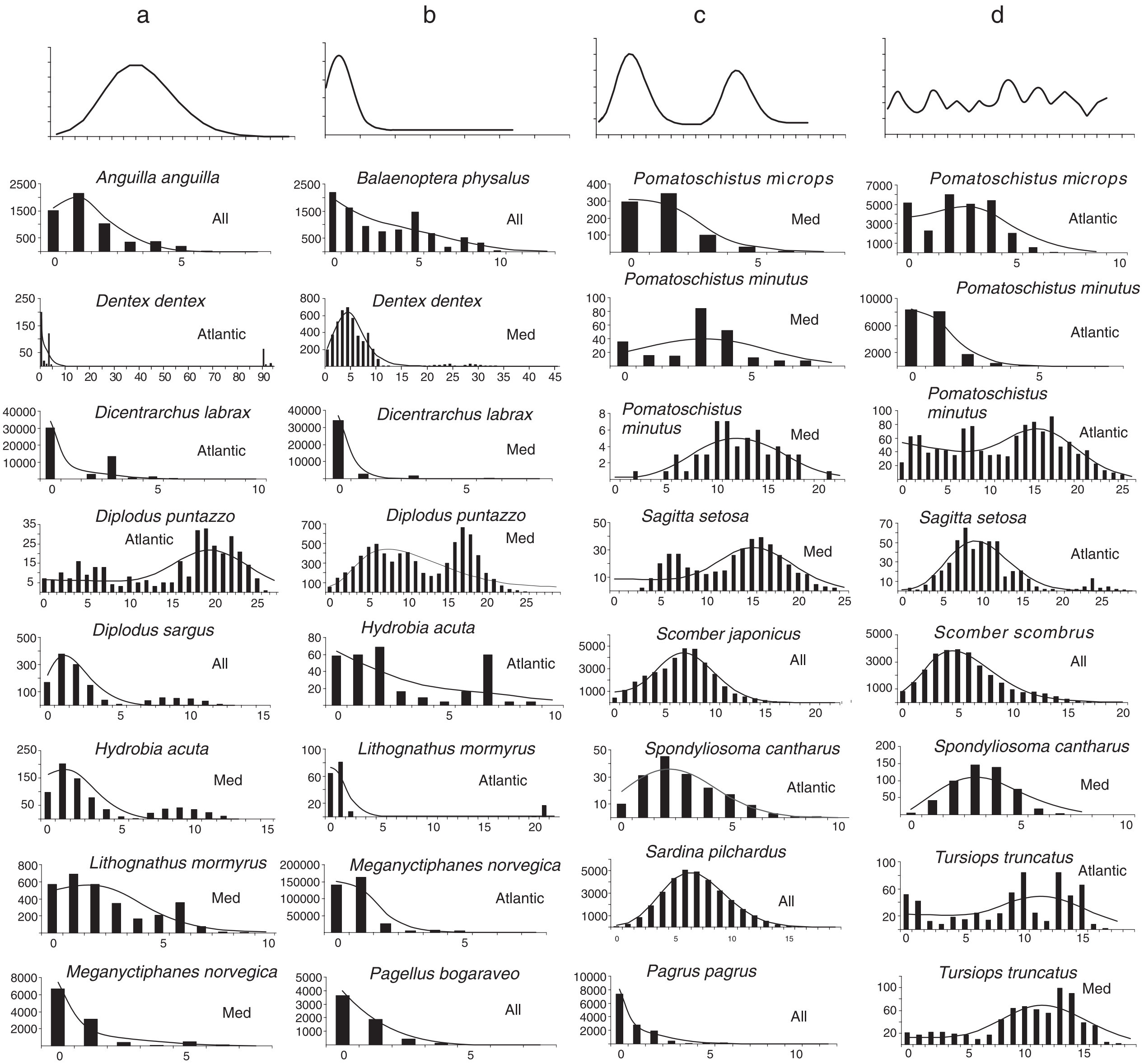

Fig. 3 Mismatch distribution for the significant geographical partitions of each species. The inserted top figure indicates the main types of mismatch distributions observed: (a) unimodal distribution associated with a typical sudden population expansion; (b) skewed unimodal distribution related to recent bottleneck or sudden population expansion; (c) bimodal distribution usually linked to constant population size (but see text); (d) ragged distribution associated with constant population size. Geographical partitions: all, no partition; Atl, Atlantic; medical, Mediterranean (the Mediterranean considered without subdivision). Bars represent the observed frequencies of nucleotide differences between pairs of individuals, curves correspond to the distribution fitted to the data under a model of population expansion.

(1999). The formula $t = \tau/(2\mu k)$ was used to estimate the timing of population expansions (t), where μ is the mutation rate per site per year and k is the sequence length. Mutation rates were those proposed for each species in the original papers (summarized in Table S3, Supplementary material).

Tajima's *D* statistic (Tajima 1989) and Fu's *Fs* test (Fu & Li 1993; Fu 1997) for selective neutrality were calculated by ARLEQUIN. For neutral markers, these tests can be used to detect changes in population size. Significant negative *D* and *Fs* values can be interpreted as signatures of population expansion. The neutrality tests used may not have sufficient statistical power to detect population growth under certain circumstances (Ramos-Onsins & Rozas 2002), and the authors have chosen to use four mismatch curve patterns (top of Fig. 3) to classify signatures of demographic history of significant geographical partitions. In one case, bimodality was due to extremely far apart peaks (*Dentex dentex*), in those situations the authors decided to consider only the first peak.

Results of the meta-analysis

Genetic analysis of the geographical partitioning by species revealed four types of structure: panmixis (*Balaenoptera physalus, Diplodus sargus, Pagellus bogaraveo, Pagrus pagrus* and *Scomber japonicus*), Atlantic–Mediterranean geographical partitioning (*Dentex dentex, Dicentrarchus labrax, Diplodus puntazzo, Hydrobia acuta, Lithognathus mormyrus, Meganyctiphanes norvegica, Pomatoschistus microps, P. minutus, Spondyliosoma cantharus* and *Tursiops truncatus*) with a possible further subdivision between East and West Mediterranean (*Sagitta setosa*) and structures other than panmixis and Atlantic–Mediterranean geographical partitioning (*Anguilla anguilla, Sardina pilchardus* and *Scomber scombrus*) (Table S1, Supplementary material).

We inferred the historical demography pattern of each species from the observed mismatch distribution in combination with Tajima's and Fu's statistical tests for neutrality, the sum of squared deviations (SSD) test and the raggedness index (Table S2, Supplementary material). Mismatch analysis of the 32 data sets resulting from the geographical partition of the 20 selected species (for details see Table S2) exposed several patterns of frequency distributions (Fig. 3). Most frequency distributions are classified into four types. Type A (nine cases) has a unimodal distribution, associated with a sudden population expansion; the mean moves further away from the *y*-axis with time since expansion (e.g. *Sa. pilchardus, Sc. japonicus and Sc. scombrus*). Type B (eight cases) has a negative binomial curve, which provides evidence for a very recent bottleneck or expansion (e.g. *D. labrax*, *M. norvegica*). Type C (six cases) represents a bimodal frequency distribution, which may be interpreted as evidence for population stasis (Zlojutro *et al*. 2006). However, in some instances, the bimodal distribution points to the presence of two distinct phylogroups (e.g. Alvarado-Bremer *et al*. 2005). In our study, inspection of the haplotype networks (not shown) suggested that in all cases the two lineages are more or less differentiated from each other. The most recent mode depicts intraspecific pairwise differences and the second mode may point to more ancestral and emerging interspecific differences (e.g. *De. dentex* Atlantic and *H. acuta* Mediterranean). Finally, type D (nine cases) has a ragged distribution, associated with stable size populations (e.g. *Tur. truncatus* and *Dip. puntazzo*-Atlantic). The combined interpretation of all demographic indicators evidenced that 20 cases out of 32 could be considered as populations which experiences an expansion (Table S2).

The neutrality tests yield a range of patterns in the investigated samples. All Tajima's *D* values were negative but only 14 out of 32 showed significant departures from neutrality. The Fu's *Fs* test turned out to be much more sensitive to detect departures from mutation-drift equilibrium, as 20 *Fs* values were significant in the same set of 32 samples. The two tests were congruent in detecting significant departures from neutrality in only 13 cases; correlation between the two values is in fact weak ($r = 0.583$). There was no general trend as in some instances, Mediterranean populations did not appear in equilibrium when compared to the Atlantic populations (e.g. *De. dentex* and *Dip. puntazzo*). In other species the opposite was true, such as for *M. norvegica*, the northern krill, and the two species of gobies (*Pom. minutus* and *Pom. microps*).

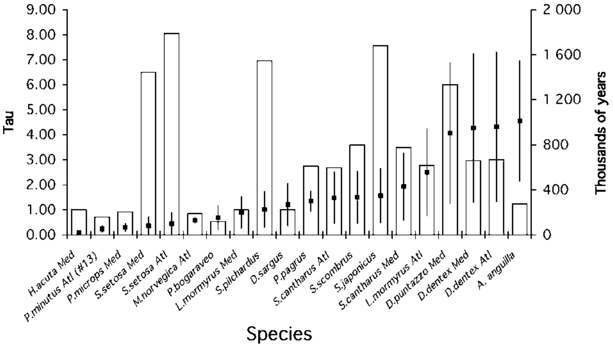

Fig. 4 Expansion time and tau value for all species in which the sudden expansion hypothesis was not rejected. Minimum and maximum expansion times estimated from a minimum and maximum mutation rate (details in Table S3). Only significant geographical partitions were considered. Vertical line, bottom expansion time estimated with the minimum substitution rate value; top, expansion time estimated with the maximum substitution rate value; square, expansion time estimated with the mean substitution rate value (details in Table S3). Bars represent τ-values, except in the cases of *Dentex dentex* (Atl), *Diplodus puntazzo* (Medical), *Diplodus sargus* (All), *Hydrobia acuta* (Medical), *Lithognathus mormyrus* (Medical) and *Sagitta setosa* (Medical) where the mode value is used as an approximation of the τ-value because of the bimodality of these particular cases.

The timeframe analysis of the 20 sets used for qualifying an expansion comprised 16 species (13 fish species, one mollusk, one crustacean and one arrow worm) (Tables S2 and S3). Substitution rates varied from 0.94% to 3.05%/million years (Myr) (Van Houdt *et al*. 2003) for fish cytochrome *b*, 2%/Myr (standard mitochondrial clock) (Brown *et al*. 1979) and 11%/Myr (Bargelloni *et al*. 2003) for fish control region, 2%/Myr and 193.3%/Myr (Peijnenburg *et al*. 2004) for *S. setosa*, 0.88% and 1.3%/Myr for *M. norvegica* (Papetti *et al*. 2005) and 1.62–2.04%/Myr (Wilke & Pfenninger 2002) for *H. acuta*. Tau values obtained from the mismatch analysis vary from 0.53 (*P. bogaraveo*) to 7.56 (*Sc. japonicus*). Average expansion values vary from 0.08 (*Pom. minutus*) to 1.62 Ma (*De. dentex*). Remarkably, in all cases, population expansion was calculated to be no older than the Pleistocene, a period characterized by strong climate cycling (Fig. 4).

The biological and genetic features of the 20 species were investigated using principal component analysis (PCA) (StatSoft Inc. 2001), which provides an alternative to reduce large data sets and search for an association among taxa on the basis of a certain number of characteristics. We took into account both biological and genetic features. Biological characteristics (retrieved from the databank Fishbase, Froese & Pauly 2006) were: range of distribution both horizontal (geographical) and vertical (depth), lifestyle (pelagic, demersal, benthic), age at first maturity (years), generation time (years), trophic niche, sex (separate sexes, hermaphrodite-proterandric, hermaphrodite-proteroginic), type of fertilization (internal or external) and type of eggs (dispersal ability). Genetic measures were based on the re-calculated genetic data and included: AMOVA, percentage of genetic variation, mean mismatch value, τ, θ_0, θ_1, Tajima's *D* and Fu's *Fs* tests.

The eigenvalues of the PCA corresponding to the first two principal components were 3.203 and 1.709, explaining 40.03% and 21.37%, respectively, of the total variability of the data. Exploration of the third principal component yielded an eigenvalue of 1.164% and 14.55% of additional variability. These results did not show any particular trend. In addition, there was no correlation between principal components and the set of biological and genetic variables considered.

What do we learn from the literature and data re-analysis?

A generalized picture of contrasting situations emerges, with cases of a lack of population structure, Atlantic–Mediterranean separation, and a population structure not associated with the Atlantic–Mediterranean partitioning. However, general trends and eventually, explanations for this apparent incongruence come from investigating the demographic history by mismatch analysis. Here, pairwise differences in haplotype sequence are tallied and compared to a negative binomial distribution (pointing to a stable or even decreasing population) and a uni- or multimodal Poisson distribution, pointing to a single or multiple expansions. Signatures of a bottleneck followed by population expansion seem associated with little or no population structure. *Sardina pilchardus, Scomber japonicus* and *Diplodus sargus* were characterized by the absence of geographical differentiation across the Atlantic–Mediterranean range. Interestingly, they showed a unimodal curve, typical of a population expansion. Extinction/recolonization may therefore justify the shallow population structure observed in these species. On the other hand, *Diplodus puntazzo* and *Hydrobia acuta,* differentiated between Atlantic and Mediterranean populations, were characterized by multiple population expansions (i.e. bimodal mismatch distribution) and mutation–drift equilibrium. One could generalize that species, which experienced expansion, after a (likely) drastic reduction of the effective population size erased signals of a historical population structure. Therefore, the more recent the bottleneck/expansion, the less time has passed to accumulate differences, even in the complete absence of gene flow.

Mitochondrial genes provide an advantage at inferring the history of the species, provided that a plausible evolutionary model for the gene is available (Emerson *et al.* 2001). However, particularly with mitochondrial genes, it is difficult to disentangle historical signals from present-day patterns of gene flow, as the signature of historical processes might obscure the current patterns of intraspecific processes. Low levels of mitochondrial genetic diversity might also result from genetic sweeps and not only from demographic histories. Lack of recombination in fact, makes this molecule prone to 'genetic hitch-hiking' that may erase historical signals (Bazin *et al.* 2006). Nevertheless, the extent of this genetic sweep seems to be dependent on the population size of the species (Mulligan *et al.* 2006); it is difficult to predict its impact on marine species with their large census population sizes and much smaller effective population sizes. Microsatellites, on the other hand, are believed to be more accurate in describing contemporeneous genetic relationships among populations and prove to be very sensitive to subtle genetic differences even over limited geographical scales (Zane *et al.* 2002). An important point of reference is the timing of a phenomenon. Time of population expansion as well as time of separation between divergent lineages (i.e. Mediterranean and Atlantic clades) can be estimated if a suitable mutation rate is assumed for the gene under investigation. In most vertebrate studies, the same mitochondrial locus (control region) was used for the analysis of population dynamics. Therefore, even when using inappropriate mutation rates for the D-loop, results should be comparable. The more accurate the placement of evolutionary processes such as bottleneck/expansion or vicariant separation between lineages into the appropriate timeframe, the better our ability to match them with the geological history of the area of interest. The ultimate goal is to identify along with the history of a region, geological and palaeo-oceanographical events that might have promoted evolutionary patterns shared by groups of species.

The phylogeographical patterns obtained from the data re-analysis seem at first inspection very diversified, with few indications for general conclusions. Such results are not unexpected given the diversity of the geographical regions considered, the tormented geological and climatic history of the area and the wide range of life-history traits of the taxa concerned. However, some generalities appear obvious:

- Three general structures of phylogeography are prominent: (i) Full congruence between Atlantic and Mediterranean clades (panmixia, e.g. *Sc. japonicus* — the chub mackerel, key speciea in pelagic ecology) suggests that gene flow between both seas is high, which is an intrinsic feature of fully pelagic species. However, this potential is not necessarily realized as the eutrophic Atlantic shelf and the oligotrophic Mediterranean Sea have contrasting levels of productivity and hence ecosystem structures and niches (Emig & Geistdoerfer 2004). Alternatively, ancestral Mediterranean clades were lost during environmentally less suitable periods, and an Atlantic clade re-occupied the vacant niche later on (although the opposite cannot be excluded). This might be the case for the bluefin tuna (*Thunnus thynnus*) and some sparid species (*Dip. sargus*, *Pagellus bogaraveo* and *Pagrus pagrus*). (ii) Where Atlantic and Mediterranean clades are distinct, like *Dicentrarchus labrax*, the three sparids *Dentex dentex*, *Lithognathus mormyrus*, and *Spondyliosoma cantharus*, or the cuttlefish *Sepia officinalis*. Several causes can be put forward: historic separation, recent isolation (e.g. isolation by distance) and distinct population dynamics or effective population size. Bimodal mismatch patterns (e.g. *Pomatoschistus minutus* and *Sagitta setosa*) also fit in this group. Fine-scale population analyses across the transition zone indicated the Almeria-Oran front as the Atlantic–Mediterranean phylogeographical break. (iii) The last group includes patterns different from those mentioned above, for example *Scomber scombrus* where an East Mediterranean clade is distinct from a clade inhabiting the West Mediterrnean and Atlantic Ocean. There are few taxa in this group, which points to its historical uniqueness.

- Genetic diversity does not necessarily decrease in a direction either from the Atlantic Ocean to the Mediterranean Sea or from the Mediterranean to the Adriatic, but it does decrease between the Mediterranean and the Black Sea clades. The history of the Black Sea is a progression of isolation from the Mediterranean Sea because of the presence of the Bosporus and Dardanelles sills, the variable levels of freshwater runoff and temperature fluctuations (Aksu *et al*. 2002).
- The following patterns of population dynamics were observed. (i) Congruent expansion dynamics between Atlantic and Mediterranean populations (e.g. *Anguilla anguilla*, *Sc. japonicus*, *Sc. scombrus* and *Sa. pilchardus* in the re-analysed data). The occurrence of such a pattern suggests similar environmental conditions such that populations expand and decrease in parallel. (ii) An alternative is a stronger tendency towards expansion in the Atlantic Ocean in comparison to the Mediterranean Sea like the northern krill *Meganyctiphanes norvegica* among invertebrates but it is also the case for large pelagic fish, such as bluefin tuna and swordfish (Bremer *et al*. 2005), pointing towards more optimal ecological conditions in the Atlantic Ocean. (iii) The third involves expansion in the Mediterranean unlike the Atlantic Ocean (e.g. *Dip. puntazzo*), pointing towards more optimal conditions in the Mediterranean Sea. The key factors influencing the carrying capacity of a species in the ocean (so called 'ocean triads' following Agostini & Bakun 2002) are: nutrient enrichment, plankton concentration and larval retention. Each of these factors underwent wide fluctuations over time into the Mediterranean Sea, particularly during the Quaternary age (Krom *et al*. 1999).
- In parallel with microfossil evidence (Lambeck *et al*. 2002), divergence between older marine clades coincides with the first cooling down of the region during the period 3.2–1.8 Ma. Three major timings of the expansion patterns seem relevant: a Late-Pleistocene expansion [*A. anguilla*, *Dip. sargus*, *H. acuta* (Mediterranean), *L. mormyrus* (Mediterranean), *M. norvegica* (Atlantic), *P. bogaraveo*, *Pom. microps* (Mediterranean) and *Pom. minutus* (Atlantic)], Mid-Pleistocene expansion [*De. dentex*, *L. mormyrus* (Atlantic), *Pa. pagrus*, *Sc. scombrus*, *Sp. cantharus*, *Lo. budegassa*] and an Early Pleistocene expansion [*Dip. puntazzo* (Mediterranean), *S. setosa*, *Sa. pilchardus*, *Sc. japonica, Lo. piscatorius*]. The question remains why each species only expanded during those time periods. The most appropriate answer would come from an analysis of intrageneric species pairs such as *Dip. puntazzo–Dip. sargus, Sc. scombrus–Sc. japonicus* and *Lo. budegassa–Lo. piscatorius* (see above). More than the anticipated biological similarity between pairs, dissimilarities in phylogeographical pattern and population dynamics/history seems to be the more common theme. Divergence does not seem to be directly attributable to life history, reproduction, ecological niche or other biological traits. This might argue that organismal determinism may play a far less significant role than marine biogeographers have generally believed. As Cunningham & Collins (1998) noted, it is possible that taxa share histories simply because there are a limited number of possible histories, and for purely stochastic reasons species will share them.

Species in the Mediterranean Sea do not show a uniform phylogeographical pattern. Most striking is the impact of any combination of two extreme cases: complete genetic separation between Atlantic–Mediterranean populations since the early Pliocene and complete absence of population differentiation, usually following late Pleistocene recolonization. Ideally, the phylogeographical data would match with the detailed Pleistocene micropalaeontological record. Unfortunately, our phylogeographical insights do not reach the same level of resolution. We are not yet able to provide a genome-based reconstruction of the palaeosystem, although we are moving in this direction. We do not fully grasp the role of species-specific biological traits in determining the patterns observed. Moreover, the general picture lacks information from the southern continental shelf and offshore. What is needed is a more extensive sampling coverage as well as an improved phylogeographical calibration. These represent our challenge for the future.

Acknowledgements

We dedicate this review to Dr Els Gysels, marine evolutionary biologist and data provider to this paper, who passed away in the heyday of her life during a tragic hiking accident in the Swiss Alps. The following colleagues provided original data sets for the meta-analysis: T. Atartouch (Rabat), L. Bargelloni (Padua), M. Bérubé (Berkeley), C. Lemaire (Angers), K. Peijnenburg (Dublin), S. Stefanni (Horta), T. Wilke (Washington) and R. Zardoya (Madrid). B. Ely (Columbia) and Z. Mamuris (Larissa) kindly provided data that was not included in the review. We thank anonymous referees and Dr M.H. Goldschmidt for their valuable comments to the manuscript. Research was funded by the EU Network of Excellence 'MARINE GENOMICS EUROPE' (project GOCE-CT-2004-505403) to TP and FV, the Belgian Office of Science Policy (project EV/01/25C) to FV and by FCT multiannual funding to RC. J. Raeymaekers gave expert statistical advice; T. Kouwenhoven and B. Van der Zwaan (Utrecht) advised on the palaeoceanographic interpretations.

References

Agostini VN, Bakun A (2002) Ocean Triads' in the Mediterranean Sea: physical mechanisms potentially structuring reproductive habitat suitability (example application to European anchovy, *Engraulis encrasicolus*). *Fisheries Oceanography*, **11**, 129–142.

Aksu AE, Hiscott RN, Kaminski MA *et al*. (2002) Last glacial-Holocene paleoceanography of the Black Sea and Marmara Sea: stable isotopic foraminiferal and coccolith evidence. *Marine Geology*, **190**, 119–149.

Alarcon JA, Magoulas A, Georgakopoulos T *et al*. (2004) Genetic comparison of wild and cultivated European populations of the gilthead sea bream (*Sparus aurata*). *Aquaculture*, **230**, 65–80.

Alhammoud B, Beranger K, Mortier L *et al*. (2005) Surface circulation of the Levantine Basin: comparison of model results with observations. *Progress in Oceanography*, **66**, 299–320.

Allegrucci G, Fortunato C, Sbordoni V (1997) Genetic structure and allozyme variation of sea bass (*Dicentrarchus labrax* and *D. punctatus*) in the Mediterranean Sea. *Marine Biology*, **128**, 347–358.

Almada VC, Oliveira RF, Gonçalves EJ *et al*. (2001) Patterns of diversity of the north-eastern Atlantic blennid fish fauna (Pisces: Blenniidae). *Global Ecology and Biogeography*, **10**, 411–422.

Alvarado-Bremer JR, Vinas J, Mejuto J, Ely B, Pla C (2005) Comparative phylogeography of Atlantic bluefin tuna and swordfish: the combined effects of vicariance, secondary contact, introgression, and population expansion on the regional phylogenies of two highly migratory pelagic fishes. *Molecular Phylogenetics and Evolution*, **36**, 169–187.

Andreakis N, Procaccini G, Kooistra WHCF (2004) *Asparagopsis taxiformis* and *Asparagopsis armata* (Bonnemaisoniales, Rhodophyta): genetic and morphological identification of Mediterranean populations. *European Journal of Phycology*, **39**, 273–283.

Atarhouch T, Ruber L, Gonzalez EG *et al*. (2006) Signature of an early genetic bottleneck in a population of Moroccan sardines (*Sardina pilchardus*). *Molecular Phylogenetics and Evolution*, **39**, 373.

Aurelle D, Guillemaud T, Afonso P *et al*. (2003) Genetic study of *Coris julis* (Osteichthyes, Perciformes, Labridae) evolutionary history and dispersal abilities. *Comptes Rendus Biologies*, **326**, 771–785.

Avise JC (1992) Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos*, **62**, 62–76.

Avise JC (2000) *Phylogeography: the History and Formation of Species*. Harvard University Press, Cambridge, Massachusetts.

Barber PH, Palumbi SR, Erdmann MV, Moosa MK (2000) Biogeography. A marine Wallace's line? *Nature*, **406**, 692–693.

Bargelloni L, Zane L, Derome N. *et al*. (2000) Molecular zoogeography of Antarctic euphausiids and notothenioids: from species phylogenies to intraspecific patterns of genetic variation. *Antarctic Science*, **12**, 259–268.

Bargelloni L, Alarcon JA, Alvarez MC *et al*. (2003) Discord in the family Sparidae (Teleostei): divergent phylogeographical patterns across the Atlantic-Mediterranean divide. *Journal of Evolutionary Biology*, **16**, 1149–1158.

Bargelloni L, Alarcon JA, Alvarez MC *et al*. (2005) The Atlantic-Mediterranean transition: discordant genetic patterns in two seabream species, *Diplodus puntazzo* (Cetti) and *Diplodus sargus* (L.). *Molecular Phylogenetics and Evolution*, **36**, 523–535.

Baus E, Darrock DJ, Bruford MW (2005) Gene-flow patterns in Atlantic and Mediterranean populations of the Lusitanian sea star *Asterina gibbosa*. *Molecular Ecology*, **14**, 3373–3382.

Bazin E, Glemin S, Galtier N (2006) Population size does not influence mitochondrial genetic diversity in animals. *Science*, **312**, 570–572.

Bernatchez L (2001) The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution*, **55**, 351–379.

Bérubé M, Aguilar A, Dendanto D *et al*. (1998) Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci. *Molecular Ecology*, **7**, 585–599.

Bianchi CN, Morri C (2000) Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. *Marine Pollution Bulletin*, **40**, 367–376.

Blanc F, Bonhomme F (1986) Polymorphisme génétique des populations naturelles de mollusques d'interet aquicole. In: *Selection, Hybridization and Genetic Engineering in Aquaculture, Vol. I*, (ed Tiews K), pp. 59–78. H. Heenemann, GmbH, Berlin, Germany.

Bolten AB, Bjornal KA, Martins HR *et al*. (1998) Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analysis. *Ecological Applications*, **8**, 1–7.

Bonhomme F, Naciri M, Bahri-Sfar L, Lemaire C (2002) Comparative analysis of genetic structure of two closely related sympatric marine fish species *Dicentrarchus labrax and Dicentrarchus punctatus*. *Comptes Rendus Biologies*, **325**, 213–220.

Borsa P, Blanquer A, Berrebi P (1997a) Genetic structure of the flounders *Platichthys flesus* and *P. stellatus* at different geographic scales. *Marine Biology*, **129**, 233–246.

Borsa P, Naciri M, Bahri L *et al*. (1997b) Intraspecific zoogeography of the Mediterranean: population genetic analysis on sixteen atlanto-mediterranean species (fish and invertebrates). *Vie Milieu*, **47**, 295–305.

Bremer JRA, Mejuto J, Gomez-Marquez J *et al*. (2005) Hierarchical analyses of genetic variation of samples from breeding and feeding grounds confirm the genetic partitioning of northwest Atlantic and South Atlantic populations of swordfish (*Xiphias gladius* L.). *Journal of Experimental Marine Biology and Ecology*, **327**, 167–182.

Brown WM, George M, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences, USA*, **76**, 1967–1971.

Charrier G, Chenel T, Durand JD *et al*. (2006) Discrepancies in phylogeographical patterns of two European anglerfishes (*Lophius budegassa* and *Lophius piscatorius*). *Molecular Phylogenetics and Evolution*, **38**, 742–754.

Chevolot M, Hoarau G, Rijnsdorp AD *et al*. (2006) Phylogeography and population structure of thornback rays (*Raja clavata* L., Rajidae). *Molecular Ecology*, **15**, 3693–3705.

Chikhi L, Bonhomme F, Agnese J-F (1998) Low genetic variability in a widely distributed and abundant clupeid species, *Sardinella aurita*. New empirical results and interpretations. *Journal of Fish Biology*, **52**, 861–878.

Chow S, Okamoto H, Miyabe N, Hiramatsu K (2000) Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. *Molecular Ecology*, **9**, 221–227.

Cimmaruta R, Bondanelli P, Nascetti G (2005) Genetic structure and environmental heterogeneity in the European hake (*Merluccius merluccius*). *Molecular Ecology*, **14**, 2577–2591.

Costagliola D, Robertson DR, Guidetti P *et al*. (2004) Evolution of coral reef fish *Thalassoma* spp. (Labridae). 2. Evolution of the eastern Atlantic species. *Marine Biology*, **144**, 377–383.

Coyer JA, Diekmann OE, Serrao EA *et al*. (2004) Population genetics of dwarf eelgrass *Zostera nolti* throughout its biogeographic range. *Marine Ecology-Progress Series*, **281**, 51–62.

Cunningham CW, Collins T (1998) Beyond area relationships: extinction and recolonization in marine molecular biogeography. In: *Molecular Approaches to Ecology and Evolution* (eds DeSalle R, Schierwater B), pp. 297–322. Birkhauser-Verlag, Basel, Switzerland.

Daemen E, Cross T, Ollevier F, Volckaert FAM (2001) Analysis of the genetic structure of European eel (*Anguilla anguilla*) using microsatellite DNA and mtDNA markers. *Marine Biology*, **139**, 755–764.

Dalebout ML, Robertson KM, Frantzis A *et al*. (2005) Worldwide structure of mtDNA diversity among Cuvier's beaked whales (*Ziphius cavirostris*): implications for threatened populations. *Molecular Ecology*, **14**, 3353–3371.

Dannewitz J, Maes GE, Johansson L *et al*. (2005) Panmixia in the European eel: a matter of time. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **272**, 1129–1137.

Dawson AG (1992) *Ice Age Earth: Late Quaternary Geology and Climate*. Routledge, London.

De Innocentiis S, Lesti A, Livi S *et al*. (2004) Microsatellite markers reveal population structure in gilthead sea bream *Sparus aurata* from Atlantic Ocean and Mediterranean Sea. *Fisheries Science*, **70**, 852–859.

Domingues VS, Bucciarelli G, Almada VC, Bernardi G (2005) Historical colonization and demography of the Mediterranean damselfish, Chromis chromis. *Molecular Ecology*, **14**, 4051–4063.

Duran S, Giribet G, Turon X (2004a) Phylogeographical history of the sponge *Crambe crambe* (Porifera, Poecilosclerida): range expansion and recent invasion of the Macaronesian islands from the Mediterranean Sea. *Molecular Ecology*, **13**, 109–122.

Duran S, Pascual M, Estoup A, Turon X (2004b) Strong population structure in the marine sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. *Molecular Ecology*, **13**, 511–522.

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

Duran S, Palacin C, Becerro MA *et al*. (2004d) Genetic diversity and population structure of the commercially harvested sea urchin *Paracentrotus lividus* (Echinodermata, Echinoidea). *Molecular Ecology*, **13**, 3317–3328.

Ely B, Stoner DS, Bremer JRA *et al*. (2002) Analyses of nuclear ldhA gene and mtDNA control region sequences of Atlantic northern bluefin tuna populations. *Marine Biotechnology*, **4**, 583–588.

Emerson B, Paradis E, Thebaud C (2001) Revealing the demographic histories of species using DNA sequences. *Trends in Ecology & Evolution*, **16**, 707–716.

Emig CC, Geistdoerfer P (2004) The Mediterranean deep-sea fauna: historical evolution, bathymetric variations and geographical changes. *Carnets de Géologie/Notebooks on Geology*. Article 2004/01 (CG2004_A01_CCE-PG), 10 p.

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.

Excoffier L, Laval G, Schneider S (2005) ARLEQUIN ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Froese R, Pauly D (2006) *FishBase. World Wide Web electronic publication*. Available from URL: www.fishbase.org.

Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Fu Y-X, Li W-H (1993) Statistical tests of neutrality of mutations. *Genetics*, **133**, 693–709.

Garcia-Martinez J, Moya A, Raga JA, Latorre A (1999) Genetic differentiation in the striped dolphin *Stenella coeruleoalba* from European waters according to mitochondrial DNA (mtDNA) restriction analysis. *Molecular Ecology*, **8**, 1069–1073.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics*, **48**, 361–372.

Gysels ES, Hellemans B, Pampoulie C, Volckaert FAM (2004a) Phylogeography of the common goby, *Pomatoschistus microps*, with particular emphasis on the colonization of the Mediterranean and the North Sea. *Molecular Ecology*, **13**, 403–417.

Gysels ES, Hellemans B, Patarnello T, Volckaert FAM (2004b) Current and historic gene flow of the sand goby *Pomatoschistus minutus* on the European Continental Shelf and in the Mediterranean Sea. *Biological Journal of the Linnean Society*, **83**, 561–576.

Haywood AM, Sellwood BW, Valdes PJ (2000) Regional warming: Pliocene (3 Ma) paleoclimate of Europe and the Meediterranean. *Geology*, **28**, 1063–1066.

Hamad N, Millot C, Taupier-Letage I (2005) A new hypothesis about the surface circulation in the eastern basin of the Mediterranean Sea. *Progress in Oceanography*, **66**, 287–298.

Hewitt GM (2000) The genetic legacy of the quaternary ages. *Nature*, **405**, 907–913.

Hrbek T, Meyer A (2003) Closing of the Tethys Sea and the phylogeny of Eurasian killifishes (Cyprinodontiformes: Cyprinodontidae). *Journal of Evolutionary Biology*, **16**, 17–36.

Hsü K, Ryan W, Cita M (1973) Late Miocene desiccation of the Mediterranean. *Nature*, **242**, 240–244.

Huyse T, Van Houdt JKJ, Volckaert FAM (2004) Paleoclimatic history and vicariant speciation in the 'sand goby' group (Gobiidae, Teleostei). *Molecular Phylogenetics and Evolution*, **32**, 324–336.

Karaiskou N, Triantafyllidis A, Triantaphyllidis C (2004) Shallow genetic structure of three species of the genus *Trachurus*. European waters. *Marine Ecology-Progress Series*, **281**, 193–205.

Krijgsman W, Hilgen FJ, Raffi I *et al*. (1999) Chronology, causes and progression of the Messinian salinity crisis. *Nature*, **400**, 652–655.

Krom MD, Michard A, Cliff RA (1999) Sources of sediment to the Ionian Sea and the western Levantine basin of the Eastern Mediterranean during S-1 sapropel time. *Marine Geology*, **160**, 45–61.

Krom M, Woodward E, Herut B *et al*. (2005) Nutrient cycling in the south east Levantine basin of the eastern Mediterranean: results from a phosphorus starved system. Deep-Sea Research Part II. *Topical Studies in Oceanography*, **52**, 2879–2896.

Ladoukakis ED, Saavedra C, Magoulas A, Zouros E (2002) Mitochondrial DNA variation in a species with two mitochondrial genomes: the case of *Mytilus galloprovincialis* from the Atlantic, the Mediterranean and the Black Sea. *Molecular Ecology*, **11**, 755–769.

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

Launey S, Ledu C, Boudry P *et al*. (2002) Geographic structure in the European flat oyster (*Ostrea edulis* L.) as revealed by microsatellite polymorphism. *Journal of Heredity*, **93**, 331–338.

Lemaire C, Versini JJ, Bonhomme F (2005) Maintenance of genetic differentiation across a transition zone in the sea: discordance between nuclear and cytoplasmic markers. *Journal of Evolutionary Biology*, **18**, 70–80.

Lo Brutto S, Arculeo M, Parrinelo N (2004) Congruence in genetic markers used to describe Mediterranean and Atlantic populations of European hake (*Merluccius merluccius* L. 1758). *Journal of Applied Ichthyology*, **20**, 81–86.

Longhurst A (1998) *Ecological Geography of the Sea*, 1st edn. Academic Press, Cajarc, France.

Lundy CJ, Moran P, Rico C *et al*. (1999) Macrogeographical population differentiation in oceanic environments: a case study of European hake (*Merluccius merluccius*), a commercially important fish. *Molecular Ecology*, **8**, 1889–1898.

Maes GE, Volckaert FAM (2002) Clinal genetic variation and isolation by distance in the European eel *Anguilla anguilla* (L.). *Biological Journal of the Linnean Society*, **77**, 509–521.

Magoulas A, Tsimenides N, Zouros E (1996) Mitochondrial DNA phylogeny and the reconstruction of the population history of a species: the case of the european anchovy (*Engraulis encrasicolus*). *Molecular Biology and Evolution*, **13**, 178–190.

Magoulas A, Castilho R, Caetano S *et al*. (2006) Mitochondrial DNA reveals a mosaic pattern of phylogeographical structure in Atlantic and Mediterranean populations of anchovy (*Engraulis encrasicolus*). *Molecular Phylogenetics and Evolution*, **3**, 734–746.

Malanotte-Rizzoli P, Robinson AR, Roether W *et al*. (1996) Experiment in Eastern Mediterranean probes origin of deep water masses. *EOS Transactions, American Geophysical Union*, **77**, 305–311.

Malanotte-Rizzoli P, Manca B, D'Alcala M *et al*. (1997) A synthesis of the Ionian Sea hydrography, circulation and water mass pathways during POEM Phase I. *Progress in Oceanography*, **39**, 153–204.

Meyers PA, Arnaboldi M (2005) Trans-Mediterranean comparison of geochemical paleoproductivity proxies in a mid-Pleistocene interrupted sapropel. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **222**, 313–328.

Millot C (1999) Circulation in the Western Mediterranean sea. *Journal of Marine Systems*, **20**, 423–442.

Mulligan CJ, Kitchen A, Miyamoto MM (2006) Comment on 'Population size does not influence mitochondrial genetic diversity in animals. *Science*, **312**, 570–572.

Naciri M, Lemaire C, Borsa P, Bonhomme F (1999) Genetic study of the Atlantic/Mediterranean transition in sea bass (*Dicentrarchus labrax*). *Journal of Heredity*, **90** (6), 591–596.

Natoli A, Birkun A, Aguilar A *et al*. (2005) Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*). *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **272**, 1217–1226.

Nesbø CL, Rueness EK, Iversen SA *et al*. (2000) Phylogeography and population history of Atlantic mackerel (*Scomber scombrus* L.): a genealogical approach reveals genetic structuring among the eastern Atlantic stocks. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **267**, 281–292.

Nikula R, Väinölä R (2003) Phylogeography of *Cerastoderma glaucum* (Bivalvia: Cardiidae) across Europe: a major break in the Eastern Mediterranean. *Marine Biology*, **143**, 339–350.

Nilsson T (1982) *The Pleistocene: Geology and Life in the Quaternary Age*. Ridel D. Publishing Company, Dordrecht, Holland.

Obst M, Funch P, Giribet G (2005) Hidden diversity and host specificity in cycliophorans: a phylogeographic analysis along the North Atlantic and Mediterranean Sea. *Molecular Ecology*, **14**, 4427–4440.

Olsen JL, Stam WT, Coyer JA *et al*. (2004) North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. *Molecular Ecology*, **13**, 1923–1941.

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. *Marine Biology*, **128**, 73–82.

Papadopoulos LN, Peijnenburg KTCA, Luttikhuizen PC (2005) Phylogeography of the calanoid copepods *Calanus helgolandicus* and *C. euxinus* suggests Pleistocene divergences between Atlantic, Mediterranean, and Black Sea populations. *Marine Biology*, **147**, 1353–1365.

Papetti C, Zane L, Bortolotto E *et al*. (2005) Genetic differentiation and local temporal stability of population structure in the euphausiid *Meganyctiphanes norvegica*. *Marine Ecology-Progress Series*, **289**, 225–235.

Passier H, Bosch H, Nijenhuis I *et al*. (1999) Sulphidic Mediterranean surface waters during Pliocene sapropel formation. *Nature*, **397**, 146–149.

Paterne M, Kallel N, Labeyrie L *et al*. (1999) Hydrological relationship between the North Atlantic Ocean and the Mediterranean Sea during the past 15–75 kyr. *Paleoceanography*, **14**, 626–638.

Peijnenburg KTCA, Breeuwer JAJ, Pierrot-Bults AC, Menken SBJ (2004) Phylogeography of the planktonic chaetognath *Sagitta setosa* reveals isolation in European seas. *Evolution*, **58**, 1472–1487.

Peijnenburg KT, Fauvelot C, Breeuwer JA, Menken SB (2006) Spatial and temporal genetic structure of the planktonic *Sagitta setosa* (Chaetognatha) in European seas as revealed by mitochondrial and nuclear DNA markers. *Molecular Ecology*, **15**, 3319–3338.

Penzo E, Gandolfi G, Bargelloni L *et al*. (1998) Messinian salinity crisis and the origin of freshwater lifestyle in Western Mediterranean gobies. *Molecular Biology and Evolution*, **15**, 1474–1480.

Perez-Losada M, Guerra A, Carvalho GR *et al*. (2002) Extensive population subdivision of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) around the Iberian Peninsula indicated by microsatellite DNA variation. *Heredity*, **89**, 417–424.

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

Pujolar JM, Roldan MI, Pla C (2002) A genetic assessment of the population structure of swordfish (*Xiphias gladius*) in the Mediterranean Sea. *Journal of Experimental Marine Biology and Ecology*, **276**, 19–29.

Pujolar JM, Roldan MI, Pla C (2003) Genetic analysis of tuna populations, *Thunnus thynnus thynnus* and *T. alalunga*. *Marine Biology*, **143**, 613–621.

Quesada H, Beynon CM, Skibinski DO (1995) A mitochondrial DNA discontinuity in the mussel *Mytilus galloprovincialis* Lmk: pleistocene vicariance biogeography and secondary intergradation. *Molecular Biology and Evolution*, **12**, 521–524.

Ramos-Onsins SE, Rozas J (2002) Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, **19**, 2092–2100.

Remerie T, Bourgois T, Peelaers D *et al*. (2006) Phylogeographic patterns of the mysid *Mesopodopsis slabberi* (Crustacea, Mysida) in Western Europe: evidence for high molecular diversity and cryptic speciation. *Marine Biology*, **149**, 465–481.

Rios C, Sanz S, Saavedra C, Pena JB (2002) Allozyme variation in populations of scallops, *Pecten jacobaeus* (L.) and *P. maximus* (L.) (Bivalvia: Pectinidae), across the Almeria-Oran front. *Journal of Experimental Marine Biology and Ecology*, **267**, 223–244.

Robinson AR, Leslie WG, Theocharis A, Lascaratos A (2001) Ocean circulation currents: Mediterranean Sea Circulation. In: *Encyclopedia of Ocean Sciences*, (eds Turekian KK, Thorpe SA), pp. 1689–1706. Academic Press, London.

Rogers AR (1995) Genetic evidence for a Pleistocene population growth. *Evolution*, **49**, 608–615.

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.

Rögl F (1999) Mediterranean and Paratethys. Facts and hypotheses of an Oligocene to Miocene paleogeography (short overview). *Geologica Carpathica*, **50**, 339–349.

Rögl F, Steininger F (1983) Vom Zerfall der Tethys zu Mediterran und Paratethys. *Annalen Des Naturhistorischen Museums in Wien*, **85A**, 135–163.

Rohling EJ, Mayewski PA, Abu-Zied RH *et al*. (2002) Holocene atmosphere–ocean interactions: records from Greenland and the Aegean Sea. *Climate Dynamics*, **18**, 587–593.

Roman J, Palumbi SR (2004) A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. *Molecular Ecology*, **13**, 2891–2898.

Roy K, Valentine JW, Jablonski D, Kidwell SM (1996) Scales of climatic variability and time averaging in Pleistocene biotas: implications for ecology and evolution. *Trends in Ecology & Evolution*, **11**, 458–463.

Sbaffi L, Wezel F, Kallel N *et al*. (2001) Response of the pelagic environment to palaeoclimatic changes in the central Mediterranean Sea during the Late Quaternary. *Marine Geology*, **178**, 39–62.

Schneider S, Excoffier L (1999) Estimation of past demographic parameters from the distribution of pairwise distances when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics*, **152**, 1079–1089.

Soltis DE, Morris AB, McLachlan JS *et al*. (2006) Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology*, **15**, 4261–4293.

Stamatis C, Triantafyllidis A, Moutou KA, Mamuris Z (2004) Mitochondrial DNA variation in northeast Atlantic and Mediterranean populations of Norway lobster, *Nephrops norvegicus*. *Molecular Ecology*, **13**, 1377–1390.

StatSoft Inc. (2001) *STATISTICA (Data Analysis Software System)*. StatSoft Inc., Tulsa, Oklahoma.

Stefanni S, Thorley JL (2003) Mitochondrial DNA phylogeography reveals the existence of an evolutionarily significant unit of the sand goby *Pomatoschistus minutus* in the Adriatic (Eastern Mediterranean). *Molecular Phylogenetics and Evolution*, **28**, 601–609.

Tajima F (1989) Statistical testing for the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.

Tintore J, Violette PE, Blade I, Cruzado A (1988) A study of an intense density front in the eastern alboran sea: the Almeria-Oran front. *Journal of Physical Oceanography*, **18**, 1384–1397.

Tortonese (1985) *Distribution and Ecology of Endemic Elements in the Mediterranean Fauna (Fishes and Echinoderms)*. Plenum Press, New York.

Triantafyllidis A, Apostolidis AP, Katsares V *et al*. (2005) Mitochondrial DNA variation in the European lobster (*Homarus gammarus*) throughout the range. *Marine Biology*, **146**, 223–235.

Van Houdt JKJ, Hellemans B, Volckaert FAM (2003) Phylogenetic relationships among Palearctic and Nearctic burbot (*Lota lota*): Pleistocene extinctions and recolonization. *Molecular Phylogenetics and Evolution*, **29**, 599–612.

Viñas J, Alvarado Bremer J, Pla C (2004) Phylogeography of the Atlantic bonito (*Sarda sarda*) in the northern Mediterranean: the combined effects of historical vicariance, population expansion, secondary invasion, and isolation by distance. *Molecular Phylogenetics and Evolution*, **33**, 32–42.

Waelbroeck C, Labeyrie L, Michel E *et al*. (2002) Sea-level and deep water temperature changes derived from benthic foraminifera isotopic records. *Quaternary Science Reviews*, **21**, 295–305.

Wares JP (2002) Community genetics in the Northwestern Atlantic intertidal. *Molecular Ecology*, **11**, 1131–1144.

Wares JP, Cunningham CW (2001) Phylogeography and historical ecology of the North Atlantic intertidal. *Evolution*, **55**, 2455–2469.

Weinberg JR, Dahlgren TG, Trowbridge N *et al*. (2003) Genetic differences within and between species of deep-sea crabs (*Chaceon*) from the North Atlantic Ocean. *Biological Bulletin*, **204**, 318–326.

Wilke T, Pfenninger M (2002) Separating historic events from recurrent processes in cryptic species: phylogeography of mud snails (*Hydrobia* spp.). *Molecular Ecology*, **11**, 1439–1451.

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. *Molecular Ecology*, **11**, 1–16.

Zardoya R, Castilho R, Grande C *et al*. (2004) Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the Mediterranean Sea. *Molecular Ecology*, **13**, 1785–1798.

Zlojutro M, Rubicz R, Devor EJ *et al*. (2006) Genetic structure of the Aleuts and circumpolar populations based on mitochondrial DNA sequences: a synthesis. *American Journal of Physical Anthropology*, **129**, 446–464.

Zonneveld K (1996) Palaeoclimatic reconstruction of the last deglaciation (18–8 ka BP) in the Adriatic Sea region. A land-sea correlation based on palynological evidence. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **122**, 89–106.

Tomaso Patarnello's scientific interest is focused on the evolutionary processes and speciation mechanisms taking place in the marine environment. Filip Volckaert studies the historical and contemporaneous evolution of fish populations and their parasites. Rita Castilho is head of the Biodiversity and Conservation group at the Centre for Marine Sciences (University of Algarve) and is interested in phylogeography in an array of different organisms.

Supplementary material

The following supplementary material is available for this article:

Table S1 Information on the species data sets analysed (reference, sample size and location), genetic parameters (number of nucleotides, polymorphic sites and haplotypes) main genetic partition results (genetic pool, genetic variation and genetic partitioning) and demographic parameters (values of mismatch observed mean, tau τ, theta, q_0 and q_1) (A), Significant gene pools: 0, no panmixia, but structure not associated with Atlantic–Mediterranean differentiation; 1, one gene pool; 2, Atlantic and Mediterranean; 3, Atlantic, Western Mediterranean and Eastern Mediterranean. (B), Percentage of genetic variation (from AMOVA): if gene pool < 1, source of variation, among populations; if gene pool = 2, source of variation, among groups. (C), Partition of samples: 0, one gene pool; 1, Atlantic; 2, Mediterranean; 2′, Western Mediterranean; 2″, Eastern Mediterranean. (D), No samples from Eastern Atlantic, so only two gene pool arrangement could be tested. (E), Samples from Venice not included as there is the suspicion of another taxon being involved. (F), Samples from Black Sea not included

Table S2 Species and geographical partitions used in the historical demography analysis. Data sets (#) refer to the last column of Table 1, partitions refer to Table 1 of the supplementary material, graph type relates to Figure 3. In bold significant results at the 0.05 level, except Fu's *Fs* test, which was tested at the 0.02 level

Table S3 Mitochondrial gene fragment, mutation rates (see text for references), number of nucleotides, generation time (from FISHBASE, http://www.fishbase.org), τ (tau) value and expansion times expressed in units of mutation rate (τ/2ut) and in t = 1000 years

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/
10.1111/j.1365-294X.2007.03477.x
(This link will take you to the article abstract).