A Phylogenetic Perspective on the Evolution of Mediterranean Teleost Fishes

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Abstract
The Mediterranean Sea is a highly diverse, highly studied, and highly impacted biogeographic region, yet no phylogenetic reconstruction of fish diversity in this area has been published to date. Here, we infer the timing and geographic origins of Mediterranean teleost species diversity using nucleotide sequences collected from GenBank. We assembled a DNA supermatrix composed of four mitochondrial genes (12S ribosomal DNA, 16S ribosomal DNA, cytochrome c oxidase subunit I and cytochrome b) and two nuclear genes (rhodopsin and recombination activating gene I), including 62% of Mediterranean teleost species plus 9 outgroups. Maximum likelihood and Bayesian phylogenetic and dating analyses were calibrated using 20 fossil constraints. An additional 124 species were gathered onto the chronogram according to their taxonomic affinity, checking for the effects of taxonomic coverage in subsequent diversification analyses. We then interpreted the time-line of teleost diversification in light of Mediterranean historical biogeography, distinguishing non-endemic natives, endemics and exotic species. Results show that the major Mediterranean orders are of Cretaceous origin, specifically ~100–80 Mya, and most Perciformes families originated 80–50 Mya. Two important clad origin events were detected. The first at 100–80 Mya, affected native and exotic species, and reflects a global diversification period at a time when the Mediterranean Sea did not yet exist. The second occurred during the last 50 Mya, and is noticeable among endemic and native species, but not among exotic species. This period corresponds to isolation of the Mediterranean from Indo-Pacific waters before the Messinian salinity crisis. The Mediterranean fish fauna illustrates well the assembly of regional faunas through origination and immigration, where dispersal and isolation have shaped the emergence of a biodiversity hotspot.

Introduction
The Mediterranean fish fauna is unique, characterized by a history of isolation and connectivity [1] resulting from tectonic movements and changes in ocean circulation. Isolation of the Mediterranean is reflected in its rich marine flora and fauna, with an estimated total of 17,000 species [1]. 619 fish species have been inventoried in the Mediterranean, among which 13% are endemic, 2% are introduced, and 67% are non-endemic natives. 85% of these fish are teleosts [2]. General geological and oceanographic processes such as those involved at the origin of the Mediterranean Sea have been shown to influence regional histories of fish diversity globally [3,4]. Studying the Mediterranean region may therefore illustrate mechanisms contributing to diversification of teleosts and help us understand the current distribution of diversity in the region.

During the Cretaceous (145–65 Mya), the Mediterranean was part of the Tethys Sea and was connected with the Atlantic as well as with the Indo-Pacific oceans. At this time, Africa, Europe and the Adriatic plates were coming closer together, making this ancestral Mediterranean Sea smaller and smaller, and drastically changing its shape and connectivity. By the Miocene (23–5 Mya), the Mediterranean Sea was isolated from the Indo-Pacific. Subsequently, circa 7–5 Mya, it is believed to have been isolated from the Atlantic as well, causing a period of important environmental stress characterised by high desiccation and low sea level known as the Messinian Salinity Crisis (MSC) [5,6]. During the MSC, the Mediterranean Sea was probably reduced to a series of small lakes, causing a rise in water salinity and a very important extinction crisis among the fish fauna. However, about 5 Mya, the connection with the Atlantic Ocean reopened through the Strait of Gibraltar, allowing colonization of new species into the Mediterranean [5,6]. Today, the Mediterranean Sea is...
enclosed by land, with only two small connections to other oceans: the Strait of Gibraltar, and the Suez Canal, an artificial connection to the Red Sea that was opened in 1869 [2]. Despite the Strait of Gibraltar being only 14 km wide, it largely determines water circulation and productivity patterns, especially in the western Mediterranean [7].

A dated phylogeny of teleost taxa specific to the Mediterranean Sea is crucial to understand how episodes of drastic environmental changes in water circulation, environmental conditions, and level of isolation [5] have marked the evolution of its current diversity. To date, however, no phylogenetic reconstruction of teleost fish diversification events in the region has been published. Teleost fish represent the largest vertebrate group on Earth, with an estimated 27,000–31,000 species worldwide [3] (see also FishBase, http://www.fishbase.org). Building a phylogeny of teleosts remains challenging and controversial due to the large number of species and the lack of agreement regarding classification of some major orders and families [8,9]. For example, one of its largest orders, the Perciformes, includes a mixture of fairly disparate polyphyletic taxa [8,9,10]. There are published phylogenies for some groups, such as the families Gobiidae [11], Sparidae [12,13], and Labridae [14], which include several representatives of Mediterranean species. However, the most complete dated teleost phylogeny published to date [15] includes only 16 Mediterranean species and an additional 34 genera (represented by Mediterranean congeners) that occur in the Mediterranean.

The main goal of this study was to reconstruct a dated phylogeny of Mediterranean teleost species based on available molecular data to investigate the potential biogeographic causes that underlie current fish diversity in the Mediterranean Sea. We used the inferred dated phylogeny to explore the possibility that biogeographic events have differentially affected native and exotic species, and to relate major changes in diversity to the Earth history. First, the end-Cretaceous extinction crisis and radiation described for fish at the global scale [3], should be reflected in the Mediterranean for all clades. Second, if the isolation of Atlantic and Indo-Pacific waters was important in the emergence of fish diversity in the Mediterranean Sea, we would expect a peak in clade origin among native species before and until the MSC, at the time when water circulation between these two oceans started to be restricted (~40–20 Mya). Such a diversification burst would support the idea that limited dispersal from the Atlantic may have played a major role in maintaining and generating biodiversity within the Mediterranean, though we cannot exclude a complementary contribution of other regional mechanisms such as local isolation or extreme environmental conditions. Finally, if allopatric speciation due to the formation of highly isolated lakes during the MSC was the main driver of current diversity, we would expect a more recent origin of native clades centred around the MSC (~7–5 Mya). In both cases, these peaks should be observed among native and endemic species, but not among exotic species.

Materials and Methods

Data harvesting

Nucleotide sequences for Mediterranean teleost fishes (as listed in [16] and references therein), plus 9 additional extra-Mediterranean species were downloaded from GenBank using the seqinR package in R v.2.12.1 [17]. Six loci, each represented by >50 species, were identified for further analyses (Appendix S1 and S2). This minimum taxonomic representation potentially ensured a greater resolving phylogenetic power [18]. The DNA markers selected included 4 mitochondrial genes — 12S ribosomal RNA (12S rDNA; 221 species), 16S ribosomal RNA (16S rDNA; 265 species), cytochrome c oxidase subunit I (COXI; 118 species), and cytochrome b (CYB; 235 species) –, and two nuclear genes, the intronless rhodopsin (RHO; 183 species) and the recombination activating gene I (RAG1; 80 species). These markers have been used previously to unravel phylogenetic relationships among closely and distantly related species [19,20,21,22,23,24,25]. Because mitochondrial genes display average faster evolutionary rates as compared to nuclear exons, the former provide resolving power for closely related organisms, while the latter provide better resolution for deeper nodes [4,15].

The final analysis included 363 Mediterranean teleost species (62% of the total number of teleost species in the region), representing all orders, 110 families and 237 genera present in the Mediterranean Sea, and 9 extra-Mediterranean species (see Appendix S1).

Phylogenetic analyses

Downloaded sequences were individually aligned for each gene using MAFFT [26], version 5. The resulting alignments were inspected and further refined manually. Ambiguous regions of the alignments were filtered using Gblocks [27], version 0.91b. Parameters were set so that the minimum block length was 10 sites, and the maximum number of contiguous non-conserved positions was 5, while conserving sites with a maximum of 50% of gaps. The resulting aligned sequences had the following number of positions (% of the original alignments): 297 (30%) for 12S rRNA, 376 (62%) for 16S rRNA, 622 (58%) for COXI, 1107 (97%) for CYB, 437 (57%) for RHO, and 1,424 (33%) for RAG1. Aligned sequences were then concatenated into a supermatrix of 4,263 sites, and analysed for phylogenetic reconstruction under maximum likelihood (ML) [28]. The best-fitting model of sequence evolution was selected using the Akaike information criterion and hierarchical likelihood ratio tests calculated under Modeltest version 3.7 [29]. Both criteria identified the general time reversible (GTR) model of nucleotide exchangeabilities, with a Gamma (Γ) distribution plus a fraction (I) of invariable sites to account for among-sites substitution rate heterogeneities. All GTR+I+I branch length parameters were estimated from the data.

A preliminary unconstrained analysis resulted in some widely accepted clades being polyphyletic, leading us to enforce the following topological constraints in subsequent tree searches: Clupeiformes + Danio, Gadiformes, Lampriformes, Myctophiformes, Pleuronectiformes, Stomiiformes, and Tetraodontiformes for orders [30,31,32,33], and Labridae [14] for families. The orders Scorpaeniformes and Syngnathiformes, and the family Serranidae (Perciformes) were also constrained based on FishBase classification and on the lack of published evidence that these clades would be polyphyletic. Conversely, because there is published evidence that the family Sciaenidae (Centracanthidae, Perciformes) is genuinely included within the Serranidae [34], and that the Echeneidae are nested within the Carangidae [22] we did not constrain these taxa. Moreover, we rooted the trees with elopomorphs (here Anguilliformes + Notacanthiformes) as the sister-group of the remaining teleosts.

A first tree was built using the Randomized Accelerated Maximum Likelihood algorithm RAxML [35], v7.0.4. The resulting tree was the starting point for a deeper exploration of the topological space using PAUP* [36], version 4b10. Different cycles of tree search with tree-bisection reconnection (TBR) branch swapping and model parameter re-estimation were performed. The number of TBR rearrangements was increased to 10,000, 50,000, and then 100,000. The search was stopped as no further increase in log-likelihood was observed. The highest-likelihood tree thus identified was taken as the 6-gene best ML.
phylogenetic hypothesis for subsequent analyses. The corresponding phylograms were subjected to the super-distance matrix (SDM) approach [37] to estimate the relative substitution rate among 12S rDNA, 16S rDNA, COXI, CYB, RHO and RAG1.

Node stability was estimated under ML through 400 replicates of bootstrap re-sampling of the DNA supermatrix [28]. For each replicate, PAUP* computed the highest-likelihood tree based on the re-estimation of the GTR$+\Gamma$ model parameters, with the 6-gene ML topology as a starting point, and 10,000 TBR branch swapping rearrangements. The bootstrap percentages of the consensus tree were mapped on the highest-likelihood phylogram using the bppConsense utility of the Bio++ program suite [38]. All trees were drawn using the APE library [39] within the R statistical package.

Molecular dating

Divergence times among taxa were estimated using a Bayesian relaxed molecular clock dating strategy [40]. We compiled a list of fossil records and calibrations that have been used in previous publications, and we selected 20 paleontological constraints based on the following criteria:

(1) Only primary calibrations were considered, whereas secondary calibrations, based on molecular estimates, were discarded. Following recommendations in [41], minimum and maximum bounds were based solely on fossil information.

(2) The fossil record under focus should be unambiguous. For example, a calibration at 161 Mya for Gadiformes [42] is described in [43] as “probable”, though the first certain fossil for this order dates from the Ypresian (56–48 Mya). Because of these discrepancies, we decided to leave this calibration point out.

(3) The taxonomic group involved in the calibration should be well resolved in the highest-likelihood phylogeny.

As a result, 20 nodes were constrained according to the available paleontological information (Table 1). For each calibration, we set the minimum (lower) date to the age of the geological stage corresponding to the oldest fossil record. The maximum (upper) bound corresponds to the earliest fossil record for the sister clade, as recommended in [41]. In addition, a 225–152 million years prior was used on the root age for the split between elopomorphs and the remaining teleosts [15]. Due to the incompleteness of the fossil record, all time calibrations were set as soft bounds [44], i.e., 5% of the total probability mass was allocated outside the specified bound. The log-normal rate-autocorrelated model was chosen to relax the molecular clock assumption because of its ability to reasonably fit various data sets [45]. Branch lengths were measured under the CAT mixture model [46], with a general time reversible (GTR) model of exchangeability among nucleotides, and a 4-category Gamma ($\Gamma$) distribution of substitution rates across sites to handle different substitution rates among the mitochondrial and nuclear loci. Dating estimates were computed by the Bayesian procedure implemented in the PhyloBayes software [47], version 3.2c (http://www.phylobayes.org). We used the CAT Dirichlet process with the number of components, weights and profiles all inferred from the ML topology, and a birth-death prior on divergence times. Four independent Markov Chains Monte Carlo (MCMC) were run for 4,000 cycles (i.e., 4,000,000 generations), with sampling every 5 cycles. After a burn-in of 200 cycles (i.e.,

| Node Number | Name of clade | Time constraints | Reference |
|-------------|---------------|-----------------|----------|
| 1           | Notacanthidae vs Anguilliformes | L94            | [43,69] |
| 2           | Anguilliformes | L50             | [43]    |
| 3           | Clupeiformes  | L57             | [43]    |
| 4           | Zebranthurus vs Medaka (Clupeomorpha) | L150-U165 | [70]    |
| 5           | Myctophidae   | L70             | [43]    |
| 6           | Aulopiformes  | L96-U128        | [15]    |
| 7           | Tetraodontiformes | L59-U98   | [15]    |
| 8           | Tetraodon vs Takifugu | L32-56   | [70]    |
| 9           | Sparidae      | L48             | [43]    |
| 10          | Stickleback vs (Tetraodon+Takifugu) | L97-U151 | [70]    |
| 11          | Gasterosteiformes (stickleback) | L71      | [70]    |
| 12          | Labrus vs Symphodus* | L40-U84 | [43]    |
| 13          | Gobiidae      | L40 – U84      | [15]    |
| 14          | Scombridae    | L61             | [43]    |
| 15          | Pleuronectiformes | L51-U99     | [15]    |
| 16          | Soledae, Pleuronectiformes | L40      | [43]    |
| 17          | Beloniformes  | L40             | [43]    |
| 18          | Blenniidae    | L40             | [43]    |
| 19          | Pomacentridae | L50 – U84      | [15]    |
| 20          | Medaka vs Stickleback | L97-U151 | [70]    |

*Notice that this node corresponds to the bifurcation between two genera and not to the family Labridae.

Node numbers correspond to the numbers shown in Figure 1.

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200,000 generations), log-likelihood and model parameters stabilized. We computed the maximum difference of age estimated for each node by the 4 chains. We observed that the median of these differences in divergence times did not exceed 0.7 Ma, ensuring that convergence had been reached.

Diversification events through time

We classified species into natives, exotics and endemics following [16] and references therein: exotic species are species that are found in the Mediterranean Sea and for which there are records of introduction between 1810 and 2006; endemics have a distribution restricted to the Mediterranean; and the rest are non-endemic natives.

We then pruned the full chronogram to study the frequency and timing of diversification events among endemics, non-endemic natives and exotic species by dropping taxa that did not belong to the group of interest (e.g. to build the exotic species tree we dropped all the natives and extra-Mediterranean species). Then we recorded the date of each split and plotted diversification events by time period. To check whether these patterns of diversification were different from random, we sampled the same number of species randomly and without replacement from the full tree (n = 38 for endemics, n = 60 for exotics; n = 263 for non-endemic natives). For each sampling, we recorded the diversification events and calculated their median. This random sampling was repeated 1000 times for each case. We performed a two-tailed significance test i.e. the observed value was considered significantly different from random whenever it was outside the central 95% resampled distribution. We then repeated the same randomizations using either the lower or the upper bounds in the confidence intervals of each node age.

Note that we preferred this randomization strategy over the strategy of estimating diversification rates for each group for two reasons: (1) diversification rates estimates are likely to be biased by incomplete sampling [48] and (2) we are interested here in the timing of diversification events, more than on the estimates of diversification rates that could be obtained using this separate methodology. However we also built lineages-through-time plots for each chronogram analyzed to look at a more precise time-line of diversification events, and compare results from enriched trees (see below).
Figure 2. Histograms of the ages of the diversification events. Histograms are shown for (a) all species found in the Mediterranean Sea, (b) exotics only, (c) non-endemic natives, and (d) endemics. Dashed lines indicate the median based on the mean (M) node age estimates as well as based on the lower (L) and upper (U) bounds for each node’s 95% credibility interval. Asterisks near the letters indicate significantly different ages than those expected by a random draw of the same age. Asterisks near the letters indicate significantly different ages than those expected by a random draw of the same age.

Results

Phylogenetic relationships

While we assumed the monophyly of several groups, many higher level relationships were recovered without the need of imposing constraints on nodes [15,50,51]. In this way, Notacanthus is the sister group of Anguilliformes, and Clupeiformes form a deep-branching group, followed by Gadiformes, Myctophiformes, Aulopiformes, and other younger clades (Figure 1a). By contrast, the order Perciformes is polyphyletic, with different families spread along the tree: Sparidae + Centracanthidae, and Serranidae (Figure 1b), Labridae, Gobiidae, and Scombridae (Figure 1c), and Carangidae and Blenniidae (Figure 1d). Three additional families are monophyletic and branch in the crown part of the tree: Mugilidae, Blenniidae, and Pomacentridae (Figure 1d). By contrast, two families are paraphyletic because Echeneidae (Echeneis and Remora: Figure 1d) is nested within Carangidae [22], and Centracanthidae (Figure 1b) is nested within Scombridae [34]. Within Carangidae, the monophyly of the tribes Carangini and Naufragini [52] is supported.

Molecular dating

The dated phylogeny suggests that the diversification of the Mediterranean teleosts sampled here started during the Jurassic at least 166–153 Mya (Figure 1a). The diversification of most Perciformes families was estimated to occur during the late Paleocene to mid-Eocene, while the origin of some of the most important orders such as Clupeiformes, Gadiformes and Aulopiformes were dated back to around 80–50 Mya (Figure 1a). Most large Perciformes families such as Sparidae and Gobiidae started their diversification at around 80–50 Mya (Figure 1b and 1c). Among the youngest Perciformes families we can find Callionymidae, which diversified 14–2 Mya (Figure 1b). Most terminal nodes were dated as <30 Mya, but some exceptions can be found. For example the node that separates the two Pomacentridae species Abudelfayyis gregensis and Chromis chromis was estimated at 82–57 Mya (Figure 1d).

Diversification events through time

When all species are considered together in the analysis of diversification, most splitting events took place within the last 40 Mya, with a median at 43 Mya (median at 36–31 Mya if considering upper and lower node age bounds respectively, see Figure 2a). However, this scenario varies when endemics, non-endemic natives and exotic species are considered separately. Exotic species only showed one diversification peak at 100–80 Mya, but no peak during the last 50 Mya (Figure 2b), and presented a median value for diversification events of 90 Mya (99–78 Mya). Non-endemic native species showed a primary peak at 40–20 Mya and a secondary peak at 100–80 Mya (Figure 2c), and had an overall median of 45 Mya (59–33 Mya). Endemics showed a primary peak at 100–80 Mya and a secondary peak at 40–20 Mya (Figure 2d), with an overall median value of 81 Mya (91–70 Mya). Similar diversification patterns are observed when using any of the chronograms enriched by taxon grafting (results not shown), and are supported by the lineage-through-time plots (Figure 3). The slope of the lineage-through-time plots for the grafted species were set to the age of the node where they had been attached. This resulted in four additional chronograms of increasing taxonomic coverage including 404, 416, 473 and 496 species respectively, the most complete of which includes 93% of all Mediterranean endemics and 87% of non-endemic natives (see Appendix S3 for details).

We finally repeated this diversification analysis increasing the taxonomic coverage. First, we attached to the backbone chronogram additional species for which sequence data were not available but for which congeneric species were already present in the ML topology by “grafting” them as polytomies at the most recent common ancestor [MRCA] [49]. In a first step, we attached species that had 2 congeners represented in the chronogram to the node that linked the two species in question (33 species, see Appendix S3). On a second step, we added species that had at least one congener represented in the chronogram by attaching them to the node that linked the congener with the closest (non-congener) species. Third, we attached species which had members of the same family, and finally those that were represented by members in the same order, attaching them to the node that linked all the members of the same clade. Branch lengths leading to each
endemics increases between 100–80 Mya and between 40–
10 Mya (Figure 3), as it is observed for non-endemic natives.
However, exotic species only showed an increase in the slope after
100 Mya. These patterns remain consistent whether the raw dated
phylogeny (Figure 3a), the grafted trees including congeneric
representatives (Figure 3b and 3c), or family and order represen-
tatives (Figure 3d and 3e) are considered.

To summarize, three general patterns were evidenced in all
diversification analyses. First, endemic and native species showed a
significantly younger diversification median age than expected by
a random draw of the same number of species from the phylogeny
(Figure 2). Second, diversification median age of exotic species was
not different from random (Figure 2). And finally, natives and
endemics showed a peak in diversification in the last 50 Mya that
was not found for the exotic pool (Figure 2 and 3).

Discussion

Reliability of the teleost phylogeny and timetree
estimates

Four elements are crucial to reliably approach the evolutionary
history of Mediterranean teleosts in our analysis: taxon sampling,
gene sampling, topology inferred, and divergence times. First, we
have followed a strategy of increasing taxon sampling at the
expense of the number of markers because our focus on the
understanding of the diversification patterns of Mediterranean
teleosts required a stable phylogenetic picture with a wide
taxonomic coverage and a reduced systematic error [53].
Conversely, other studies have favoured the number of genes by
comparing complete teleost mitochondrial genomes (e. g. [10]).

Second, the relative evolutionary rates among the 6 genes — as
measured by the SDM procedure [37] — showed that the slowest-
evolving marker is, as expected, the nuclear gene RAG1. Furthmore, the mitochondrial and nuclear DNA supermatrix
of ~4,300 unambiguously aligned sites combined genes with
contrasted evolutionary dynamics. This likely provided phylol-
genetic resolving power at lower taxonomic level for the faster-
evolving markers (e.g., CYB, COXI), and at deeper levels for the
slower-evolving ones (RAG1, RHO, mitochondrial rDNAs).
Certainly the resolution of additional teleost diversification events
during intermediate periods of time will require gathering
evolutionary signal in complete mitogenomes and other nuclear
markers (e.g. [10,54]). However, considering supplementary genes
would have required sequencing de novo, which was out of the
scope of this project. Third, the amount of missing character states
in our supermatrix was 59 %. This reflects our choice of sampling
incomplete taxa to maximize the taxonomic coverage. Although
this approach may decrease phylogenetic accuracy, it has been
shown that the limited availability of complete characters is more
important than the excess of missing character states [55].
Therefore, additional taxa involving a non-negligible amount of
missing data may not compromise the accuracy of the phyloge-
netic inference [56]. Fourth, as the phylogenetic tree contains the
primary information about both evolutionary rates and divergence
times, the estimation of the teleost timetree heavily relies upon the
correct measurement of branch lengths through realistic models of
sequence evolution. The CAT mixture model used here distributes
the alignment sites into categories to handle the site-specific
nucleotide preferences [46]. Thanks to its more efficient ability to
detect multiple substitutions, branch lengths estimated under the
CAT model will be less affected by saturation and will handle the heterogeneity present between nuclear and mitochondrial loci. Finally, we improved the phylogenetic resolution of our tree by securing the monophyly of widely accepted taxa, and leaving other clades unconstrained. Although it can be argued that the constrains impose an additional level of subjectivity in the analysis, as we had to decide which clades needed to be constrained or not, supplementary analyses comparing constrained versus unconstrained trees (results not shown) showed that the timing of speciation events is not influenced by these decisions and that our conclusions are robust to the phylogenetic structure presented here.

Timeline of the diversification of native and exotic species

Here we draw for the first time a timeline of origin and diversification events for the teleosts of the Mediterranean Sea. Overall, the diversification of all major clades in the Mediterranean (Figure 1) coincides with that published by Santini and colleagues [15] for teleosts at the global scale. Santini et al.’s work was based on one nuclear gene (RAG1) sampled for 225 species, and 45 calibrations. Here we used more genes to build a dated phylogeny of 372 species, and 29 calibrations. What in [15] species were chosen to maximize the number of teleost orders worldwide, we selected species according to a biogeographic criterion, i.e. their occurrence in the Mediterranean Sea. A major consequence of our strategy was that several orders and families had two or more representatives in the tree, while some others were not represented. Despite these differences in the circumscription of the taxa and phylogenetic markers, all major clades represented in [15] were sampled here. More importantly, the evolutionary history of speciation events in the Mediterranean could not be deduced from a global study such as [15] where only 34 Mediterranean genera and an additional 16 Mediterranean species were represented.

Our results show similar dates of diversification for some of the major orders and families, but they also reveal a difference in tempo between native and exotic species. The fact that median diversification age for exotic species was different from random, but those of native species was (Figure 2), suggests that speciation within the region has been affected by a succession of biogeographic events at the global but also at the local scale. However, diversification events among native species did not originate slowed down. Although the incomplete representation of the different taxa may influence our perception of speciation and extinction events, neither lineage-through-time plots (Figure 3) nor comparisons with random expectation (Figure 2) suggest any acceleration of speciation events during the MSC. By the beginning of this period the African, Arabic and European landmasses were effectively separating the Indian and Atlantic oceans. The Eocene-Oligocene transition that corresponds with this period is also marked by large global climate changes. This transition culminated with the MSC at around 6 Mya, which probably eliminated a large portion of fish diversity in the region [5,6] and
where locally surviving species were mostly neritic [63]. However, during this time, the Indian Ocean and the Atlantic fish faunas remained isolated, providing plenty of opportunities for vicariant speciation and promoting a higher diversification rate which has been suggested as the basis of the Mediterranean fish diversity today [5,6]. Therefore, both the timing of diversification events among natives (Figures 2 and 3) and the analysis of the fossil record in the Mediterranean [63,64], point to an important role of the separation between the Indian Ocean and the Atlantic Ocean as a driver of current fish diversity in the area. The fossil record also shows a wide variety of fish that are now extinct in the area, suggesting that part of this diversity has been shaped by important extinction events, and a balance between origination and extinction [64]. Adding to this evidence, paleontological analyses in the Mediterranean have already demonstrated that the picture regarding the MSC is not as simple as originally thought, i.e. that the Mediterranean was not hyper saline everywhere and that many species could have survived extinction locally [63]. In particular, biochemical analysis of sediments and fossil faunas including otoliths have shown that some interior parts of the Mediterranean, specifically in Italy, would have been connected to the Sea and would have shown salinity levels comparable to those currently present in the Mediterranean Sea [64,65,66]. Therefore, the MSC may have played a rather secondary role in speciation events leading to the current fish diversity in the Mediterranean.

Certainly our results regarding the tempo of diversification could have been influenced by our coverage of the different groups analysed. For example, in the raw dated phylogeny we represented 46% of all endemic teleosts in the Mediterranean (Figure 3a). Attaching species to the most recent common ancestors if they had at least one congener represented increases this representation to 66% (Figure 3c, see Appendix S3 for number of species added at each level). Finally, by also considering species that had a member of the same family (Figure 3d) or on the same order (Figure 3e) we increased the coverage of endemics to 92%. Although one may argue that the patterns observed in the most complete chronogram are due to an artefact of adding species to deeper family and order nodes, this argument cannot be applied to the analysis carried out adding only congeners to the backbone tree. Here, one would expect accentuated patterns that are already present in the backbone chronogram for endemic species. These analyses do not show any increase in diversification of endemics or natives during the MSC, but they always show the two above-mentioned peaks after 100 Mya and 40 Mya (Figure 3). Therefore we expect that these patterns will be robust to analyses using further gene sequencing and additional species.

Conclusions

Overall our results show that fish diversity in the Mediterranean Sea originated largely during the Cretaceous and Paleocene during episodes of global change, when the Mediterranean Sea still did not exist. They also suggest that the isolation between Atlantic and Indo-Pacific waters before the MSC had a large role in the emergence of native and endemic species diversity. Beyond the establishment of phylogenetic relationships among Mediterranean marine fish and advances in the comprehension of evolutionary history underlying this diversity, our study paves the way towards a phylogenetic perspective in the conservation of fish biodiversity at a macroecological scale [67]. In a different vein, understanding the interplay between phylogenetic diversity and environmental gradients at large biogeographic scales may also help us understand the mechanisms that are behind the emergence and maintenance of diversity [68]. This understanding is fundamental in the Mediterranean Sea where biodiversity may be at high risk under the rates of current global changes [1,2,6,67].

Supporting Information

Appendix S1 Catalog of GenBank sequences used in the phylogenetic analysis.

Appendix S2 Gene representation and saturation in the phylogenetic analysis.

Appendix S3 Species grafted at their most recent common ancestor (MRCA).

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Author Contributions

Conceived and designed the experiments: CNM NM DM. Performed the experiments: CNM NJR JD. Analysed the data: CNM NJR JD. Wrote the paper: CNM NM DM NJR. Given Mediterranean fish database: DM.

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### Appendix Table S1: GenBank sequence catalog.

| ORDER          | FAMILY            | SPECIES                                      | 12S   | 16S   | COXI  | CytB  | Rhod | RAG1  |
|----------------|-------------------|----------------------------------------------|-------|-------|-------|-------|------|-------|
| Anguilliformes | Anguillidae       | *Anguilla anguilla* (Linnaeus 1758)           | AF266494 | AB021749 | AP007233 | AB021776 | L78007 |
| Muraenesocidae | Muraenidae        | *Muraenox cinereus* (Forsskål 1775)          | AF417318 | EF607449 | AU295080 |
|                |                   | *Enchelycore anatina* (Lowe 1838)             |        |        |        |        |      |
|                |                   | *Gymnothorax unicolor* (Delarohe 1809)       |        |        |        |        |      |
|                |                   | *Muraena helena* (Linnaeus 1758)             |        |        |        |        |      |
|                | Nemichthyidae     | *Nemichthys scolopaceus* Richardson 1848     | AB049989 | AY952481 | EU148262 | AB038418 |
|                | Nettastomatidae   | *Nettastoma melanurum* Rafinesque 1810      | DQ645673 | DQ645712 |      |
|                | Ophichthidae      | *Echelus myrus* (Linnaeus 1758)              | DQ645651 | DQ645690 |      |
| Aulopiformes   | Aulopidae         | *Aulopus filamentosus* (Bloch 1792)         |       |        |        |        |      |
| Chlorophthalmidae |               | *Chlorophthalmus agassizi* Bonaparte 1840    | AP002918 | DQ027906 | DQ027975 | EF439508 | EF439358 | FJ896455 |
|                | Ipnopidae         | *Bathypterois dubius* Vaillant 1888          | AY141326 | AY141396 | AY141257 |      |
|                | Synodontidae      | *Bathypterois grallator* (Goode & Bean 1886) |        |        |        |        |      |
|                | Synodus saurus    |                                                |        |        |        |        |      |
|                | Sudis hyalina     | Rafinesque 1810                              |        |        |        |        |      |
| Synodontidae   | Adrianichthyidae  | *Oryzias latipes* (Temminck & Schlegel 1846) | AP008948.1 | AP008948.1 | AP008948.1 | AP008948.1 | NM_001104 | EF095641.695 |
| Order              | Family             | Genus                | Species                      | Accessions                  |
|--------------------|--------------------|----------------------|------------------------------|-----------------------------|
| Beryciformes       | Belonidae          | Belone belone        | gracilis (Linnaeus 1761)     | AF231541, AF231514, EU036423, AY141268 |
|                    |                    | Belone               | svtovidovi Collette & Parin 1970 | AF243956, AF243880          |
|                    |                    | Tylosurus acus       | (Lacepède 1803)               | AF231571, AF231528, AF231656, EF427530 |
|                    | Exocoetidae        | Exocoetis volitans   | Linnaeus 1758                 | AP002933, AP002933, AP002933, AP002933 |
|                    | Hemiramphidae      | Hemiramphus far      | (Forsskål 1775)               | AY693487, EU148546, AY693516 |
|                    |                    | Hyporhamphus affinis| (Günther 1866)                | EF609376                    |
|                    | Scomberesocidae    | Scomberesox saurus   | (Walbaum 1792)                | AF243984, AB355963, AY308771 |
|                    | Berycidae          | Beryx splendens      | Lowe 1834                     | AF092197, AF100909, EF609297, AB108491, AY141265, EF095636 |
|                    | Holocentridae      | Sargocentron         | rubrum (Forsskål 1775)        | AP004432, AP004432, AP004432, AP004432 |
|                    |                    | Trachichthyidae      | Geophybroberyx darwini        | DQ108100                    |
|                    |                    | Hoplostethus         | mediterraneus Cuvier 1829     | AY141335, DQ885093, AY141264, EF095635 |
|                    | Clupeiformes       | Clupeidae            | Alosa alosa (Linnaeus 1758)   | AP009131, AP009131, AP009131, EU224046, EU224142 |
|                    |                    | Alosa fallax         | (Lacepède, 1803)              | EU552737, EU552574, EU491985 |
|                    |                    | Dussumieria acuta    | Valenciennes 1847             | EU014222                    |
|                    |                    | Dussumieria elopsoides| Bleeker 1849                 | EU364556, EF607361          |
|                    |                    | Etrumeus teres       | (DeKay 1842)                  | DQ912038, DQ912073, AP009139, EU552621, DQ912110 |
|                    |                    | Pellonula leonensis  | Boulenger 1916                | NC_009591.1, NC_009591.1, NC_009591.1, NC_009591.1, DQ912130 |
|                    |                    | Sardina pilchardus   | (Walbaum 1792)                | DQ912053, DQ912088, EF609451, AF472582, EF439304 |
|                    |                    | Sardinella aurita    | Valenciennes 1847             | DQ912032, DQ912067, AM911173, EU552619, EF439427, DQ912104 |
|                    |                    | Sardinella maderensis| (Lowe 1838)                   | AP009143, AM911205, AM911175, AF472583, EF439303 |
| Order                  | Family         | Species                          | GenBank Accession Numbers | EMBL Accession Numbers | NCBI Accession Numbers |
|------------------------|----------------|----------------------------------|---------------------------|------------------------|------------------------|
| Engraulidae            | Spratelloides | *Spratelloides delicatulus* (Bennett 1832) | DQ912058 | DQ912093 | AP009144 | AP009144 | DQ912128 |
|                        |                | *Sprattus sprattus* (Linnaeus 1758) | AP009234 | AM911201 | AM911177 | AF472581 | EU491991 |
| Cypriniformes          | Engraulis     | *Engraulis encrasicolus* (Linnaeus 1758) | DQ912031 | DQ912066 | AM911182 | EU552563 | EU224151 | DQ912103.1 | U71093 |
|                        | Cyprinidae    | *Danio rerio* (Hamilton 1822) | NC_002333.2 | NC_002333.2 | NC_002333.2 | NC_002333.2 | NM_131084.1 |
| Cyprinodontiformes     | Aphanius     | *Aphanius dispar* (Rüppell 1829) | | | | ADU05964 | | |
|                        |                | *Aphanius fasciatus* (Valenciennes 1821) | | | | AFU05965 | AF299273 |
|                        |                | *Aphanius iberus* (Valenciennes 1846) | | | | | AF299274 |
| Poesiliidae            | Gambusia      | *Gambusia affinis* (Baird & Girard 1853) | NC_004388.1 | NC_004388.1 | NC_004388.1 | NC_004388.1 | | |
| Dactylopteriformes     | Dactylopterus | *Dactylopterus volitans* (Linnaeus 1758) | AF150006 | | | EF439514 | AY141282 |
| Gadiformes             | Gadidae       | *Gadiculus argenteus* Guichenot 1850 | | | | | EU224053 | EU224201 |
|                        |                | *Merlangius merlangus* (Linnaeus 1758) | | | | | | |
|                        |                | *Micromesistius poutassou* (Risso 1827) | | | | | | |
|                        |                | *Trisopterus luscus* (Linnaeus 1758) | | | | | | |
|                        |                | *Trisopterus minutus* (Linnaeus 1758) | | | | | | |
| Lotidae                | Gaidropsarus  | *Gaidropsarus bicayensis* (Collett 1890) | | | | | | |
|                        |                | *Gaidropsarus mediterraneus* (Linnaeus 1758) | | | | | | |
|                        |                | *Gaidropsarus vulgaris* (Cloquet 1824) | | | | | | |
|                        |                | *Molva dypterygia* (Pennant 1784) | | | | | | |
| Macrouridae            | Coryphaenoides| *Coryphaenoides guentheri* (Vaillant 1888) | | | | | | |

Continued...
| Family               | Genus                  | Species                  | Author                  | Accession Numbers |
|---------------------|------------------------|--------------------------|-------------------------|-------------------|
| Hynemocephalus      | italicus               | Giglioli 1884            |                         | FJ215246          |
| Nezumia             | aequalis               | Günther 1878             |                         | FJ215280          |
| Trachyrincus        | scabrus                | (Rafinesque 1810)        |                         | FJ215298          |
| Merlucciidae        | Merluccius             | merluccius 1758          | Linnaeus                 | DQ274008          |
|                     |                        |                          |                         | EF609408          |
|                     |                        |                          |                         | DQ174062          |
|                     |                        |                          |                         | EF439400          |
| Meridae             | Guttigadus             | latifrons 1908           | Holt & Byrne             | EU148219          |
| Mora moro           | moro                   | (Risso 1810)             |                         | AY368285          |
|                     |                        |                          |                         | AY368307          |
|                     |                        |                          |                         | EF609410          |
|                     |                        |                          |                         | DQ197964          |
|                     |                        |                          |                         | AY368322          |
| Phycidae            | Phycis                 | blennoides 1768          | Brünnich                  | AY845393          |
|                     |                        |                          |                         | AY850365          |
|                     | Phycis                 | phycis 1766              | Linnaeus                  | DQ197978          |
|                     |                        |                          |                         | DQ197880          |
| Gasterostiformes    | Gasterosteidae         | Gasterosteus             | aculeatus 1758           | NC_003174.1       |
|                     |                        |                          | Linnaeus                  | NC_003174.1       |
|                     |                        |                          |                         | NC_003174.1       |
|                     |                        |                          |                         | EU637962.1        |
|                     |                        |                          |                         | AB445183          |
|                     | Puigitus               | pungitus 1758            | Linnaeus                  | NC_011571.1       |
|                     |                        |                          |                         | NC_011571.1       |
|                     | Spinachia              | spinachia 1758           | Linnaeus                  | NC_011582.1       |
|                     |                        |                          |                         | NC_011582.1       |
| Hypoptychidae       | Hypoptychus            | dybowskii 1880           | Steindachner              | NC_004400         |
| Gobiesociformes     | Apletodon              | dentatus 1887            | Facciola                  | AF549200           |
|                     |                        |                          |                         | AF549207           |
|                     | Diplocogaster         | bimaculata 1788          | Bonnaterre                | AF549197           |
|                     |                        |                          |                         | AF549205           |
|                     | Gouania               | willdenowi 1810          | Risso                     | EF363030           |
|                     |                        |                          |                         | EF363032           |
|                     | Lepadogaster           | candollei 1810           | Risso                     | AY036588           |
|                     |                        |                          |                         | AF549203           |
|                     | Lepadogaster           | lepadogaster 1788        | Bonnaterre                | AY036589           |
|                     |                        |                          |                         | AF549202           |
|                     | Lepadogaster           | purpurea 1788            | Bonnaterre                | AY036599           |
|                     |                        |                          |                         | AF549201           |
|                     | Opeatogenys            | gracilis 1864            | Canestrini                 | AF549196           |
|                     |                        |                          |                         | AF549206           |
| Lampriformes        | Lampridae              | Lampris                 | guttatus 1878             | AF049726           |
|                     |                        |                          |                         | AF049736           |
|                     |                        |                          |                         | DQ885096           |
|                     |                        |                          |                         | DQ197959           |
|                     |                        |                          |                         | AY308764           |
| Taxonomic Order | Family       | Species                        | Author/Year | Accession Numbers |
|-----------------|-------------|--------------------------------|-------------|-------------------|
| Lophotidae      | Lophotus    | *lacepede* Giorni 1809          | AY036616    | AY036618          | FJ896461         |
| Regalecidae     | Regalecus  | *glesne* Ascanius 1772          | AF049728    | EU099465          | AY368328         | EF107625         |
| Trachipteridae  | Zu         | *cristatus* (Bonelli 1819)      | AY652748    | AY652749          | FJ896462         |
| Lophiiformes    | Lophius    | *budegassa* Spinola 1807        | EF095552    |                   |                   |
|                 | piscatorius| Linnaeus 1758                  | AY368294    |                   |                   |
| Myctophiformes  | Myctophidae| *Benthosema* glaciale (Reinhardt 1837) | DQ532843 | EU148098          | EU366728         |
|                 | Ceratosepelus maderensis (Lowe 1839) |           |               |                   |                   |
|                 | *Diaphus* metopoclampus (Cocco 1829) |           |               |                   |                   |
|                 | *Diaphus rafinesquii* (Cocco 1838) |           |               |                   |                   |
|                 | *Diogenichthys atlanticus* (Tåning 1928) |           |               |                   |                   |
|                 | *Electrona risso* (Cocco 1829) |           |               |                   |                   |
|                 | *Gonichthys coco* (Cocco 1829) |           |               |                   |                   |
|                 | *Hygophum benoiti* (Cocco 1838) |           |               |                   |                   |
|                 | *Hygophum hygorn* (Lütken 1892) |           |               |                   |                   |
|                 | *Lobianchia dolineini* (Zugmayer 1911) |           |               |                   |                   |
|                 | *Lobianchia gemellarii* Cocco 1838 |           |               |                   |                   |
|                 | *Myctophum punctatum* Rafinesque 1810 |           |               |                   |                   |
|                 | *Notoscopecus bolini* Nafpaktitis 1975 |           |               |                   |                   |
|                 | *Symbolophorus veranyi* (Moreau 1888) |           |               |                   |                   |
| Notacanthiformes | Notacanthidae | *Notacanthus bonaparte* Risso 1840 | X99182 | X99181 | EU148274 |
| Order                  | Family            | Species                              | Author                  | Accession Numbers | GenBank Accession Numbers |
|-----------------------|-------------------|--------------------------------------|-------------------------|-------------------|--------------------------|
| Osmeriformes          | Argentinidae      | *Argentina sphyraena* Linnaeus 1758  |                         | EU492324          | EU492231                 |
| Perciformes           | Acropomatidae     | *Synagrops japonicus* (Döderlein 1883) |                         |                   |                          |
| Apogonidae            |                   | *Apogon imberbis* (Linnaeus 1758)    | Linnaeus 1758           | AM158282          | FJ462721                 |
| Blenniidae            |                   | *Aidablennius sphynx* (Valenciennes 1836) |                         |                   |                          |
|                       |                   | *Aidablennius ocellaris* (Linnaeus 1758) |                         |                   |                          |
|                       |                   | *Coryphoblennius galerita* (Linnaeus 1758) |                         |                   |                          |
|                       |                   | *Lipophrys adriaticus* (Steindachner & Kolombatovic 1883) |                         |                   |                          |
|                       |                   | *Lipophrys canevae* (Vinciguerra 1880) | Linnaeus 1758           |                   |                          |
|                       |                   | *Lipophrys dalmatius* (Steindachner & Kolombatovic 1883) |                         |                   |                          |
|                       |                   | *Lipophrys nigriceps* (Vinciguerra 1883) |                         |                   |                          |
|                       |                   | *Lipophrys pholis* (Linnaeus 1758)    | Linnaeus 1758           |                   |                          |
|                       |                   | *Lipophrys trigloides* (Valenciennes 1836) |                         |                   |                          |
|                       |                   | *Omobranchus punctatus* (Valenciennes 1836) |                         | OPU90393          |                          |
|                       |                   | *Parablennius gattorugine* (Linnaeus 1758) |                         | AF414715          | DQ160198                 |
|                       |                   | *Parablennius incognitus* (Bath 1968) | Linnaeus 1758           | AY098784          | AY098829                 |
|                       |                   | *Parablennius pilicornis* (Cuvier 1829) |                         | AY098795          | AY098831                 |
|                       |                   | *Parablennius rouxi* (Cocco 1833)     | Linnaeus 1758           | AY098781          | AY098833 AJ872148        |
|                       |                   | *Parablennius sanguinolentus* (Pallas 1814) |                         | AF414697          | AF428241                 |
|                       |                   | *Parablennius tentacularis* (Brünnich 1768) |                         | AY098780          | AY098838                 |
|                       |                   | *Parablennius zvonimiri* (Kolombatovic 1892) |                         | AY098790          | AY098840                 |
| Scientific Name                  | Accession Numbers  |
|---------------------------------|--------------------|
| *Salaria pavo* (Risso 1810)     | AY098798           |
| *Scartella cristata* (Linnaeus 1758) | AY098803           |
| *Brama brama* (Bonnaterre 1788) |                    |
| *Callanthias ruber* (Rafinesque 1810) | EF120863           |
| *Callionymus lyra* Linnaeus 1758 | AY141344           |
| *Callionymus maculatus* Linnaeus 1810 |                    |
| *Callionymus reticulatus* Valenciennes 1837 | EU491962           |
| *Alectis alexandrinus* (Geoffroy Saint-Hilaire 1817) | AF363738           |
| *Alepes djedaba* (Forsskål 1775) | EF613269           |
| *Caranx cryos* (Mitchell 1815)  |                    |
| *Caranx hippos* (Linnaeus 1766) | AY050717           |
| *Caranx rhonchus* Geoffroy Saint-Hilaire 1817 | AY050733           |
| *Elagatis bipinnulata* (Quoy & Gaimard 1825) | EU014213           |
| *Lichia amia* (Linnaeus 1758)   | EF392593           |
| *Pseudocaranx dentex* (Bloch & Schneider 1801) | EF609442           |
| *Seriola carpenteri* Mather 1971 | EF392607.1         |
| *Seriola dumerili* (Risso 1810) | EF607552           |
| *Seriola fasciata* (Bloch 1793)  | AY050748           |
| *Seriola rivoliana* Valenciennes 1833 | AB264297           |
| *Trachinotus ovatus* (Linnaeus 1758) | AY141388           |
| Family                  | Species                        | GenBank Accession Numbers | NCBI Accession Numbers |
|------------------------|--------------------------------|---------------------------|------------------------|
| Trachurus mediterraneus | Steindachner 1868              | AF487412                  | AY526548 EU036619      |
| Trachurus picturatus    | Bowdich 1825                   | AF487415 EU148351         | AY526546 EF439329      |
| Trachurus trachurus     | Linnaeus 1758                  | AB108498 AB108498         | AY526533 EU491981      |
| Centracanthidae         | Centracanthus cirrus Rafinesque 1810 |                           | EU167766               |
|                        | Spicara flexuosa Rafinesque 1810 |                           | EU036502 EU036606 EU167804 |
|                        | Spicara maena (Linnaeus 1758)  | AP009164 AP009164         | AY247343 EU036610 EU167805 |
|                        | Spicara smaris (Linnaeus 1758) |                           | EF439599 EF439465      |
| Centrolophidae          | Centrolophus niger (Gmelin 1789)| AB205412 AB205434 AB205456 EF439348 |
|                        | Schedophilus ovalis (Cuvier 1833) | AB205413 AB205435 AB205457 EF427506 |
| Cepolidae               | Cepola macrophthalmalma (Linnaeus 1758) | DQ027923 DQ027993         | EF439350 EU167817      |
| Chaetodontidae          | Chaetodon hoefleri Steindachner 1881 | EF616824 EF616908        |                        |
| Cichlidae               | Cichlasoma bimaculatum (Linnaeus 1758) | EF432874 AY263863 AF145128 EU706368 |
| Coryphaenidae           | Coryphaena equiselis Linnaeus 1758 | DQ874715 AY579555 DQ885087 AY050761 DQ874824 EU167822 |
|                        | Coryphaena hippurus Linnaeus 1758 | DQ874715 AY579555 DQ885087 AY050761 DQ874824 EU167822 |
| Echeneidae              | Echeneis naucrates Linnaeus 1758 | AY141389 DQ532869         | AY050763 AY141315 EU167829 |
|                        | Remora osteochir (Cuvier 1829)  | EU574934                  |                        |
|                        | Remora remora (Linnaeus 1758)   | AY836584 EU403077         |                        |
| Epigonidae              | Epigonus constanciae (Giglioli 1880) | EF120867                  |                        |
|                        | Epigonus telescopus (Risso 1810) | EF609350 DQ197949 DQ197851 EU167904 |
| Gempylidae              | Ruvettus pretiosus Cocco 1833   | EU003538 DQ874736         | EU003556 DQ80265 DQ874813 |
| Gobiidae                      | Species                                      | Accession 1  | Accession 2  |
|-------------------------------|----------------------------------------------|--------------|--------------|
|                               | *Aphia minuta* (Risso 1810)                  | EF218623     | EF218638     |
|                               | *Buenia affinis* Iljin 1930                   | EF218628     | EF218643     |
|                               | *Crystallogobius linearis* (Düben 1845)       | EF218635     | EF218650     |
|                               | *Gobius auratus* Risso 1810                   | AF067254     | AF067267     |
|                               | *Gobius bucchichi* Steindachner 1870          | EF218627     | EF218642     |
|                               | *Gobius cobitis* Pallas 1814                  | EF218629     | EF218644     |
|                               | *Gobius cruentatus* Gmelin 1789               | EF218626     | EF218641     |
|                               | *Gobius niger* Linnaeus 1758                  | EF218630     | EF218645     | AY884591  |
|                               | *Gobius paganellus* Linnaeus 1758             | EF218636     | AF518216     |
|                               | *Gobius xanthocephalus* Heymer & Zander 1992 | DQ382237     |              |
|                               | *Knipowitschia panizzea* (Verga 1841)        | AF067259     | AJ616812     |
|                               | *Lesueurigobius friesii* (Malm 1874)         | EF218624     | EF218639     |
|                               | *Lesueurigobius suerii* (Risso 1810)         | EF218625     | EF218640     |
|                               | *Pomatoschistus canestrinii* (Ninni 1883)    | AJ616818     | AJ616835     |
|                               | *Pomatoschistus knerii* (Steindachner 1861)  | EF218632     | EF218647     |
|                               | *Pomatoschistus marmoratus* (Risso 1810)     | AF067262     | AF067275     |
|                               | *Pomatoschistus microps* (Krøyer 1838)       | AJ616811     | AJ616828     | AJ550471  |
|                               | *Pomatoschistus minutus* (Pallas 1770)        | EF218633     | EF218648     | AY940726  |
|                               | *Pomatoschistus norvegicus* (Collett 1902)   | AJ616814     | AJ616831     |
|                               | *Pomatoschistus pictus* (Malm 1865)          | AJ616807     | AJ616834     |
Pomatoschistus quagga (Heckel 1837)  
AF067264  AF067277

Pseudaphya ferreri (de Buen & Fage 1908)  
EF218631  EF218646

Zebenus zebrus (Risso 1827)  
AF067266  AF067279

Zosterisessor ophiocephalus (Pallas 1814)  
EF218634  EF218649  AY884592

Haemulidae
Parapristipoma octolineatum (Valenciennes 1833)  
DQ197977  DQ197879  HQ676666

Plectorhinchus mediterraneus (Guichenot 1850)  
DQ197979  DQ197881

Pomadasys incisus (Bowdich 1825)  
EU410417  DQ197981  DQ197883  HQ676679

Pomadasys stridens (Forsskål 1775)  
HQ676685

Istiophoridae
Tetrapturus albidus Poey 1860  
DQ854632  DQ882009

Tetrapturus belone Rafinesque 1810  
DQ854640  DQ882010

Tetrapturus georgii Lowe 1841  
DQ854642  DQ882011

Labridae
Acantholabrus palloni (Risso 1810)  
AF517587  DQ197923  DQ197825

Centrolabrus exoletus (Linnaeus 1758)  
AF414200  AY092041

Coris julis (Linnaeus 1758)  
AJ810130  AY092042  AY328856  EU167885

Ctenolabrus rupestris (Linnaeus 1758)  
AJ810131  AF517586

Labrus merula Linnaeus 1758  
AJ810141  AF517592

Labrus viridis Linnaeus 1758  
AJ810142  AF517593

Lappanella fasciata (Cocco 1833)  
AF517589

Symphodus baillonii (Valenciennes 1839)  
AY092052  AY092037

Symphodus cinereus (Bonnaterre 1788)  
AJ810147  AY092036
| Species                                      | Accession Numbers |
|----------------------------------------------|-------------------|
| *Symphodus doderleini* 1890                  | AF517602          |
| *Symphodus mediterraneus* (Linnaeus 1758)    | AJ810148          |
| *Symphodus melanocercus* (Risso 1810)        | AF517595          |
| *Symphodus melops* (Linnaeus 1758)           | AF517601          |
| *Symphodus ocellatus* (Linnaeus 1758)        | AJ810150          |
| *Symphodus roissali* (Risso 1810)            | AY092039          |
| *Symphodus rostratus* (Bloch 1791)           | AY092040          |
| *Symphodus tinca* (Linnaeus 1758)            | AF517596          |
| *Thalassoma pavo* (Linnaeus 1758)            | AY328877.1        |
| *Xyrichtys novacula* (Linnaeus 1758)         | EF439246          |
| *Lutjanus argentimaculatus* (Forsskål 1775)  | DQ900672          |
| *Luvarus imperialis* Rafinesque 1810         | AB276966          |
| *Dicentrarchus labrax* (Linnaeus 1758)       | AY141370          |
| *Dicentrarchus punctatus* (Bloch 1792)       | AF247437          |
| *Chelon labrosus* (Risso 1827)               | DQ016292          |
| *Liza aurata* (Risso 1810)                   | EF437077          |
| *Liza ramado* (Risso 1827)                   | EF437079          |
| *Liza saliens* (Risso 1810)                  | EF437081          |
| *Mugil cephalus* Linnaeus 1758               | DQ225777          |
| *Oedalechilus labeo* (Cuvier 1829)           | Z71995            |
| Family          | Genus                  | Species                  | Authors          | Accession Numbers                  |
|-----------------|------------------------|--------------------------|------------------|------------------------------------|
| Mullidae        | Mullus                 | barbatus Linnaeus 1758   | EF439552         |                                    |
|                 |                        | surmuletus Linnaeus 1758 | EF439143         |                                    |
|                 | Upeneus                | moluccensis (Bleeker 1855)| AF227675         |                                    |
| Nomeida         | Psenes                 | pellucidus Lütken 1880   | AB205425         |                                    |
|                 |                        |                          | AB205447         |                                    |
|                 |                        |                          | AB205469         |                                    |
| Pinguipedidae   | Pinguipes              | brasilianus Cuvier 1829  | EU074542         |                                    |
| Polyprionidae   | Polyprion              | americanus (Bloch &      | AM158291         |                                    |
|                 |                        | Schneider 1801)          |                  |                                    |
| Pomacentridae   | Abudefduf              | vaigiensis (Quoy &       | AF436880         |                                    |
|                 |                        | Gaimard 1825)            |                  |                                    |
|                 | Chromis                | chromis (Linnaeus 1758)  | AF517577         |                                    |
|                 |                        |                          |                  |                                    |
| Pomatomidae     | Pomatomus              | saltatrix (Linnaeus 1766)| AF055612         |                                    |
| Priacanthidae   | Priacanthus             | hamrur (Forsskål 1775)  | DQ885115         |                                    |
|                 |                        |                          |                  |                                    |
| Rachycentridae  | Rachycentron           | canadum (Linnaeus 1766)  | DQ532949         |                                    |
| Scaridae        | Scarus                 | ghobban Forsskål 1775    | EF609452         |                                    |
|                 | Sparsisoma             | cretense (Linnaeus 1758) | SCU95777         |                                    |
|                 |                        |                          |                  |                                    |
| Sciaenidae      | Argyrosomus            | regius (Asso 1801)       | DQ197924         |                                    |
|                 | Umbrina                | canariensis Valenciennes 1843 | EF392637   |                                    |
|                 |                        | cirrosa (Linnaeus 1758)  | AF143198         |                                    |
| Scombridae      | Acanthocybium          | solandri (Cuvier 1832)   | DQ854648         |                                    |
|                 |                        | rochei (Risso 1810)      | AB176810         |                                    |
|                 | Euthynus               | alletteratus (Rafinesque 1810) | AB176806 |                                    |
|                 |                        | pelamis (Linnaeus 1758)  | AB176808         |                                    |

Accession numbers: EF439552, EF439143, DQ197965, EF095617, EF095658, AF227675, EU167747, AB205425, AB205447, AB205469, EU074542, AM158291, DQ107915, EF392605, EF427493, AF436880, DQ006016, AY208557, AF517577, AY208527, AY208640, AF055612, DQ080341, DQ080430, EU167741, DQ885115, DQ885111, EU167865, DQ532949, EF609446, AB292793, EU167910, SCU95777, AF517578, DQ198004, DQ197906, DQ457040, DQ854648, DQ874727, DQ835838, DQ080324, DQ874804, AB176810, DQ835852, DQ835838, DQ835838, DQ080311, DQ080400, AB176806, DQ874730, DQ835903, DQ080308, DQ80398, AB176808, DQ874729, DQ835922, DQ080315, DQ80410.
| Family   | Species                                      | Accession Numbers                      |
|----------|----------------------------------------------|----------------------------------------|
| Serranidae |  |                                            |                                        |
|          |  | **Rastrelliger kanagurta** (Cuvier 1816)   | DQ497857                               |
|          |  | **Sarda sarda** (Bloch 1793)               | DQ874691  DQ874723  DQ835917  DQ080300  DQ874800 |
|          |  | **Scomber japonicus** Houttuyn 1782         | AB241442  EF458394  EF433288  AB018996  AY141311 |
|          |  | **Scomber scombrus** Linnaeus 1758          | AB241438  DQ874720  DQ835839  DQ080334  DQ874797  EU477493 |
|          |  | **Scomberomorus commerson** (Lacepède 1800) | EF095579  EF095607  DQ107670  DQ497865  EF095634  EF095676 |
|          |  | **Scomberomorus tritor** (Cuvier 1832)     | AF231582  AF231539  AF231666 |
|          |  | **Thunnus alalunga** (Bonnaterre 1788)     | AB176804  DQ835820  DQ080289  DQ080389 |
|          |  | **Thunnus thynus** (Linnaeus 1758)         | AY507951  DQ835876  DQ080266  DQ080358 |
|          |  | **Epinephelus aeneus** (Geoffroy Saint-Hilaire 1817) | AY141367  AY947593  DQ197950  AY141291 |
|          |  | **Epinephelus caninus** (Valenciennes 1843) | AM158294  AY947585  AJ420204 |
|          |  | **Epinephelus coioides** (Hamilton 1822)   | AY947608  DQ107891  DQ354156 |
|          |  | **Epinephelus hafensis** (Ben-Tuvia 1853)  | AJ420207 |
|          |  | **Epinephelus malabaricus** (Bloch & Schneider 1801) | DQ067309  DQ107871  |
|          |  | **Epinephelus marginatus** (Lowe 1834)      | AM158299  AY947595  AB179759  DQ197854 |
|          |  | **Mycteroperca rubra** (Bloch 1793)        | AM158292  AY947587  DQ197969  DQ197871 |
|          |  | **Serranus atricauda** Günther 1874        | AM158286  DQ197999  EF439313 |
|          |  | **Serranus cabrilla** (Linnaeus 1758)      | AM158283  DQ198000  EF439445 |
|          |  | **Serranus hepatus** (Linnaeus 1758)       | AM158289  EF439586  EF439449 |
|          |  | **Serranus scriba** (Linnaeus 1758)        | AM158288  DQ198001  EF439451 |
| Siganidae |  | **Siganus luridus** (Rüppell 1829)         | DQ532959  DQ898056 |
| Family       | Species                                                   | GenBank Accession Numbers |
|-------------|-----------------------------------------------------------|--------------------------|
| Siganidae   | *Siganus rivulatus* Forsskål & Niebuhr 1775              | DQ898115, DQ898075       |
|             | *Sillago sihama* (Forsskål 1775)                         | EU257812, EU257202, EF607562, EU167874 |
| Sparidae    | *Boops boops* (Linnaeus 1758)                            | AF247396, DQ197932, EF439263, EU167763 |
|             | *Crenidens crenidens* (Forsskål 1775)                    | AF247397, AF240699        |
|             | *Dentex dentex* (Linnaeus 1758)                          | DQ532863, AF143197, EF427464 |
|             | *Dentex gibbosus* (Rafinesque 1810)                      | AJ247272, DQ197941, DQ197843 |
|             | *Dentex macrourus* (Bloch 1791)                          | AJ247273, EF392580, EF427466 |
|             | *Dentex macrourus* Valenciennes 1830                     | EU410413, DQ197942, DQ197844 |
|             | *Diplodus annularis* (Linnaeus 1758)                     | AJ247286, EF392581, EF427467 |
|             | *Diplodus bellottii* (Steindachner 1882)                 | AJ247288                 |
|             | *Diplodus cervinus* (Lowe 1838)                          | AF247420, AF240723, DQ197847 |
|             | *Diplodus puntazzo* (Walbaum 1792)                       | AJ247291, EF392585, EF427471 |
|             | *Diplodus sargus* (Linnaeus 1758)                        | AF365354, EF427554, DQ197848 |
|             | *Diplodus vulgaris* (Geoffroy Saint-Hilaire 1817)        | AJ247294, DQ197947, DQ197849 |
|             | *Lithognathus mormyrus* (Linnaeus 1758)                  | AF247410, AF240712, DQ197863, EU167782 |
|             | *Oblada melanura* (Linnaeus 1758)                        | AF247399, AF240701, EF439410, EU167786 |
|             | *Pagellus acarne* (Risso 1827)                           | AF247411, AF240713, DQ197872 |
|             | *Pagellus bellottii* Steindachner 1882                   | AF247412, DQ197971, DQ197873 |
|             | *Pagellus bogaraveo* (Brünnich 1768)                     | DQ197972, DQ197874        |
|             | *Pagellus erythrinus* (Linnaeus 1758)                    | AJ247284, DQ197973, EF439417, EU167790 |
| Scientific Name                                      | Linnaeus Year | Accession Numbers |
|-----------------------------------------------------|---------------|-------------------|
| *Pagrus auriga*                                     | 1843          | AY178433 AF247425  DQ197974 DQ197876 EU167788 |
| *Pagrus caeruleostictus*                            | (1830)        | AJ247276          DQ197975 DQ197877 EU167789 |
| *Pagrus pagrus*                                     | (1758)        | AY178431 AF247426  DQ197976 DQ197878 EU167791 |
| *Sarpa salpa*                                       | (1758)        | AF247402          DQ197992 EF439306 HQ676686 |
| *Sparus aurata*                                     | (1758)        | EF095565 AF247432  AF240735 EU224181 EF095657 |
| *Spondyliosoma cantharus*                           | (1758)        | AF247403          AF240705 EF439321 |
| *Sphyraena sphyraena*                               | (1758)        | AY141386 DQ532964  DQ080263 AY141312 |
| *Sphyraena viridens*                                | Cuvier 1829   |                   |
| *Sphyraenidae*                                      |               | AY141386 DQ532964  DQ080263 AY141312 |
| **Stromateidae**                                    |               |                  |
| *Pampus argenteus*                                  | Euphrasen 1788 | AY141383 AY141453  DQ107596 AY141309 |
| *Sphyraenidae*                                      |               |                  |
| *Tetragonurus cuvieri*                              | Risso 1810    | AB205429 AB205451  AB205473 |
| *Trachinidae*                                       |               |                  |
| *Echiichthys vipera*                                | Cuvier 1829   | EU492114 EU492019  |
| *Trachinus draco*                                   | Linnaeus 1758 | AY141378 AF518227  EF439610 AY141304 |
| *Trachinus radiatus*                                | Cuvier 1829   | DQ198015 EF439480  |
| **Trachinidae**                                     |               |                  |
| *Lepidopus caudatus*                                | Euphrasen 1788 | AF100917          DQ080261 DQ080352 |
| *Trichiurus lepturus*                               | Linnaeus 1758 | DQ874687 AB201821  EF607600 DQ364151 DQ874796 EU167903 |
| **Trachinidae**                                     |               |                  |
| *Tripterygion delaisi*                               | Cadenat & Blache 1970 | AY098809 AY098849  AJ872120 |
| *Tripterygion melanurus*                             | Guichenot 1850 | AJ868524 AJ872145  |
| *Tripterygion tripteronotus*                         | Risso 1810    | AF324198 AJ872130  |
| **Trachyuridae**                                    |               |                  |
| *Lepidopus caudatus*                                | Euphrasen 1788 | AF100917          DQ080261 DQ080352 |
| *Trichiurus lepturus*                               | Linnaeus 1758 | DQ874687 AB201821  EF607600 DQ364151 DQ874796 EU167903 |
| **Triterygiidae**                                   |               |                  |
| *Tripterygion delaisi*                               | Cadenat & Blache 1970 | AY098809 AY098849  AJ872120 |
| *Tripterygion melanurus*                             | Guichenot 1850 | AJ868524 AJ872145  |
| *Tripterygion tripteronotus*                         | Risso 1810    | AF324198 AJ872130  |
| **Trachyuridae**                                    |               |                  |
| *Lepidopus caudatus*                                | Euphrasen 1788 | AF100917          DQ080261 DQ080352 |
| *Trichiurus lepturus*                               | Linnaeus 1758 | DQ874687 AB201821  EF607600 DQ364151 DQ874796 EU167903 |
| **Uranoscopidae**                                   |               |                  |
| *Uranoscopus scaber*                                | Linnaeus 1758 | AF518213          DQ198017 EU036628  |
| **Xiphiidae**                                       |               |                  |
| *Xiphias gladius*                                   | Linnaeus 1758 | DQ854646 DQ874734  DQ107623 DQ080249 DQ874811  |
| Family         | Genus                     | Species Name                           | GenBank ID 1 | GenBank ID 2 | GenBank ID 3 | GenBank ID 4 |
|---------------|---------------------------|----------------------------------------|--------------|--------------|--------------|--------------|
| Pleuronectiformes | Bothidae                  | *Arnoglossus imperialis* (Rafinesque 1810) | AF542209     | AY359651     | AY141283     |
|               |                           | *Arnoglossus laterna* (Walbaum 1792)    | AF542210     | AY359653     | EU224096     |
|               |                           | *Arnoglossus thori* Kyle 1913          | AF542208     | AY157329     | AY029189     |
|               |                           | *Bothus podas* (Delaroche 1809)         | AF542221     | AY157326     | AF324334     | AY368313     |
| Citharidae    |                           | *Citharus linguatula* (Linnaeus 1758)   | AF542220     | AY157325     | EF439510     | AY141323     |
| Pleuronectidae |                           | *Platichthys flesus* (Linnaeus 1758)    | AB125244     | AY359670     | EU524278     | AB125334     | EU492025     |
|               |                           | *Pleuronectes platessa* Linnaeus 1758   | AF542207     | AY157328     | EU224075     | EU224175     |
| Scophthalmidae|                           | *Lepidorhombus boscii* (Risso 1810)     | AM931031     | DQ304652     | EF439534     | EF439124     |
|               |                           | *Lepidorhombus whiffiagonis* (Walbaum 1792) | AY998042     | DQ195533     | EF427570     | EF439125     |
|               |                           | *Psetta maxima* (Linnaeus 1758)         | AF517557     | AY359664     | AY164471     | EU224174     |
|               |                           | *Scophthalmus rhombus* (Linnaeus 1758)  | AY998044     | AY359665     | EF427597     | EF439439     |
| Soleidae      |                           | *Bathysolea profundicola* (Vaillant 1888) |              |              | AY359659     |              |
|               |                           | *Buglossidium luteum* (Risso 1810)      |              |              | EU492126     | EU492030     |
|               |                           | *Dicologlossa cuneata* (Moreau 1881)    | AB125241     | AY157321     | AB125331     | EF456044     |
|               |                           | *Microchirus azevia* (de Brito Capello 1867) | AB125238     | AY157318     | AB125329     | EF427488     |
|               |                           | *Microchirus boscanion* (Chabanaud 1926) | AB125239     | AY125250     | AB125330     |              |
|               |                           | *Microchirus hexophthalmus* (Bennett 1831) | AB125242     | AY125253     | AB125332     |              |
|               |                           | *Microchirus ocellatus* (Linnaeus 1758)  |              |              |              |              |
|               |                           | *Microchirus variegatus* (Donovan 1808)  | AF542218     | AY157327     | AF113198     |              |
|               |                           | *Pegusa impar* (Bennett 1831)           | AF542215     | AY141429     | EF427582     | AY141284     | AF113192     |
Pegusa lascaris (Risso 1810)  AB125234  AB125245  AB125325  EF427491
Solea aegyptiaca Chabanaud 1927  AF289718
Solea senegalensis Kaup 1858  AB125235  AY359661  AB125326  EF439167
Solea solea (Linnaeus 1758)  AF488492  AF488442  AB125327  EU224131  EF095644
Synaptura lusitanica de Brito Capello 1868  AB125243  AB125254  AB125333  EF439470
Synapturichthys kleinii (Risso 1827)  AB125237  AB125248  AB125328  EF439468

Scorpaeniformes  Cottidae

Taurulus bubalis (Euphrasen 1786)  AY141363  EU492317  EU492224

Scorpaenidae

Pontinus kuhlii (Bowdich 1825)  DQ197983  DQ197885
Pterois miles (Bennett 1828)  DQ125237  AJ429402  EU148593  EF209664
Scorpaena elongata Cadenat 1943  EF456020  EF456081
Scorpaena maderensis Valenciennes 1833  DQ197996  DQ197898
Scorpaena notata Rafinesque 1810  DQ125235  AF518222  DQ197997  DQ197899
Scorpaena porcus Linnaeus 1758  DQ125238  EF392615  EU036590
Scorpaena scrofa Linnaeus 1758  DQ125234  AF518223  EU036494  EF439442
Scorpaenodes arenai Torchio 1962  DQ125239

Sebastidae

Helicolenus dactylopterus (Delaroche 1809)  DQ125236  EU410418  EF609371  DQ197956  DQ197858
Trachyscorpia cristulata (Goode & Bean 1896)  AY538980

Triglidae

Chelidonichthys lucernus (Linnaeus 1758)  AY141362  EF120859  EF609323  EF427548  AY141287
Eutrigla gurnardus (Linnaeus 1758)  EF427560  EF439111
Lepidotrigla cavillone (Lacepède 1801)  EF439536  EF439389
**Stomiiformes**

**Gonostomatidae**

*Trigla lyra* Linnaeus 1758

*Trigloporus lastoviza* (Bonnaterre 1788)

*Cyclothone braueri* Jespersen & Tånig 1926

*Cyclothone pygmaea* Jespersen & Tånig 1926

*Gonostoma denudatum* Rafinesque 1810

**Phosichthyidae**

*Ichthyococcus ovatus* (Cocco 1838)

**Stomiidae**

*Chauliodus sloani* Bloch & Schneider 1801

*Stomias boa boa* (Risso 1810)

**Syngnathiformes**

**Centriscidae**

*Macroramphus scolopax* (Linnaeus 1758)

**Fistulariidae**

*Fistularia commersonii* Rüppell 1838

*Fistularia petimba* Lacepède 1803

**Syngnathidae**

*Entelurus aequoreus* (Linnaeus 1758)

*Hippocampus fuscus* Rüppell 1838

*Hippocampus hippocampus* (Linnaeus 1758)

*Hippocampus ramulosus* Leach 1814

*Nerophis ophidion* (Linnaeus 1758)
| Species                                      | GenBank Accession Numbers                                      |
|----------------------------------------------|----------------------------------------------------------------|
| Syngnathus abaster Risso 1827                | AF354959 AF355010 AF356060                                     |
| Syngnathus acus Linnaeus 1758                 | AF354940 AF354991 AF356040                                     |
| Syngnathus rostellatus Nilsson 1855          | AF354941 AF354992 AF356041                                     |
| Syngnathus taenionotus Canestrini 1871       | AF354960 AF355011 AF356061                                     |
| Syngnathus typhle Linnaeus 1758              | AF354960 AF354992 AF356041                                     |
| Capros aper (Linnaeus 1758)                  | EF095553 DQ532846 EU148107 AP009159 AY141262 EF095638         |
| Mola mola (Linnaeus 1758)                    | AY700258 DQ532911 AP006238 AY940835 AF137215 EF095643         |
| Ranzania laevis (Pennant 1776)               | AP006047 AP006047 DQ521011 EF392608 EF427496                  |
| Lagocephalus sceleratus (Gmelin 1789)        | AB194240 EF362414                                            |
| Lagocephalus spadiceus (Richardson 1845)     | EF60741 9                                                  |
| Sphoeroides pachyaster (Müller & Troschel 1848) | AP006745 AB194239 EU074598 EF392642 EF427517         |
| Sphoeroides spengleri (Bloch 1785)          | AY700284 AY679668 AY700354                                     |
| Takifugu rubripes (Temminck & Schlegel 1850) | NC_004299.1 NC_004299.1 NC_004299.1 NC_004299.1 AF137214.1 AY700363 |
| Tetraodon nigroviridis Marion de Procé 1822 | NC_007176.1 NC_007176.1 NC_007176.1 NC_007176.1 AJ293018.1 |
| Zeus faber Linnaeus 1758                     | AF149993 DQ027916 EF609496 DQ198019 EF439493 FJ215202         |

For each species represented in the phylogeny we have listed the GenBank accession number of each gene used in the phylogenetic analysis. An empty cell represents a gene that was not included in the analysis. Species names, corresponding name authorities and classification follow FishBase version 02/2011 (http://www.fishbase.org/).
Appendix 2 Summary of gene representation and saturation in the phylogenetic analysis.

In this appendix we provide a summary of representation for each gene, as well as an analysis of saturation by gene.

*Gene representation*

Even though the percent of species represented solely by mitochondrial genes is large, more than half of the species in the phylogeny are represented by some combination of nuclear and mitochondrial genes (Table A2.1). The least represented gene is RAG1 with 80 species, followed by COXI, with 118 species (Figure A2.1). The best represented gene is 16S, with 265 species (Figure A2.1). The phylogeny contains a total of 373 species, so these numbers correspond to a minimum of 21 % and a maximum of 71 % respectively. Moreover, whereas 16% of the species are represented by only 1 gene, and 5 % are represented by all 6 genes, the vast majority are represented by at least 2 genes (84 %) (Figure A2.2).

**Table A2.1 Number of cases and corresponding percent (based on the total number of species in the phylogeny) where the species was represented by nuclear versus mitochondrial genes.**

| Model                                      | Number of species | Percent |
|--------------------------------------------|-------------------|---------|
| Only mitochondrial genes                   | 154               | 41.3    |
| Only nuclear genes                         | 13                | 3.5     |
| Some combination of nuclear and mitochondrial genes | 206               | 55.2    |
| Only 1 nuclear gene                        | 175               | 46.9    |
| Both RAG1 and RHOD genes                   | 44                | 11.8    |
Figure A2.1
Number of species represented for each gene (based only on the 373 species represented in the phylogeny).

- **12S**: 152 represented, 221 not represented
- **16S**: 108 represented, 265 not represented
- **COXI**: 255 represented, 118 not represented
- **CytB**: 138 represented, 235 not represented
- **RHOD**: 190 represented, 183 not represented
- **RAG1**: 293 represented, 80 not represented
Figure A2.2
Number of species represented by 1, 2, 3, 4, 5 or 6 genes (based on the 373 species represented in the phylogeny), irrespective of whether they are nuclear or mitochondrial.

Saturation information by gene

Here we compared saturation of the nucleotide substitutions in the two nuclear recombination activating gene 1 (RAG1) and rhodopsin (RHO) markers and 4 mitochondrial cytochrome b (CYB), 12S rRNA, 16S rRNA, and cytochrome c oxidase subunit 1 (COX1) markers when inferring the phylogeny of Mediterranean teleosts.

To evaluate whether the slower-evolving RAG1 and RHO and the faster-evolving CYB, 12S rRNA, 16S rRNA, and COX1 saturated when reconstructing the teleost phylogeny, we constructed saturation-plots of the number of maximum likelihood inferred substitutions between any pair of taxa (i.e., patristic distances measured on the highest-likelihood phylogram reconstructed from each of the 6 alignments) against the corresponding observed (apparent) number of nucleotide differences in the 6 alignments. The slope of the regression lines for example suggest that the saturation level of the RAG1 marker is moderate, whereas the COX1 display stronger saturation. The former will provide phylogenetic information for deeper nodes in the Mediterranean teleost tree, whereas the latter will provide information for terminal nodes.
Saturation plot of the RAG1 marker.
Dashes correspond to the regression line through the origin (slope = 0.52).

Saturation plot of the RHO marker.
Dashes correspond to the regression line through the origin (slope = 0.26).

Saturation plot of the CYB marker.
Dashes correspond to the regression line through the origin (slope = 0.28).

Saturation plot of the 12S rRNA marker.
Dashes correspond to the regression line through the origin (slope = 0.28).
Saturation plot of the 16SrRNA marker.
Dashes correspond to the regression line through the origin (slope = 0.16).

Saturation plot of the COX1 marker.
Dashes correspond to the regression line through the origin (slope = 0.09).

The straight line indicates the absence of saturation, i.e., the situation for which the number of inferred substitutions is equal to the number of observed differences in the alignment. Note the difference of X-axis scale between the six plots.
Appendix S3: Species attached to their most recent common ancestor (MRCA). Table S3.1 shows a summary of the level at which the species was attached to the raw chronogram, whereas Table S3.2 shows the list of species attached. Name authorities were taken from FishBase v02/2011.

Table S3.1 Summary of number of species attached to the raw chronogram.

|                     | At least two congeners (C2) | At least one congener (C1) | Family | Order | Total |
|---------------------|----------------------------|---------------------------|--------|-------|-------|
| All Species         | 33                         | 10                        | 57     | 24    | 124   |
| Endemics            | 9                          | 2                         | 16     | 4     | 31    |
| Non-endemic natives | 16                         | 6                         | 28     | 12    | 62    |
| Exotics             | 8                          | 2                         | 13     | 8     | 31    |
| Order          | Family           | Species Name                                  | Status | Attachment Level |
|---------------|------------------|-----------------------------------------------|--------|------------------|
| Anguilliformes| Chlopsidae       | *Chlopsis bicolor* Rafinesque 1810            | Native | O                |
|               | Congridae        | *Ariosoma balearicum* (Delaroche 1809)        | Native | O                |
|               |                  | *Conger conger* (Linnaeus 1758)               | Native | O                |
|               |                  | *Gnathophis mystax* (Delaroche 1809)          | Native | O                |
|               | Heterenchelyidae | *Panturichthys fowleri* (Ben-Tuvia 1953)      | Endemic| O                |
|               | Ophichthidae     | *Apterichtus anguiformis* (Peters 1877)        | Native | F                |
|               |                  | *Apterichtus caecus* (Linnaeus 1758)          | Native | F                |
|               |                  | *Dalophys imberbis* (Delaroche 1809)          | Native | F                |
|               |                  | *Ophichthus rufus* (Rafinesque 1810)          | Endemic| F                |
|               |                  | *Ophisurus serpens* (Linnaeus 1758)           | Native | F                |
|               | Synaphobranchidae| *Dysomma brevirostre* (Facciolà 1887)         | Native | O                |
| Aulopiformes  | Evermannellidae  | *Evermannella balbo* (Risso 1820)             | Native | O                |
|               | Paralepididae    | *Paralepis speciosa* Belloti 1878             | Endemic| F                |
| Beloniformes  | Exocoetidae      | *Cheilopogon furcatus* (Mitchill 1815)        | Exotic | F                |
|               |                  | *Cheilopogon heterurus* (Rafinesque 1810)     | Native | F                |
|               |                  | *Exocoetus obtusirostris* Günther 1866        | Native | F                |
|               |                  | *Parexocoetus mento* (Valenciennes 1847)      | Exotic | F                |
| Clupeiformes  | Clupeidae        | *Hyrporhamphus picarti* (Valenciennes 1847)   | Native | C1               |
| Gadiformes    | Macrouridae      | *Nezumia sclerorhynchus* (Valenciennes 1838)  | Native | F                |
|               | Moridae          | *Eretmophorus kleinembergi* Giglioli 1889     | Native | F                |
|               |                  | *Gadella maraldi* (Risso 1810)                | Native | F                |
|               |                  | *Lepidon guentheri* (Giglioli 1880)           | Exotic | F                |
|               |                  | *Lepidon lepidion* (Risso 1810)               | Endemic| F                |
|               |                  | *Physiculus dalwigki* Kaup 1858               | Native | F                |
| Phylogenetic Group | Family | Species Name | Date of Description | Origin | Category |
|--------------------|--------|--------------|---------------------|--------|----------|
| Lophiiformes       | Chaunacidae | Chaunax pictus Lowe 1846 | Exotic | O |
| Mugiliformes       | Mugilidae  | Liza carinata (Valenciennes 1836) | Exotic | C2 |
| Myctophiformes     | Myctophidae | Diaphus holti Tåning 1918 | Native | C2 |
|                    |         | Lampanyctus crocodilus (Risso 1810) | Native | F |
|                    |         | Lampanyctus pusillus (Johnson 1890) | Native | F |
|                    |         | Notoscopelus elongatus (Costa 1844) | Endemic | C1 |
| Myctophiformes     | Myctophidae | Diaphus holti Tåning 1918 | Native | C2 |
| Osmeriformes       | Alepocephalidae | Alepocephalus rostratus Risso 1820 | Native | O |
|                    | Argentinidae | Glossanodon leioGLOSSUS (Valenciennes 1848) | Native | F |
|                    | Microstomatidae | Nansenia iberica Matallanas 1985 | Endemic | O |
|                    | Microstomatidae | Nansenia oblata (Facciolà, 1887) | Native | O |
|                    | Microstomatidae | Microstoma microstoma (Risso 1810) | Native | O |
| Perciformes        | Apogonidae | Apogon pharaonis (Belloti 1874) | Exotic | C1 |
|                    | Blenniidae | Hypleurochilus bananensis (Poll 1959) | Native | F |
|                    | Blenniidae | Salaria basilisca (Valenciennes 1836) | Endemic | C1 |
|                    | Callionymidae | Callionymus fasciatus Valenciennes 1837 | Native | C2 |
|                    | Callionymidae | Callionymus filamentosus Valenciennes 1837 | Exotic | C2 |
|                    | Callionymidae | Callionymus pusillus Delaroche 1809 | Native | C2 |
|                    | Callionymidae | Callionymus risso Lesueur 1814 | Native | C2 |
|                    | Callionymidae | Synchiropus phaeton ( Günther 1861) | Native | F |
|                    | Carangidae | Campogramma glaycos (Lacepède 1801) | Native | F |
|                    | Carangidae | Nau cares ductor (Linnaeus 1758) | Native | F |
|                    | Centracanthidae | Centracanthus cirrus Rafinesque 1810 | Native | F |
|                    | Centrolophidae | Schedophilus medusophagus (Cocco 1839) | Native | F |
|                    | Echeneidae | Remora brachyptera (Lowe 1839) | Native | C2 |
|                    | Epigonidae | Epigonus denticulatus Dieuzeide 1950 | Native | C2 |
|                    | Epigonidae | Microichthys cocii Rüppell 1852 | Endemic | F |
|                    | Epigonidae | Microichthys sanzoi Sparta 1950 | Endemic | F |
|                    | Gobiidae | Buenia jeffreysii ( Günther 1867) | Native | C1 |
|                    | Gobiidae | Chromogobius quadrivittatus (Steindachner 1863) | Endemic | F |
|                    | Gobiidae | Chromogobius zebratus ( Kolombatovic 1891) | Endemic | F |
Corcyrogobius liechtensteini (Kolombatovic 1891)  Endemic  F
Deltentosteus collonianus (Risso 1820)  Native  F
Deltentosteus quadrimaculatus (Valenciennes 1837)  Native  F
Didogobius bentuvi Miller 1966  Endemic  F
Didogobius schlieweni Miller 1993  Endemic  F
Didogobius splechtnai Ahnelt & Patzner 1995  Endemic  F
Gammogobius steinitzi Bath 1971  Endemic  F
Gobius ater Bellotti 1888  Endemic  C2
Gobius couchi Miller & El-Tawil 1974  Exotic  C2
Gobius fallax Sarato 1889  Endemic  C2
Gobius geniporus Valenciennes 1837  Endemic  C2
Gobius roulei de Buen 1928  Native  C2
Gobius strictus Fage 1907  Endemic  C2
Gobius vittatus Vinciguerra 1883  Endemic  C2
Lebetus guilleti (Le Danois 1913)  Native  F
Millerigobius macrocephalus (Kolombatovic 1891)  Endemic  F
Monishia ochetica (Norman 1927)  Exotic  F
Odondebuenia balearica (Pellegrin & Fage 1907)  Endemic  F
Oxyurichthys papuensis (Valenciennes 1837)  Exotic  F
Pomatoschistus bathi Miller 1982  Endemic  C2
Pomatoschistus tortonesei Miller 1969  Endemic  C2
Silhouettea aegyptia (Chabanaud 1933)  Exotic  F
Speleogobius trigloides Zander & Jelinek 1976  Endemic  F
Thorogobius ephippiatus (Lowe 1839)  Native  F
Thorogobius macrolepis (Kolombatovic 1891)  Endemic  F
Vanneaugobius pruvoti (Fage 1907)  Native  F

Labridae  Pteragogus pelycus Randall 1981  Exotic  F
Mullidae  Pseudupeneus prayensis (Cuvier 1829)  Exotic  F
Nomeidae  Cubiceps capensis (Smith 1845)  Native  F
| Family          | Species                                      | Author               | Status | Code |
|-----------------|----------------------------------------------|----------------------|--------|------|
| Sciaenidae      | *Sciaena umbra* Linnaeus 1758                | Native               | F      |
|                 | *Umbrina ronchus* Valenciennes 1843          | Native               | C2     |
| Scombridae      | *Orcynopsis unicolor* (Geoffroy Saint-Hilaire 1817) | Native               | F      |
| Serranidae      | *Anthias anthias* (Linnaeus 1758)            | Native               | F      |
|                 | *Epinephelus alexandrinus* (Forsskål 1775)  | Native               | C2     |
| Sparidae        | *Rhabdosargus haffa* (Forsskål 1775)         | Exotic               | F      |
| Sphyraenidae    | *Sphyraena chrysotaenia* Klunzinger 1884     | Exotic               | C2     |
|                 | *Sphyraena flavicauda* Rüppell 1838          | Exotic               | C2     |
| Trachinidae     | *Trachinus araneus* Cuvier 1829              | Native               | C2     |
| Pleuronectiformes | *Arnoglossus kessleri* Schmidt 1915          | Endemic              | C2     |
|                 | *Arnoglossus rueppellii* (Cocco 1844)        | Endemic              | C2     |
| Cynoglossidae   | *Cynoglossus sinasarabici* (Chabanaud 1931)  | Exotic               | O      |
|                 | *Symphurus ligulatus* (Cocco 1844)           | Native               | O      |
|                 | *Symphurus nigrescens* Rafinesque 1810       | Native               | O      |
| Pleuronectidae  | *Platichthys flesus* (Linnaeus 1758)         | Native               | C2     |
| Soleidae        | *Pegusa nasuta* (Pallas 1814)                | Native               | C2     |
| Scorpaeniformes | *Eutelicthys leptochirus* Tortonese 1959     | Endemic              | O      |
|                 | *Paraliparis murieli* Matallanas 1984        | Endemic              | O      |
| Liparidae       | *Peristedion cataphractum* (Linnaeus 1758)   | Native               | O      |
| Peristediidae   | *Papilloculiceps longiceps* (Cuvier 1829)    | Exotic               | O      |
|                 | *Platycephalus indicus* (Linnaeus 1758)      | Exotic               | O      |
|                 | *Sorsogona prionota* (Sauvage 1873)         | Exotic               | O      |
| Scorpaenidae    | *Scorpaena lopeii* Cadenat 1943              | Native               | C2     |
|                 | *Scorpaena stephanica* Cadenat 1943          | Exotic               | C2     |
| Triglidae       | *Lepidotrigla dieuzeidei* Blanc & Hureau 1973 | Native               | C1     |
| Stomiiformes    | *Vinciguerria attenuata* (Cocco 1838)        | Native               | C1     |
| Phosichthyidae  | *Valenciennellus tripunctulatus* (Esmark 1871) | Native               | F      |
| Sternopychidae  | *Bathophilus nigerrimus* Giglioli 1882       | Native               | F      |
| Stomiidae       | *Nerophis maculatus* Rafinesque, 1810        | Native               | C1     |
| Syngnathiformes | *Minyichthys sentus* Dawson, 1982            | Native               | F      |
Species grafted into the final chronogram next to their nearest closest relative for the diversification analyses. Status: species were classified as endemic, (non-endemic) native or exotic. Attachment level: species were attached to a congener if there were at least two congeners present in the phylogeny (C2); if there was only one congener present in the phylogeny (C2), they were attached to the nearest node joining the congener and the closest species in the phylogeny; if no congener was present, the new species was attached to the most recent common ancestor of the same family (F) or of the same order (O), i.e. to the node joining all members of the same family or order. Each one of these attachments levels was carried out sequentially one after the other, in four different and increasingly more species rich chronograms. Species names, the corresponding name authorities and classification follow FishBase version 02/2011 (http://www.fishbase.org/).