A Phylogenetic Perspective on the Evolution of Mediterranean Teleost Fishes

Christine N. Meynard[1,2]*¤, David Mouillot[3], Nicolas Mouquet[1], Emmanuel J. P. Douzery[1]

1 Institut des Sciences de l'Evolution, UMR 5554-CNRS-IRD, Université de Montpellier II, Place Eugene Bataillon, CC065, Montpellier, France, **2** INRA, UMR CBGP (INRA/IRD/Cirad/Montpellier SupAgro), Campus international de Baillarguet, CS 30016, Montferrier-sur-Lez, France, **3** Ecosystèmes Lagunaires, UMR 5119 CNRS-UM2-IFREMER-IRD, Place Eugene Bataillon, Montpellier, France

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 grafted 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 clade 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.

Citation: Meynard CN, Mouillot D, Mouquet N, Douzery EJP (2012) A Phylogenetic Perspective on the Evolution of Mediterranean Teleost Fishes. PLoS ONE 7(5): e36443. doi:10.1371/journal.pone.0036443

Editor: Michael Knapp, University of Otago, New Zealand

Received January 28, 2012; **Accepted** April 4, 2012; **Published** May 8, 2012

Funding: This work has been supported by the bioinformatics cluster of the Institut des Sciences de l'Evolution de Montpellier (ISE-M), the computer grid of University Montpellier 2, by the Agence Nationale de la Recherche "Domaines Emergents" (ANR-08-EMER-011 "PhylAriane"), "Biologie Systémique" (BIOSYS06_136906 "MITOSYS"), and "6ème extinction" (ANR-09-PEXT-01102 "EVORANGE"), by two projects from the "Fondation pour la Recherche sur la Biodiversité" (FABIO and BIODIVMED), and by the "Fondation TOTAL." DM received funding from the "Institut Universitaire de France." The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have the following interest: Nicolas Mouquet is currently an editor with PLoS ONE. This does not alter the authors' adherence to all the PLoS ONE policies on sharing data and materials.

* E-mail: Christine.Meynard@supagro.inra.fr

¤ Current address: INRA, UMR CBGP (INRA/IRD/Cirad/Montpellier SupAgro), Campus international de Baillarguet, CS 30016, Montferrier-sur-Lez, France

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 [8] (see alsoFishBase, 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 COX1, 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+Γ+I and 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 *Spicara* (Centracanthidae, Perciformes) is genuinely included within the Sparidae [34], and that the Echeneidae are nested within the Carangidae [22] we did not constrained 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

Table 1. Nodes used for calibration in the phylogeny.

Node Number	Name of clade	Time constraints	Reference
1	Notacanthidae vs Anguilliformes	L94	[43,69]
2	Anguilliformes	L50	[43]
3	Clupeiformes	L57	[43]
4	Zebrafish 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	Soleidae, 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.
doi:10.1371/journal.pone.0036443.t001

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+Γ+I 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 (Γ) 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 3.2e (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.,

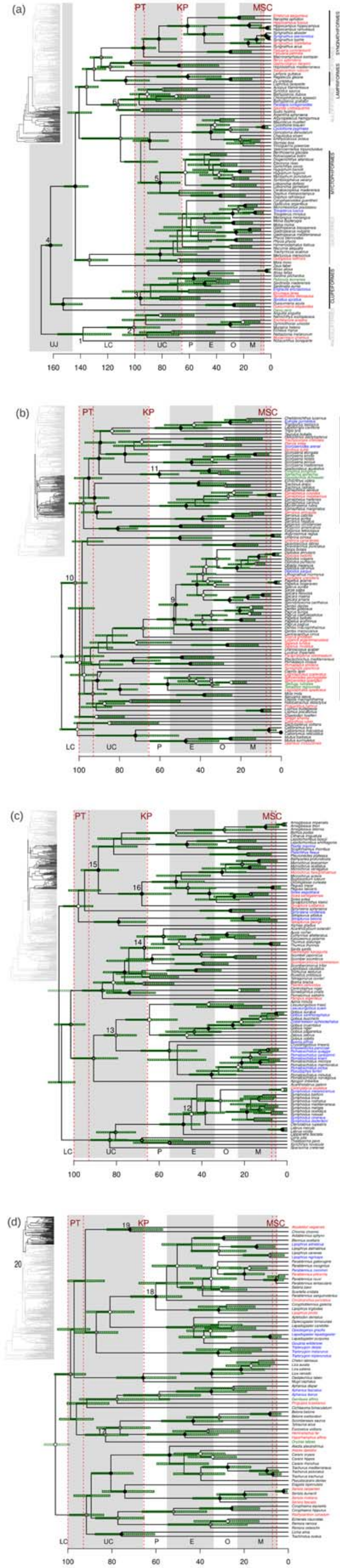


Figure 1. Dated phylogeny of Mediterranean teleost fishes. Phylogenetic relationships and divergence time estimates of Mediterranean teleosts, inferred from a supermatrix of 6 mitochondrial and nuclear genes. The global phylogeny is given on the upper left, with black branches indicating the part of the tree that is represented on the right panel. Color codes on species names indicate the origin of the species: green = extra-Mediterranean species; red = exotic species; blue = endemic species; black = non-endemic native. The green dashed boxes around nodes indicate the 95% credibility interval for the estimated node age. Maximum likelihood node bootstrap support is indicated using different types of circles: back circle = >90%, double circle = 70–90%, single white circle = 50–70%, no circle = <50%. Letters at the bottom indicate geologic time references: UJ = Upper Jurassic; LC = Lower Cretaceous; UC = Upper Cretaceous; P = Paleocene; E = Eocene; O = Oligocene; M = Miocene. Numbers in the phylogeny correspond to the calibration described in Table 1. Note that node 20 appears on the left panel of Figure 1d as this node links Figure 1b and 1d. We also show with dashed red lines important biogeographic events: peak temperatures (PT) that occurred during the Cenomanian (93–100 Mya); the Cretaceous-Paleogene (KP) mass extinction some 65 Mya, and the Messinian salinity crisis (MSC) some 7–5 Mya. On the right hand side, names in upper case correspond to teleost orders while names in lower case correspond to families. For clarity, names alternate between black and grey.
doi:10.1371/journal.pone.0036443.g001

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).

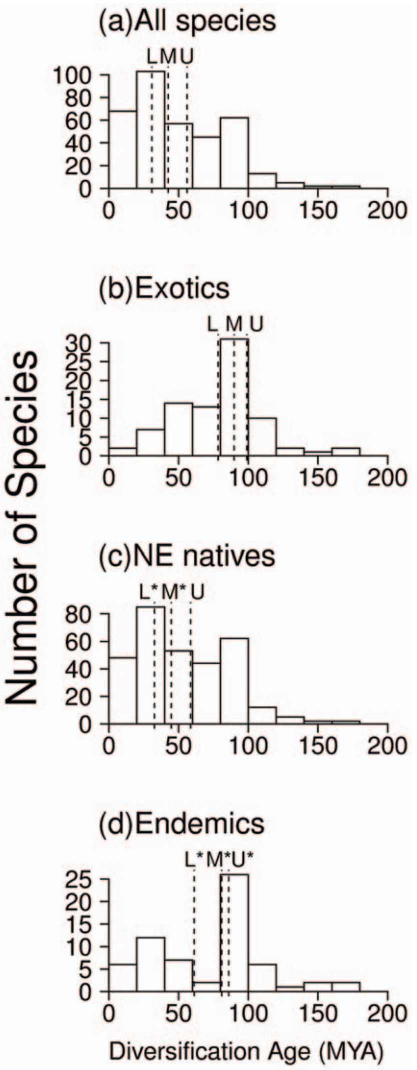


Figure 2. Histograms of the ages of the diversification events. Histograms are shown for (a) all species found in the Mediterranean Sea, (b) exotics only, (c) non-endemic natives, and (d) endemics. Dashed lines indicate the median based on the mean (M) node age estimates as well as based on the lower (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 number of species from the global chronogram ($P<0.05$, two-tailed bootstrap test).
doi:10.1371/journal.pone.0036443.g002

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 of 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).

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 Sparidae [34]. Within Carangidae, the monophyly of the tribes Carangini and Naucratini [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 the Cretaceous (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 *Abudefduf vaigiensis* 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 56–31 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

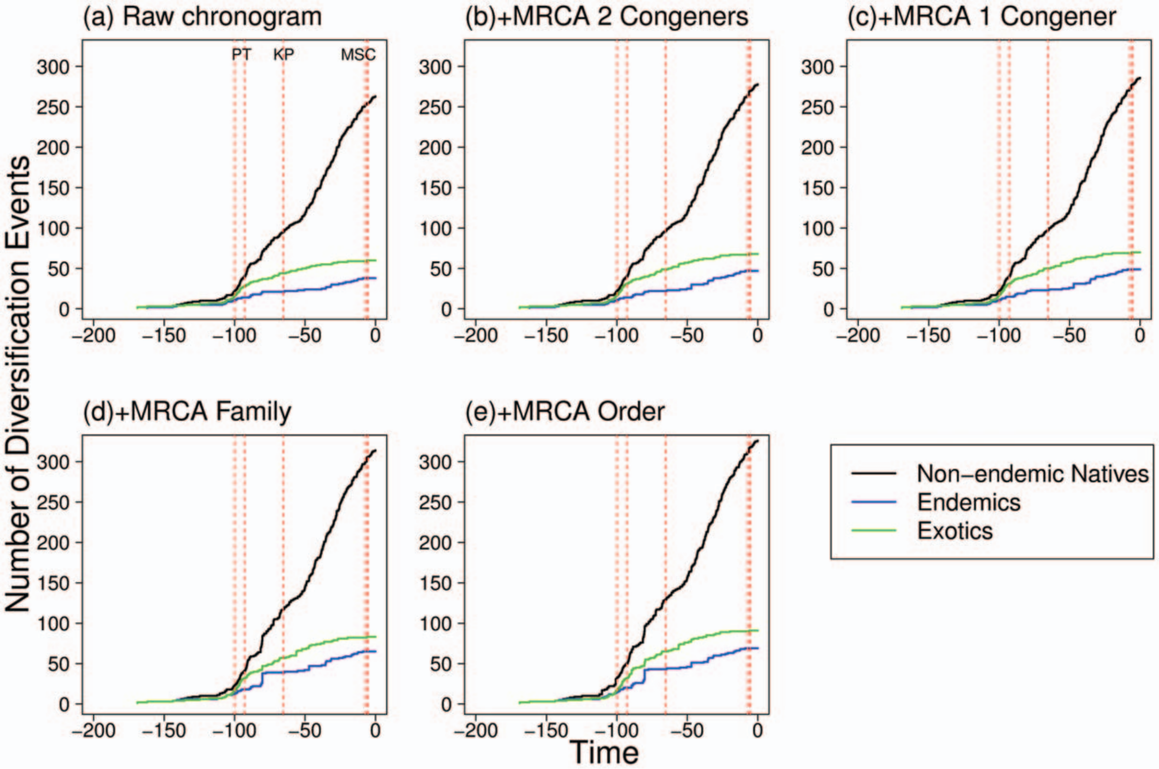


Figure 3. Lineage-through-time plots. Shown for (a) the backbone raw dated phylogeny; (b) adding species represented by at least two congeners in the backbone; (c) adding species represented by at least one congener; (d) adding species represented by a member of its family and (e) adding species represented by a member of its order. We also show with dashed red lines important biogeographic events: PT = peak temperatures during the Cenomanian (93–100 Mya); KP = Cretaceous-Paleogene mass extinction some 65 Mya; MSC = Messinian Salinity Crisis (7–5 Mya).
doi:10.1371/journal.pone.0036443.g003

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 representatives (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. Furthermore, the mitochondrial and nuclear DNA supermatrix of ~4,300 unambiguously aligned sites combined genes with contrasted evolutionary dynamics. This likely provided phylogenetic 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 phylogenetic 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 20 calibrations. While 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 not 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 correspond to the MSC, which occurred at around 6 Mya. In fact, natives showed an older diversification peak at 100–80 Mya, and a peak at 40–20 Mya. Lineage-through-time plots (Figure 3) suggest that between these periods of time the number of clades been originated 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 6 Mya. However they both support two important diversification events for native species (100 Mya and 40 Mya), while only one relevant diversification event for exotics (100 Mya).

According to our diversification estimates, the deepest clades of Mediterranean teleosts would have originated roughly 160 Mya, the Anguilliformes having originated 100 Mya (confidence interval: 120–81 Mya) and the node between Clupeiformes + Danio and the rest of euteleosts been placed at 160 Mya (confidence interval: 166–154 Mya) (Figure 1a). This would place the origin of Mediterranean teleosts shortly after the origin of teleosts globally. Actually, Santini *et al.* [15] found that teleost diversification occurred some 193 Mya (with confidence intervals between 173 and 214 Mya). The first fossil record for teleosts dates back to the upper Jurassic at 151 Mya [4,57], which is slightly more recent than our molecular estimate for the origin of Mediterranean teleosts. In contrast, some of the molecular calibrations based on mitogenomic data would place the origin of teleosts much earlier, up to 326 Mya [10,19,58]. This would imply that Mediterranean teleosts could have a much more recent origin as compared to the global pool, which would also translate into major orders and families originating later. As discussed earlier [4], there is no independent fossil or climatological event that would suggest such an early origin of teleosts. Furthermore, a recent analysis of fish fossil skeletons and otoliths also supports the idea that there is no evidence for such an early origin of teleost fish worldwide [3].

At least two factors may explain the difference in molecular estimates mentioned above [4,15]. First, previous dating efforts have used a mixed calibration strategy, where the minimum bound was set by the fossil record, whereas the maximum bound incorporated previous molecular estimates. This strategy can have a detrimental effect on the quality of divergence time estimates [59]. Second, studies that have used only mitochondrial genes tend to find older node estimates than those based on nuclear genes [4]. This may be due to differences in substitution rates between nuclear and mitochondrial genes. Here we used a combination of two nuclear and 4 mitochondrial genes analysed under a mixture model to mitigate the relative effects of both types of markers, and we have calibrated our tree based only on fossil records, potentially reducing the pitfalls mentioned above. More importantly, our timeline seems to be in closer agreement with the fossil record and morphological diversification studies [3]. For example, Figure 1 shows that several clades such as Tetraodontiformes, Aulopiformes, Myctophiformes, Clupeiformes and Anguilliformes originated and diversified during the Cretaceous. This is consistent with recent analyses of the fossil record at the global level [3,60,61,62] as well as within the Tethys Sea [60]. In all cases, important radiation events have been observed during the Cretaceous, coinciding with the chronology shown in Figure 1 for the diversification of major fish orders. Such radiations were associated to a global increase in sea surface temperature at the beginning of the Cretaceous, presumably allowing for the evolution and emergence of new clades, followed by the massive extinction at the Cretaceous/Paleogene boundary, which may have triggered the actual diversification and occupation of newly available empty niches [62]. The peak in diversification events after 100 Mya, which can be seen in all fish groups (Figures 2 and 3) is therefore consistent with the idea that high sea level as well as high temperatures during this period of time created new opportunities for speciation and diversification [3,61,62]. The fact that native as well as exotic species show this peak (Figure 2) suggests that these global changes that occurred well before the closing of the Mediterranean Sea, had important consequences for the origin of Mediterranean diversity as well. This is also supported by the origination of major Perciformes families during the Paleocene, coinciding with the major morphological diversification of teleosts [3].

Native species originated mostly during the last 50 Mya (Figure 2c–d), by a process that seemed to have started before the MSC. By the beginning of this period the African, Arabic and Eurasian plates were coming closer together, and water flow was slowly stagnating to form what is today the Mediterranean Sea, and 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 Meditarranean 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 faunal fossils 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.
(DOC)

Appendix S2 Gene representation and saturation in the phylogenetic analysis.
(DOC)

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

Acknowledgments

We are greatly thankful to Ylenia Chiari for useful comments on earlier versions of this manuscript. We are also in debt with David M. Kaplan for giving us access to different clusters, and with Laure Velez for adding name authorities in the appendices. This publication is contribution No 2012-004 of the Institut des Sciences de l'Evolution de Montpellier (UMR 5554 – CNRS-IRD).

Author Contributions

Conceived and designed the experiments: CNM NM DM. Performed the experiments: CNM EJPD. Analyzed the data: CNM EJPD. Wrote the paper: CNM NM DM EJPD. Provided Mediterranean fish database: DM.

References

1. Coll M, Piroddi C, Steenbeek J, Kaschner K, Lasram FB, et al. (2010) The biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. Plos One 5, e11842.
2. Lasram FB, Guilhaumon F, Mouillot D (2009) Fish diversity patterns in the Mediterranean Sea: deviations from a mid-domain model. Marine Ecology-Progress Series 376: 253–267.
3. Friedman M (2010) Explosive morphological diversification of spiny-finned teleost fishes in the aftermath of the end-Cretaceous extinction. Proceedings of the Royal Society B-Biological Sciences 277: 1675–1683.
4. Hurley IA, Mueller RL, Dunn KA, Schmidt EJ, Friedman M, et al. (2007) A new time-scale for ray-finned fish evolution. Proceedings of the Royal Society B-Biological Sciences 274: 489–498.
5. Bianchi CN, Morri C (2000) Marine biodiversity of the Mediterranean Sea: Situation, problems and prospects for future research. Marine Pollution Bulletin 40: 367–376.
6. Lejeusne C, Chevaldonné P, Pergent-Martini C, Boudouresque CF, Pérez T (2010) Climate change effects on a miniature ocean: the highly diverse, highly impacted Mediterranean Sea. Trends in Ecology and Evolution 25: 250–260.
7. Pinardi N, Massetti E (2000) Variability of the large scale general circulation of the Mediterranean Sea from observations and modelling: a review. Palaeogeography, Palaeoclimatology, Palaeoecology 158: 153–173.
8. Nelson JS (2006) Fishes of the world. New Jersey, USA: John Wiley & Sons.
9. Stiassny ML, Wiley EO, Johnson GD, de Carvahlo MR (2004) Gnathostome Fishes. In: Cracraft J, Donoghue MJ, eds. Assembling the tree of life. New York, USA: Oxford University Press.
10. Miya M, Takeshima H, Endo H, Ishiguro NB, Inoue JG, et al. (2003) Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 26: 121–138.
11. Giovannotti M, Cerioni PN, La Mesa M, Caputo V (2007) Molecular Phylogeny of the three paedomorphic Mediterranean gobies (Perciformes: Gobiidae). Journal of Experimental Zoology Part B-Molecular and Developmental Evolution 308B: 722–729.
12. De la Herran R, Rejon CR, Rejon MR, Garridos-Ramos MA (2001) The molecular phylogeny of the Sparidae (Pisces, Perciformes) based on two satellite DNA families. Heredity 87: 691–697.

13. Orrell TM, Carpenter KE (2004) A phylogeny of the fish family Sparidae (porgies) inferred from mitochondrial sequence data. Molecular Phylogenetics and Evolution 32: 425–434.
14. Westneat MW, Alfaro ME (2005) Phylogenetic relationships and evolutionary history of the reef fish family Labridae. Molecular Phylogenetics and Evolution 36: 370–390.
15. Santini F, Harmon LJ, Carnevale G, Alfaro ME (2009) Did genome duplication drive the origin of teleosts? A comparative study of diversification in ray-finned fishes. BMC Evolutionary Biology 9, 194.
16. Lasram FB, Mouillot D (2009) Increasing southern invasion enhances congruence between endemic and exotic Mediterranean fish fauna. Biological Invasions 11: 697–711.
17. R Development Core Team (2011) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org.
18. Lecointre G, Philippe H, Lê HLV, Le Guyader H (1993) Species sampling has a major impact on phylogenetic inference. Molecular Phylogenetics and Evolution 2: 205–224.
19. Miya M, Kawaguchi A, Nishida M (2001) Mitogenomic exploration of higher teleostean phylogenies: A case study for moderate-scale evolutionary genomics with 38 newly determined complete mitochondrial DNA sequences. Molecular Biology and Evolution 18: 1993–2009.
20. Venkatesh B, Erdmann MV, Brenner S (2001) Molecular synapomorphies resolve evolutionary relationships of extant jawed vertebrates. Proceedings of the National Academy of Science USA 98: 11382–11387.
21. Chen W-J, Bonillo C, Lecointre G (2003) Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. Molecular Phylogenetics and Evolution 26: 262–288.
22. Li B, Dettaï A, Cruaud C, Couloux A, Desoutter-Meniger M, et al. (2009) RNF213, a new nuclear marker for acanthomorph phylogeny. Molecular Phylogenetics and Evolution 50: 345–363.
23. Sevilla RG, Diez A, Norén M, Mouchel O, Jérôme M, et al. (2007) Primers and polymerase chain reaction conditions for DNA barcoding teleost fish based on the mitochondrial cytochrome *b* and nuclear rhodopsin genes. Molecular Ecology Notes 7: 730–734.
24. Wright TF, Schirtzinger EE, Matsumoto T, Eberhard JR, Graves GR, et al. (2008) A multilocus molecular phylogeny of the parrots (Psittaciformes): Support for a Gondwanan origin during the Cretaceous. Molecular Biology and Evolution 25: 2141–2156.
25. Zheng LP, Yang JX, Chen XY, Wang WY (2010) Phylogenetic relationships of the Chinese Labeoninae (Teleostei, Cypriniformes) derived from two nuclear and three mitochondrial genes. Zoologica Scripta 39: 559–571.
26. Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33: 511–518.
27. Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17: 540–552.
28. Felsenstein J (2004) Inferring phylogenies: Sinauer Associates Incorporated.
29. Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.
30. Yamanoue Y, Miya M, Matsuura K, Katoh M, Sakai H, et al. (2008) A new perspective on phylogeny and evolution of tetraodontiform fishes (Pisces: Acanthopterygii) based on whole mitochondrial genome sequences: Basal ecological diversification? BMC Evolutionary Biology 8, 212.
31. Yamanoue Y, Miya M, Matsuura K, Yagishita N, Mabuchi K, et al. (2007) Phylogenetic position of tetraodontiform fishes within the higher teleosts: Bayesian inferences based on 44 whole mitochondrial genome sequences. Molecular Phylogenetics and Evolution 45: 89–101.
32. Roa-Varon A, Orti G (2009) Phylogenetic relationships among families of Gadiformes (Teleostei, Paracanthopterygii) based on nuclear and mitochondrial data. Molecular Phylogenetics and Evolution 52: 688–704.
33. Azevedo MFC, Oliveira C, Pardo BG, Martinez P, Foresti F (2008) Phylogenetic analysis of the order Pleuronectiformes (Teleostei) based on sequences of 12S and 16S mitochondrial genes. Genetics and Molecular Biology 31: 284–292.
34. Chiba SN, Iwatsuki Y, Yoshino T, Hanzawa N (2009) Comprehensive phylogeny of the family Sparidae (Perciformes: Teleostei) inferred from mitochondrial gene analyses. Genes & Genetic Systems 84: 153–170.
35. Stamatakis A, Ludwig T, Meyer H (2005) RAxML-III: a fast program for maximum likelihood-based inference of large phylogenies. Bioinformatics 21: 456–463.
36. Swofford DL (1998) PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Version 4. Sunderland, Massachussetts: Sinauer Associates.
37. Criscuolo A, Berry V, Douzery EJP, Gascuel O (2006) SDM: A fast distance-based approach for (super) tree building in phylogenomics. Systematic Biology 55: 740–755.
38. Dutheil J, Gaillard S, Bazin E, Glémin S, Ranwez V, et al. (2006) Bio++: a set of C++ libraries for sequence analysis, phylogenetics, molecular evolution and population genetics. BMC Bioinformatics 7: 188.
39. Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20: 289–290.
40. Thorne JL, Kishino H (2002) Divergence time and evolutionary rate estimation with multilocus data. Systematic Biology 51: 689–702.
41. Benton MJ, Donoghue PC, Asher RJ (2009) Calibrating and constraining molecular clocks. In: Hedges SB, Kumar S, eds. The time tree of life. Oxford: Oxford University Press. pp 35–86.
42. Yamanoue Y, Miya M, Inoue JG, Matsuura K, Nishida M (2006) The mitochondrial genome of spotted green pufferfish Tetraodon nigroviridis (Teleostei: Tetraodontiformes) and divergence time estimation among model organisms in fishes. Genes & Genetic Systems 81: 29–39.
43. Patterson C (1993) Osteichthyes: Teleostei. In: Benton MJ, ed. The fossil record 2. London: Chapman & Hall. pp 621–656.
44. Yang Z, Rannala B (2006) Bayesian estimation of species divergence times under a molecular clock using multiple fossil calibrations with soft bounds. Molecular Biology and Evolution 23: 212–226.
45. Lepage T, Bryant D, Philippe H, Lartillot N (2007) A general comparison of relaxed molecular clock models. Molecular Biology and Evolution 24: 2669–2680.
46. Lartillot N, Philippe H (2004) A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process. Molecular Biology and Evolution 21: 1095–1109.
47. Lartillot N, Lepage T, Blanquart S (2009) PhyloBayes 3: a Bayesian software package for phylogenetic reconstruction and molecular dating. Bioinformatics 25: 2286–2288.
48. Morlon H, Potts MD, Plotkin JB (2010) Inferring the Dynamics of Diversification: A Coalescent Approach. Plos Biology 8, e1000493.
49. Strauss SY, Webb CO, Salamin N (2006) Exotic taxa less related to native species are more invasive. Proceedings of the National Academy of Science USA 103: 5841–5845.
50. Li CH, Lu GQ, Orti G (2008) Optimal data partitioning and a test case for ray-finned fishes (Actinopterygii) based on ten nuclear loci. Systematic Biology 57: 519–539.
51. Peng Z, He S, Wang J, Wang W, Diogo R (2006) Mitochondrial molecular clocks and the origin of the major Otocephalan clades (Pisces: Teleostei): A new insight. Gene 370: 113–124.
52. Reed DL, Carpenter KE, deGravelle MJ (2002) Molecular systematics of the Jacks (Perciformes: Carangidae) based on mitochondrial cytochrome b sequences using parsimony, likelihood, and Bayesian approaches. Molecular Phylogenetics and Evolution 23: 513–524.
53. Delsuc F, Brinkmann H, Philippe H (2005) Phylogenomics and the reconstruction of the tree of life. Nature Reviews Genetic 6: 361–375.
54. Li JT, Che J, Murphy RW, Zhao H, Zhao EM, et al. (2009) New insights to the molecular phylogenetics and generic assessment in the Rhacophoridae (Amphibia: Anura) based on five nuclear and three mitochondrial genes, with comments on the evolution of reproduction. Molecular Phylogenetics and Evolution 53: 509–522.
55. Wiens JJ (2003) Missing data, incomplete taxa, and phylogenetic accuracy. Systematic Biology 52: 528–538.
56. Philippe H, Snell EA, Bapteste E, Lopez P, Holland PWH, et al. (2004) Phylogenomics of eukaryotes: impact of missing data on large alignments. Molecular Biology and Evolution 9: 1740–1752.
57. Arratia G (2000) Phylogenetic relationships of Teleostei: past and present. Estudios Oceanologicos 19: 19–51.
58. Inoue JG, Miya M, Tsukamoto K, Nishida M (2003) Basal actinopterygian relationships: a mitogenomic perspective on the phylogeny of the "ancient fish". Molecular Phylogenetics and Evolution 26: 110–120.
59. Graur D, Martin W (2004) Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. Trends in Genetics 20: 80–86.
60. Cavin L (2008) Paleobiogeography of Cretaceous Bony Fishes (Actinistia, Dipnoi and Actinopterygii). In: Cavin L, Longbottom A, Richter M, eds. Fishes and the breakup of Pangea. London: Geological Society of London, Special Publication 295. pp 165–183.
61. Cavin L, Forey PL (2007) Using ghost lineages to identify diversification events in the fossil record. Biology Letters 3: 201–204.
62. Cavin L, Forey PL, Lecuyer C (2007) Correlation between environment and Late Mesozoic ray-finned fish evolution. Palaeogeography Palaeoclimatology Palaeoecology 245: 353–367.
63. Girone A, Nolf D, Cavallo O (2010) Fish otoliths from the pre-evaporitic (Early Messinian) sediments of northern Italy: their stratigraphic and palaeobiogeographic significance. Facies 56: 399–432.
64. Landini W, Sorbini C (2005) Evolutionary trends in the Plio-Pleistocene ichthyofauna of the Mediterranean Basin: nature, timing and magnitude of the extinction events. Quaternary International 131: 101–107.
65. Carnevale G, Landini W, Sarti G (2006) Mare versus Lago-mare: marine fishes and the Mediterranean environment at the end of the Messinian Salinity crisis. Journal of the Geological Society 163: 75–80.
66. Carnevale G, Longinelli A, Caputo D, Barbieri M, Landini W (2008) Did the Mediterranean marine reflooding precede the Mio-Pliocene boundary? Paleontological and geochemical evidence from upper Messinian sequences of Tuscany, Italy. Palaeogeography Palaeoclimatology Palaeoecology 257: 81–105.
67. Mouillot D, Albouy C, Guilhaumon F, Ben Rais Lasram F, Coll M, et al. (2011) Protected and threatened components of fish biodiversity in the Mediterranean Sea. Current Biology 21: 1044–1050.
68. Meynard C, Devictor V, Mouillot D, Thuiller W, Jiguet F, et al. (2011) Beyond taxonomic diversity patterns: how do α, β and γ components of bird functional

and phylogenetic diversity respond to environmental gradients across France? Global Ecology and Biogeography 20: 893–903.

69. Inoue JG, Kumazawa Y, Miya M, Nishida M (2009) The historical biogeography of the freshwater knifefishes using mitogenomic approaches: a Mesozoic origin of the Asian notopterids (Actinopterygii: Osteoglossomorpha). Molecular Phylogenetics and Evolution 51: 486–499.
70. Benton MJ, Donoghue PCJ (2007) Paleontological evidence to date the tree of life. Molecular Biology and Evolution 24: 26–53.


(a)
PT
KP
MSC
Entelurus aequoreus
Nerophis ophidion
Hippocampus fuscus
Hippocampus hippocampus
Hippocampus ramulosus
Syngnathus abaster
Syngnathus taenionotus
Syngnathus typhle
Syngnathus rostellatus
Syngnathus acus
Fistularia commersonii
Fistularia petimba
Macroramphosus scolopax
Beryx splendens
Gephyroberyx darwinii
Hoplostethus mediterraneus
Sargocentron rubrum
Lampris guttatus
Regalecus glesne
Zu cristatus
Lophotus lacepede
Aulopus filamentosus
Synodus saurus
Bathypterois dubius
Chlorophthalmus agassizi
Bathypterois grallator
Paralepis coregonoides
Saurida undosquamis
Sudis hyalina
Argentina sphyraena
Argyropelecus hemigymnus
Maurolicus muelleri
Cyclothone braueri
Cyclothone pygmaea
Gonostoma denudatum
Chauliodus sloani
Ichthyococcus ovatus
Stomias boa
Vinciguerria poweriae
Valenciennellus tripunctulatus
Benthosema glaciale
Notoscopelus bolini
Diogenichthys atlanticus
Electrona risso
Gonichthys cocco
Hygophum benoiti
Hygophum hygomii
Myctophum punctatum
Symbolophorus veranyi
Lobianchia dofleini
Lobianchia gemellarii
Ceratoscopelus maderensis
Diaphus metopoclampus
Diaphus rafinesquii
Coryphaenoides guentheri
Gadiculus argenteus
Micromesistius poutassou
Trisopterus luscus
Trisopterus minutus
Merlangius merlangus
Molva dypterygia
Molva molva
Gaidropsarus biscayensis
Gaidropsarus vulgaris
Gaidropsarus mediterraneus
Phycis blennoides
Phycis phycis
Hymenocephalus italicus
Nezumia aequalis
Trachyrincus scabrus
Merluccius merluccius
Guttigadus latifrons
Mora moro
Zeus faber
Alosa alosa
Alosa fallax
Sardina pilchardus
Pellonula leonensis
Sardinella maderensis
Sardinella aurita
Engraulis encrasicolus
Etrumeus teres
Spratelloides delicatulus
Sprattus sprattus
Dussumieria acuta
Dussumieria elopsoides
Danio rerio
Anguilla anguilla
Nemichthys scolopaceus
Enchelycore anatina
Gymnothorax unicolor
Muraena helena
Echelus myrus
Nettastoma melanurum
Muraenesox cinereus
Notacanthus bonaparte
SYNGNATHIFORMES
BERYCIFORMES
LAMPRIFORMES
AULOPIFORMES
MYCTOPHIFORMES
GADIFORMES
CLUPEIFORMES
ANGUILLIFORMES
6
5
4
3
2
1
UJ
LC
UC
P
E
O
M
160
140
120
100
80
60
40
20
0
(b)
PT
KP
MSC
Chelidonichthys lucernus
Eutrigla gurnardus
Trigloporus lastoviza
Lepidotrigla cavillone
Trigla lyra
Taurulus bubalis
Helicolenus dactylopterus
Trachyscorpia cristulata
Pterois miles
Scorpaenodes arenai
Pontinus kuhlii
Scorpaena elongata
Scorpaena scrofa
Scorpaena notata
Scorpaena porcus
Scorpaena maderensis
Gasterosteus aculeatus
Pungitius pungitius
Spinachia spinachia
Hypoptychus dybowskii
Echiichthys vipera
Trachinus draco
Trachinus radiatus
Epinephelus aeneus
Epinephelus coioides
Epinephelus malabaricus
Epinephelus haifensis
Epinephelus caninus
Mycteroperca rubra
Epinephelus marginatus
Serranus atricauda
Serranus cabrilla
Serranus scriba
Serranus hepatus
Epigonus constanciae
Polyprion americanus
Epigonus telescopus
Argyrosomus regius
Umbrina cirrosa
Umbrina canariensis
Dicentrarchus labrax
Dicentrarchus punctatus
Boops boops
Diplodus annularis
Diplodus bellottii
Diplodus vulgaris
Diplodus puntazzo
Oblada melanura
Diplodus cervinus
Diplodus sargus
Lithognathus mormyrus
Crenidens crenidens
Pagellus acarne
Pagellus bogaraveo
Sparus aurata
Sarpa salpa
Spicara flexuosa
Spicara maena
Spicara smaris
Spondyliosoma cantharus
Dentex dentex
Dentex gibbosus
Pagrus auriga
Pagrus caeruleostictus
Pagellus bellottii
Pagellus erythrinus
Pagrus pagrus
Dentex macrophthalmus
Dentex maroccanus
Centracanthus cirrus
Scarus ghobban
Lutjanus argentimaculatus
Siganus luridus
Siganus rivulatus
Uranoscopus scaber
Luvarus imperialis
Parapristipoma octolineatum
Plectorhinchus mediterraneus
Pomadasys incisus
Pomadasys stridens
Synagrops japonicus
Capros aper
Lagocephalus sceleratus
Sphoeroides pachygaster
Sphoeroides spengleri
Takifugu rubripes
Tetraodon nigroviridis
Lagocephalus spadiceus
Mola mola
Ranzania laevis
Cepola macrophthalma
Halobatrachus didactylus
Priacanthus hamrur
Lophius budegassa
Lophius piscatorius
Chaetodon hoefleri
Sillago sihama
Callanthias ruber
Dactylopterus volitans
Callionymus lyra
Callionymus maculatus
Callionymus reticulatus
Mullus barbatus
Mullus surmuletus
Upeneus moluccensis
SCORPAENIFORMES
GASTEROSTEIFORMES
Serranidae
Sparidae 1
Centracanthidae
Sparidae 2
TETRAODONTIFORMES
11
10
9
7
LC
UC
P
E
O
M
100
80
60
40
20
0
(c)
PT
KP
MSC
Arnoglossus imperialis
Arnoglossus thori
Arnoglossus laterna
Bothus podas
Citharus linguatula
Lepidorhombus boscii
Lepidorhombus whiffiagonis
Psetta maxima
Scophthalmus rhombus
Platichthys flesus
Pleuronectes platessa
Bathysolea profundicola
Microchirus boscanion
Microchirus ocellatus
Microchirus variegatus
Microchirus hexophthalmus
Microchirus azevia
Buglossidium luteum
Dicologlossa cuneata
Pegusa impar
Pegusa lascaris
Solea aegyptiaca
Solea senegalensis
Solea solea
Synapturichthys kleinii
Synaptura lusitanica
Sphyraena sphyraena
Sphyraena viridensis
Tetrapturus albidus
Tetrapturus belone
Tetrapturus georgii
Xiphias gladius
Acanthocybium solandri
Auxis rochei
Euthynnus alletteratus
Katsuwonus pelamis
Thunnus alalunga
Thunnus thynnus
Sarda sarda
Rastrelliger kanagurta
Scomber japonicus
Scomber scombrus
Scomberomorus commerson
Scomberomorus tritor
Lepidopus caudatus
Trichiurus lepturus
Ruvettus pretiosus
Tetragonurus cuvieri
Brama brama
Psenes pellucidus
Centrolophus niger
Schedophilus ovalis
Pomatomus saltatrix
Pampus argenteus
Aphia minuta
Lesueurigobius friesii
Lesueurigobius suerii
Gobius auratus
Gobius xanthocephalus
Gobius bucchichi
Zosterisessor ophiocephalus
Gobius cruentatus
Gobius niger
Gobius paganellus
Zebrus zebrus
Gobius cobitis
Buenia affinis
Crystallogobius linearis
Knipowitschia panizzae
Pomatoschistus quagga
Pomatoschistus canestrinii
Pomatoschistus knerii
Pomatoschistus microps
Pomatoschistus marmoratus
Pomatoschistus pictus
Pseudaphya ferreri
Pomatoschistus minutus
Pomatoschistus norvegicus
Apogon imberbis
Acantholabrus palloni
Centrolabrus exoletus
Symphodus melanocercus
Symphodus bailloni
Symphodus tinca
Symphodus rostratus
Symphodus mediterraneus
Symphodus melops
Symphodus ocellatus
Symphodus roissali
Symphodus cinereus
Symphodus doderleini
Ctenolabrus rupestris
Labrus merula
Labrus viridis
Lappanella fasciata
Coris julis
Thalassoma pavo
Xyrichthys novacula
Sparisoma cretense
PLEURONECTIFORMES
Scombridae
Gobiidae
Labridae
15
16
14
13
12
LC
UC
P
E
O
M
100
80
60
40
20
0
(d)
PT
KP
MSC
Abudefduf vaigiensis
Chromis chromis
Aidablennius sphynx
Blennius ocellaris
Lipophrys adriaticus
Lipophrys dalmatinus
Lipophrys canevae
Lipophrys nigriceps
Parablennius gattorugine
Parablennius incognitus
Parablennius zvonimiri
Parablennius pilicornis
Parablennius rouxi
Parablennius tentacularis
Salaria pavo
Scartella cristata
Parablennius sanguinolentus
Omobranchus punctatus
Coryphoblennius galerita
Lipophrys trigloides
Lipophrys pholis
Apletodon dentatus
Diplecogaster bimaculata
Lepadogaster candollei
Opeatogenys gracilis
Lepadogaster lepadogaster
Lepadogaster purpurea
Gouania willdenowi
Tripterygion delaisi
Tripterygion melanurus
Tripterygion tripteronotus
Chelon labrosus
Liza aurata
Liza saliens
Liza ramado
Oedalechilus labeo
Mugil cephalus
Aphanius dispar
Aphanius fasciatus
Aphanius iberus
Gambusia affinis
Pinguipes brasilianus
Cichlasoma bimaculatum
Belone belone
Belone svetovidovi
Scomberesox saurus
Tylosurus acus
Exocoetus volitans
Hemiramphus far
Hyporhamphus affinis
Oryzias latipes
Alectis alexandrinus
Alepes djedaba
Caranx crysos
Caranx hippos
Caranx rhonchus
Trachurus mediterraneus
Trachurus picturatus
Trachurus trachurus
Pseudocaranx dentex
Elagatis bipinnulata
Seriola carpenteri
Seriola dumerili
Seriola rivoliana
Seriola fasciata
Coryphaena equiselis
Coryphaena hippurus
Rachycentron canadum
Echeneis naucrates
Remora remora
Remora osteochir
Lichia amia
Trachinotus ovatus
Pomacentridae
Blenniidae
GOBIESOCIFORMES
Mugilidae
CYPRINODONTIFORMES
BELONIFORMES
Carangidae 1
Carangidae 2
20
19
18
17
LC
UC
P
E
O
M
100
80
60
40
20
0

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	*Muraenesox cinereus* (Forsskål 1775)	AF417318		EF607449	AY295080		
	Muraenidae	*Enchelycore anatina* (Lowe 1838)				EF439251	EF427475	
		Gymnothorax unicolor (Delarohe 1809)					AY862128	
		Muraena helena Linnaeus 1758				AY862092	AY862118	
	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)					EF439259	EU366688
	Chlorophthalmidae	*Chlorophthalmus agassizi* Bonaparte 1840	AP002918	DQ027906	DQ027975	EF439508	EF439358	FJ896455
	Ipnopidae	*Bathypterois dubius* Vaillant 1888	AY141326	AY141396			AY141257	
		Bathypterois grallator (Goode & Bean 1886)						EU366690
	Paralepididae	*Paralepis coregonoides* Risso 1820						EU366708
		Sudis hyalina Rafinesque 1810	EU574933					
	Synodontidae	*Saurida undosquamis* (Richardson 1848)	AP002920	AB297971	AP002920	AP002920		EU366712
		Synodus saurus (Linnaeus 1758)	AF049723	AF049733		DQ198009	DQ197911	
Batrachoidiformes	Batrachoididae	*Halobatrachus didactylus* (Bloch & Schneider 1801)		AY368308		AF165351	AY368323	
Beloniformes	Adrianichthyidae	*Oryzias latipes* (Temminck & Schlegel 1846)	AP008948.1	AP008948.1	AP008948.1	AP008948.1	NM_001104695.1	EF095641

	Belonidae	*Belone belone gracilis* (Linnaeus 1761)	AF231541	AF231514		EU036423	AY141268	
		Belone svetovidovi Collette & Parin 1970		AF243956		AF243880		
		Tylosurus acus (Lacepède 1803)	AF231571	AF231528		AF231656	EF427530	
	Exocoetidae	*Exocoetus 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
Beryciformes	Berycidae	*Beryx splendens* Lowe 1834	AF092197	AF100909	EF609297	AB108491	AY141265	EF095636
	Holocentridae	*Sargocentron rubrum* (Forsskål 1775)	AP004432	AP004432	AP004432	AP004432		
	Trachichthyidae	*Gephyroberyx darwinii* (Johnson 1866)			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	

		Spratelloides delicatulus (Bennett 1832)	DQ912058	DQ912093	AP009144	AP009144		DQ912128
		Sprattus sprattus (Linnaeus 1758)	AP009234	AM911201	AM911177	AF472581	EU491991	
	Engraulidae	*Engraulis encrasicolus* (Linnaeus 1758)	DQ912031	DQ912066	AM911182	EU552563	EU224151	DQ912103.1
Cypriniformes	Cyprinidae	*Danio rerio* (Hamilton 1822)	NC_002333.2	NC_002333.2	NC_002333.2	NC_002333.2	NM_131084.1	U71093
Cyprinodontiformes	Cyprinodontidae	*Aphanius dispar* (Rüppell 1829)		ADU05964				
		Aphanius fasciatus (Valenciennes 1821)		AFU05965		AF299273		
		Aphanius iberus (Valenciennes 1846)				AF299274		
	Poesiliidae	*Gambusia affinis* (Baird & Girard 1853)	NC_004388.1	NC_004388.1	NC_004388.1	NC_004388.1		
Dactylopteriformes	Dactylopteridae	*Dactylopterus volitans* (Linnaeus 1758)	AF150006			EF439514	AY141282	
Gadiformes	Gadidae	*Gadiculus argenteus* Guichenot 1850				EU224053	EU224201	
		Merlangius merlangus (Linnaeus 1758)	AY141330		EF609406	DQ174057	AY141260	FJ215265
		Micromesistius poutassou (Risso 1827)	AY842451	AY850366		DQ174068	EU492215	
		Trisopterus luscus (Linnaeus 1758)			EF609486	DQ174081	EU224138	
		Trisopterus minutus (Linnaeus 1758)	AY845395	AY862158		DQ174083	EU036622	
	Lotidae	*Gaidropsarus biscayensis* (Collett 1890)				EF427562	EF439115	
		Gaidropsarus mediterraneus (Linnaeus 1758)				EF427563	EF439117	
		Gaidropsarus vulgaris (Cloquet 1824)				DQ174050		
		Molva dypterygia (Pennant 1784)				AF469625	EF439140	
		Molva molva (Linnaeus 1758)				DQ174071	EF439141	FJ215275
	Macrouridae	*Coryphaenoides guentheri* (Vaillant 1888)			EU148125			

		Hymenocephalus italicus Giglioli 1884						FJ215246
		Nezumia aequalis (Günther 1878)						FJ215280
		Trachyrincus scabrus (Rafinesque 1810)						FJ215298
	Merlucciidae	*Merluccius merluccius* (Linnaeus 1758)	DQ274008		EF609408	DQ174062	EF439400	
	Moridae	*Guttigadus latifrons* (Holt & Byrne 1908)			EU148219			
		Mora moro (Risso 1810)	AY368285	AY368307	EF609410	DQ197964	AY368322	
	Phycidae	*Phycis blennoides* (Brünnich 1768)	AY845393	AY850365		DQ174072	AY368321	
		Phycis phycis (Linnaeus 1766)				DQ197978	DQ197880	
Gasterosteiformes	Gasterosteidae	*Gasterosteus aculeatus* Linnaeus 1758	NC_003174.1	NC_003174.1	NC_003174.1	NC_003174.1	EU637962.1	EF033039
		Pungitius pungitius (Linnaeus 1758)	NC_011571.1	NC_011571.1	NC_011571.1	NC_011571.1		AB445183
		Spinachia spinachia (Linnaeus 1758)	NC_011582.1	NC_011582.1	NC_011582.1	NC_011582.1	AY141281.1	AB445184
	Hypoptychidae	*Hypoptychus dybowskii* Steindachner 1880	NC_004400	NC_004400	NC_004400	NC_004400		AB445176
Gobiesociformes	Gobiesocidae	*Apletodon dentatus* (Facciolà 1887)	AF549200	AF549207			AY141274	
		Diplecogaster bimaculata (Bonnaterre 1788)	AF549197	AF549205				
		Gouania willdenowi (Risso 1810)	EF363030	EF363032				
		Lepadogaster candollei Risso 1810	AY036588	AF549203				
		Lepadogaster lepadogaster (Bonnaterre 1788)	AY036589	AF549202			AY141273	
		Lepadogaster purpurea (Bonnaterre 1788)	AY036599	AF549201				
		Opeatogenys gracilis (Canestrini 1864)	AF549196	AF549206				
Lampriformes	Lampridae	*Lampris guttatus* (Brünnich 1788)	AF049726	AF049736	DQ885096	DQ197959		AY308764

	Lophotidae	*Lophotus lacepede* Giorna 1809	AY036616	AY036618				FJ896461
	Regalecidae	*Regalecus glesne* Ascanius 1772	AF049728	EU099465			AY368328	EF107625
	Trachipteridae	*Zu cristatus* (Bonelli 1819)	AY652748	AY652749				FJ896462
Lophiiformes	Lophiidae	*Lophius budegassa* Spinola 1807	EF095552			EF427574	EF095608	EF095637
		Lophius piscatorius Linnaeus 1758	AY368294			EF427575	AY368325	
Myctophiformes	Myctophidae	*Benthosema glaciale* (Reinhardt 1837)		DQ532843	EU148098			EU366728
		Ceratoscopelus maderensis (Lowe 1839)			EU148109			
		Diaphus metopoclampus (Cocco 1829)			EU148149			
		Diaphus rafinesquii (Cocco 1838)			EU148152			
		Diogenichthys atlanticus (Tåning 1928)		AB042178				
		Electrona risso (Cocco 1829)			EU148157			
		Gonichthys cocco (Cocco 1829)			EU148175			
		Hygophum benoiti (Cocco 1838)		AB024912	EU148202			
		Hygophum hygomii (Lütken 1892)	AF049724	AB024915	EU148205			EF094947
		Lobianchia dofleini (Zugmayer 1911)		DQ532898				
		Lobianchia gemellarii Cocco 1838		AB042159				
		Myctophum punctatum Rafinesque 1810		AF221864	EU148251			
		Notoscopelus bolini Nafpaktitis 1975			EU148276			
		Symbolophorus veranyi (Moreau 1888)			EU148340			
Notacanthiformes	Notacanthidae	*Notacanthus bonaparte* Risso 1840	X99182	X99181	EU148274			

Osmeriformes	Argentinidae	*Argentina sphyraena* Linnaeus 1758				EU492324	EU492231
Perciformes	Acropomatidae	*Synagrops japonicus* (Döderlein 1883)		EF120861		AB104919	
	Apogonidae	*Apogon imberbis* (Linnaeus 1758)	AM158282	FJ462721			
	Blenniidae	*Aidablennius sphynx* (Valenciennes 1836)	AF549191	AF549193			
		Blennius ocellaris Linnaeus 1758	AY098746	AY098815			
		Coryphoblennius galerita (Linnaeus 1758)	AY098748	AY098816			
		Lipophrys adriaticus (Steindachner & Kolombatovic 1883)	AY098758	AF324188			
		Lipophrys canevae (Vinciguerra 1880)	AF414713	AY098821			
		Lipophrys dalmatinus (Steindachner & Kolombatovic	AY098756	AY098823			
		Lipophrys nigriceps (Vinciguerra 1883)	AF414714	AY098824			
		Lipophrys pholis (Linnaeus 1758)	AY098761	AY098825			
		Lipophrys trigloides (Valenciennes 1836)	AY098768	AY987024			
		Omobranchus punctatus (Valenciennes 1836)	OPU90393				
		Parablennius gattorugine (Linnaeus 1758)	AF414715	DQ160198			AY141271
		Parablennius incognitus (Bath 1968)	AY098784	AY098829			
		Parablennius pilicornis (Cuvier 1829)	AY098795	AY098831			
		Parablennius rouxi (Cocco 1833)	AY098781	AY098833	AJ872148		
		Parablennius sanguinolentus (Pallas 1814)	AF414697	AF428241			
		Parablennius tentacularis (Brünnich 1768)	AY098780	AY098838			
		Parablennius zvonimiri (Kolombatovic 1892)	AY098790	AY098840			

	Salaria pavo (Risso 1810)	AY098798	AY098842				
	Scartella cristata (Linnaeus 1758)	AY098803	AY098845				
Bramidae	*Brama brama* (Bonnaterre 1788)			EF609300	DQ197933	DQ197835	
Callanthiidae	*Callanthias ruber* (Rafinesque 1810)		EF120863			EU637945	
Callionymidae	*Callionymus lyra* Linnaeus 1758	AY141344	AY141414			AY141270	
	Callionymus maculatus Linnaeus 1810					EU491964	
	Callionymus reticulatus Valenciennes 1837					EU491962	
Carangidae	*Alectis alexandrinus* (Geoffroy Saint-Hilaire 1817)				AF363738		EU167759
	Alepes djedaba (Forsskål 1775)		EF613269	EF607307	EF512295		
	Caranx crysos (Mitchill 1815)				AY050717	EF427461	
	Caranx hippos (Linnaeus 1766)		DQ532847		AY050720		EU167758
	Caranx rhonchus Geoffroy Saint-Hilaire 1817				AY050733		
	Elagatis bipinnulata (Quoy & Gaimard 1825)			EU014213	AY050734		
	Lichia amia (Linnaeus 1758)				EF392593	EF427481	
	Pseudocaranx dentex (Bloch & Schneider 1801)			EF609442	EF392607.1	EF439301	
	Seriola carpenteri Mather 1971				EF392617	EF427512	
	Seriola dumerili (Risso 1810)			EF607552	AB292794	EF439446	
	Seriola fasciata (Bloch 1793)				AY050748	EF439317	
	Seriola rivoliana Valenciennes 1833				AB264297	EF427516	
	Trachinotus ovatus (Linnaeus 1758)	AY141388	DQ027921	DQ027991	AY050750	AY141314	

	Trachurus mediterraneus (Steindachner 1868)		AF487412		AY526548	EU036619	
	Trachurus picturatus (Bowdich 1825)		AF487415	EU148351	AY526546	EF439329	
	Trachurus trachurus (Linnaeus 1758)	AB108498	AF487410	AB108498	AY526533	EU491981	
Centracanthidae	*Centracanthus cirrus* Rafinesque 1810						EU167766
	Spicara flexuosa Rafinesque 1810				EU036502	EU036606	EU167804
	Spicara maena (Linnaeus 1758)	AP009164	AF247434	AP009164	AF240737	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 macrophthalma* (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				DQ080244	DQ080342	
	Coryphaena hippurus Linnaeus 1758	DQ874715	AY857955	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	DQ080265	DQ874813	

Gobiidae	*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 panizzae (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				
	Zebrus 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 bailloni (Valenciennes 1839)	AY092052	AY092037				
	Symphodus cinereus (Bonnaterre 1788)	AJ810147	AY092036				

	Symphodus doderleini Jordan 1890		AF517602				
	Symphodus mediterraneus (Linnaeus 1758)	AJ810148	AF517601				
	Symphodus melanocercus (Risso 1810)	AJ810149	AF517595				
	Symphodus melops (Linnaeus 1758)	AF414197	AY092038				
	Symphodus ocellatus (Linnaeus 1758)	AJ810150	AF517603				
	Symphodus roissali (Risso 1810)	AJ810151	AY092039				
	Symphodus rostratus (Bloch 1791)	AF414198	AY092040				
	Symphodus tinca (Linnaeus 1758)	AJ810152	AF517596				
	Thalassoma pavo (Linnaeus 1758)				AY328877.1	DQ197913	
	Xyrichtys novacula (Linnaeus 1758)				EF439246	EF439331	
Lutjanidae	*Lutjanus argentimaculatus* (Forsskål 1775)	AY484978	DQ444481	DQ885104	DQ900672		EU627659
Luvaridae	*Luvarus imperialis* Rafinesque 1810	AY057234	AY264587	AP009161	AB276966		EF530099
Moronidae	*Dicentrarchus labrax* (Linnaeus 1758)	AY141370	AY141440		EF427553	EU492059	AH008179
	Dicentrarchus punctatus (Bloch 1792)		AF247437		AF143191	DQ197846	
Mugilidae	*Chelon labrosus* (Risso 1827)	DQ016292	AY169697		DQ197935	DQ197837	
	Liza aurata (Risso 1810)	EF437077	AY169698		EF427572	EF439127	
	Liza ramado (Risso 1827)	EF437079	AY169700		EU224058	EU224158	
	Liza saliens (Risso 1810)	EF437081	AY169702		Z70774		
	Mugil cephalus Linnaeus 1758	DQ225772	DQ307686	EF607446	DQ225777	EF095609	EF095639
	Oedalechilus labeo (Cuvier 1829)	Z71995	AY169705		Z70777		

Mullidae	*Mullus barbatus* Linnaeus 1758				EF439552	EF439143	
	Mullus surmuletus Linnaeus 1758	EF095566	EF095594		DQ197965	EF095617	EF095658
	Upeneus moluccensis (Bleeker 1855)				AF227675		EU167747
Nomeidae	*Psenes pellucidus* Lütken 1880		AB205425	AB205447	AB205469		
Pinguipedidae	*Pinguipes brasilianus* Cuvier 1829			EU074542			
Polyprionidae	*Polyprion americanus* (Bloch & Schneider 1801)	AM158291	AY947616	DQ107915	EF392605	EF427493	
Pomacentridae	*Abudefduf vaigiensis* (Quoy & Gaimard 1825)	AF436880	AY365120	AP006016	AY208557		
	Chromis chromis (Linnaeus 1758)		AF517577		AY208527		AY208640
Pomatomidae	*Pomatomus saltatrix* (Linnaeus 1766)		AF055612	DQ885112	DQ080341	DQ080430	EU167741
Priacanthidae	*Priacanthus hamrur* (Forsskål 1775)			DQ885115			EU167865
Rachycentridae	*Rachycentron canadum* (Linnaeus 1766)		DQ532949	EF609446	AB292793		EU167910
Scaridae	*Scarus ghobban* Forsskål 1775			EF609452			
	Sparisoma cretense (Linnaeus 1758)	SCU95777	AF517578		DQ198004	DQ197906	DQ457040
Sciaenidae	*Argyrosomus regius* (Asso 1801)				DQ197924	DQ197826	
	Umbrina canariensis Valenciennes 1843				EF392637	EF427532	
	Umbrina cirrosa (Linnaeus 1758)				AF143198		
Scombridae	*Acanthocybium solandri* (Cuvier 1832)	DQ854648	DQ874727	DQ835838	DQ080324	DQ874804	
	Auxis rochei (Risso 1810)	AB176810		DQ835852	DQ080311	DQ080400	
	Euthynnus alletteratus (Rafinesque 1810)	AB176806	DQ874730	DQ835903	DQ080308	DQ080398	
	Katsuwonus pelamis (Linnaeus 1758)	AB176808	DQ874729	DQ835922	DQ080315	DQ080410	

	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 thynnus (Linnaeus 1758)	AY507951		DQ835876	DQ080266	DQ080358	
Serranidae	*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 haifensis (Ben-Tuvia 1853)				AJ420207		
	Epinephelus malabaricus (Bloch & Schneider 1801)		DQ067309	DQ107871			AY551565
	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		

	Siganus rivulatus Forsskål & Niebuhr 1775		DQ898115		DQ898075		
Sillaginidae	*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 macrophthalmus (Bloch 1791)		AJ247273		EF392580	EF427466	
	Dentex maroccanus 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)	AY178432			DQ197972	DQ197874	
	Pagellus erythrinus (Linnaeus 1758)		AJ247284		DQ197973	EF439417	EU167790

	Pagrus auriga Valenciennes 1843	AY178433	AF247425		DQ197974	DQ197876	EU167788
	Pagrus caeruleostictus (Valenciennes 1830)		AJ247276		DQ197975	DQ197877	EU167789
	Pagrus pagrus (Linnaeus 1758)	AY178431	AF247426		DQ197976	DQ197878	EU167791
	Sarpa salpa (Linnaeus 1758)		AF247402		DQ197992	EF439306	HQ676686
	Sparus aurata Linnaeus 1758	EF095565	AF247432		AF240735	EU224181	EF095657
	Spondyliosoma cantharus (Linnaeus 1758)		AF247403		AF240705	EF439321	
Sphyraenidae	*Sphyraena sphyraena* (Linnaeus 1758)	AY141386	DQ532964		DQ080263	AY141312	
	Sphyraena viridensis Cuvier 1829				DQ080262	DQ080353	
Stromateidae	*Pampus argenteus* (Euphrasen 1788)	AY141383	AY141453	DQ107596		AY141309	
Tetragonuridae	*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	
Trachiuridae	*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			
Uranoscopidae	*Uranoscopus scaber* Linnaeus 1758	AF518213			DQ198017	EU036628	
Xiphiidae	*Xiphias gladius* Linnaeus 1758	DQ854646	DQ874734	DQ107623	DQ080249	DQ874811	

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)		AY359663		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	AB125250		AB125330	
		Microchirus hexophthalmus (Bennett 1831)	AB125242	AB125253		AB125332	
		Microchirus ocellatus (Linnaeus 1758)	AF542218	AY157327		AF113198	
		Microchirus variegatus (Donovan 1808)	AF542215	AY141429		EF427582	AY141284
		Pegusa impar (Bennett 1831)				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	

		Trigla lyra Linnaeus 1758				EF439617	EF439485	
		Trigloporus lastoviza (Bonnaterre 1788)				EF427546	EF439098	
Stomiiformes	Gonostomatidae	*Cyclothone braueri* Jespersen & Tåning 1926	CY2MTSS04					
		Cyclothone pygmaea Jespersen & Tåning 1926	CY2MTSS15	CY2MTLS31				
		Gonostoma denudatum Rafinesque 1810	AB026027	AB026039				
	Phosichthyidae	*Ichthyococcus ovatus* (Cocco 1838)			EU148211			GQ860317
		Vinciguerria poweriae (Cocco 1838)						GQ860320
	Sternoptychidae	*Argyropelecus hemigymnus* Cocco 1829		EU099497	EU148087			
		Maurolicus muelleri (Gmelin 1789)		AJ277245	EU148246			
		Valenciennellus tripunctulatus (Esmark 1871)						GQ860310
	Stomiidae	*Chauliodus sloani* Bloch & Schneider 1801	AP002915	AP002915	EU148112	AP002915		GQ860327
		Stomias boa boa (Risso 1810)			EU148335			
Syngnathiformes	Centriscidae	*Macroramphosus scolopax* (Linnaeus 1758)	AY141354	AY141424	AP005988	AP005988	AY141280	
	Fistulariidae	*Fistularia commersonii* Rüppell 1838	AP005987	AP005987	EF607383	AY786435		
		Fistularia petimba Lacepède 1803	AY141355	AY141425			AY141324	
	Syngnathidae	*Entelurus aequoreus* (Linnaeus 1758)	AF354944	DQ437522	EU148160	AF356044		
		Hippocampus fuscus Rüppell 1838		DQ288371		DQ288354		
		Hippocampus hippocampus (Linnaeus 1758)		DQ288358		AF192665		
		Hippocampus ramulosus Leach 1814	AY368288	AY368310			AY368330	
		Nerophis ophidion (Linnaeus 1758)	AF354943	AF354994		AF356043		

		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	AY368291	AF354993		AF356042	AY368326	
Tetraodontiformes	Caproidae	*Capros aper* (Linnaeus 1758)	EF095553	DQ532846	EU148107	AP009159	AY141262	EF095638
	Molidae	*Mola mola* (Linnaeus 1758)	AY700258	DQ532911	AP006238	AY940835	AF137215	EF095643
		Ranzania laevis (Pennant 1776)	AP006047	AP006047	DQ521011	EF392608	EF427496	
	Tetraodontidae	*Lagocephalus sceleratus* (Gmelin 1789)		AB194240		EF362414		
		Lagocephalus spadiceus (Richardson 1845)			EF60741 9			
		Sphoeroides pachygaster (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	
Zeiformes	Zeidae	*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.

	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).

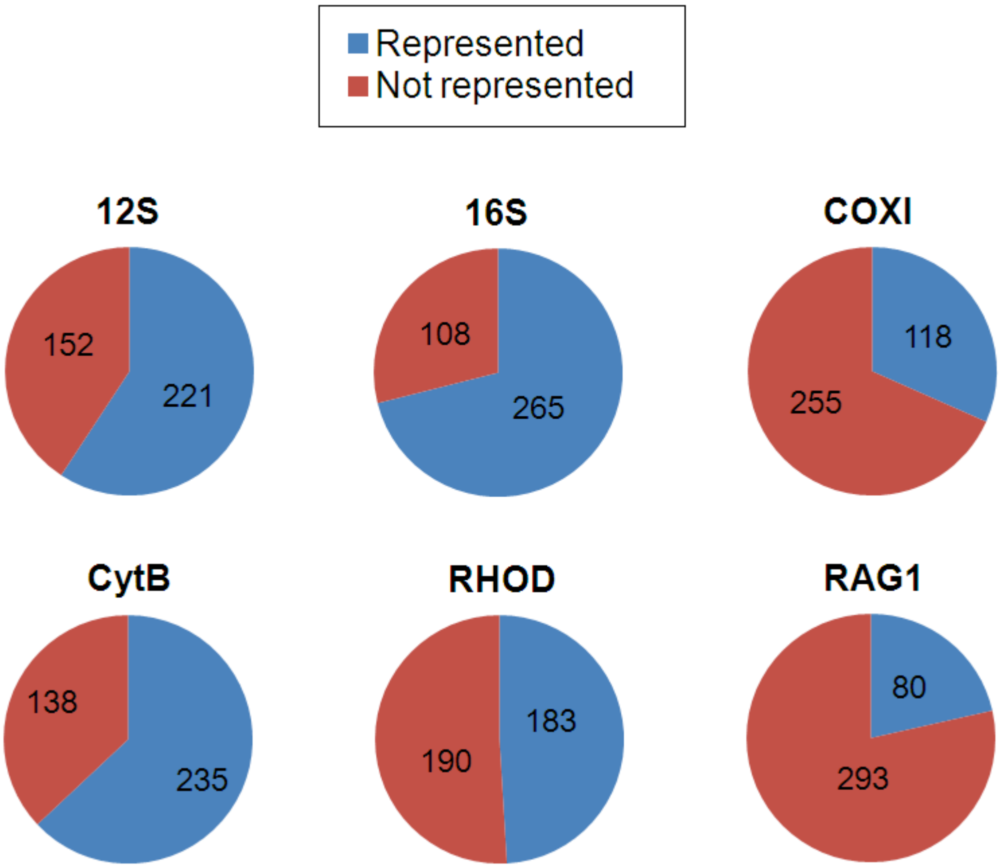
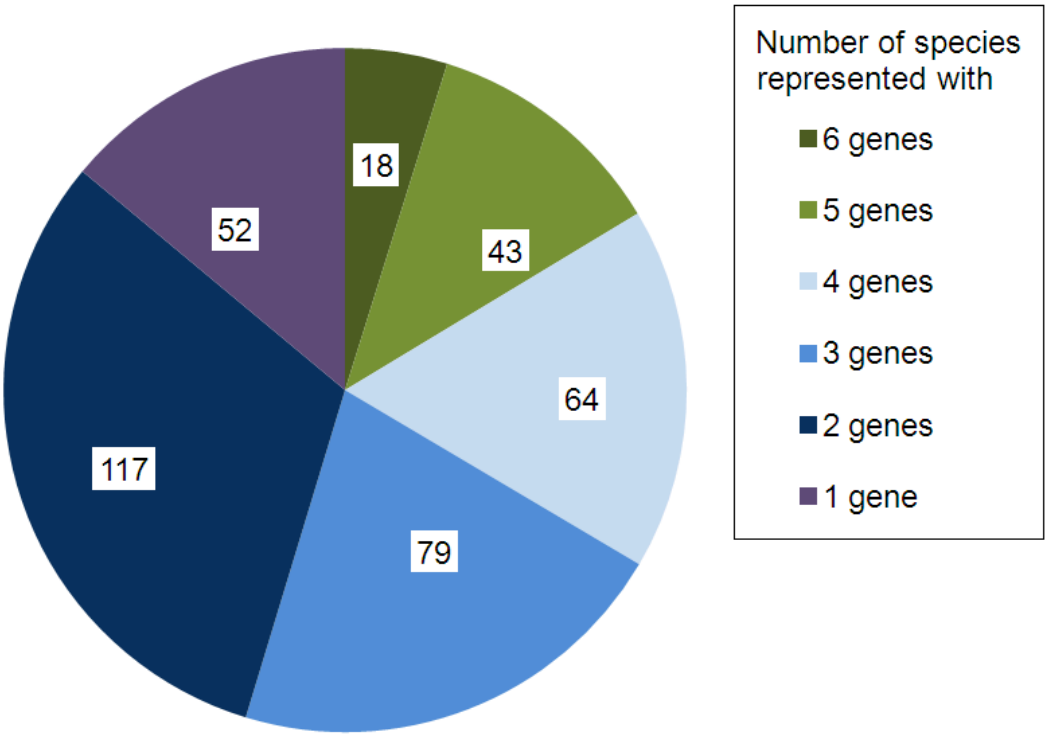


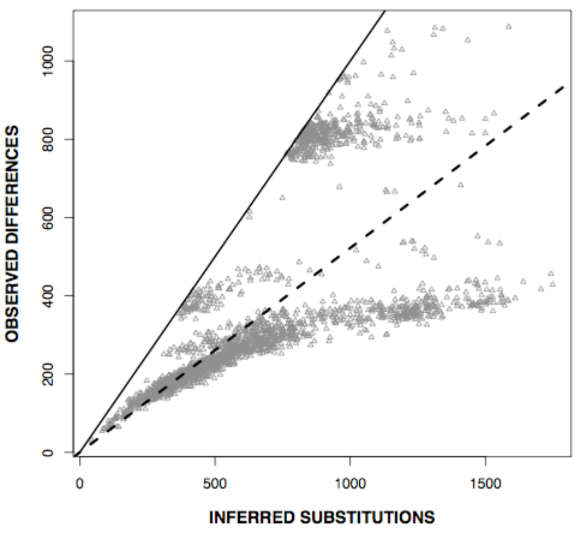
Figure A2.2
Number of species represented by 1, 2, 3, 4, 5 or 6 genes (based on the 373 species represented in the phylogeny), irrespective of whether they are nuclear or mitochondrial.



Saturation information by gene

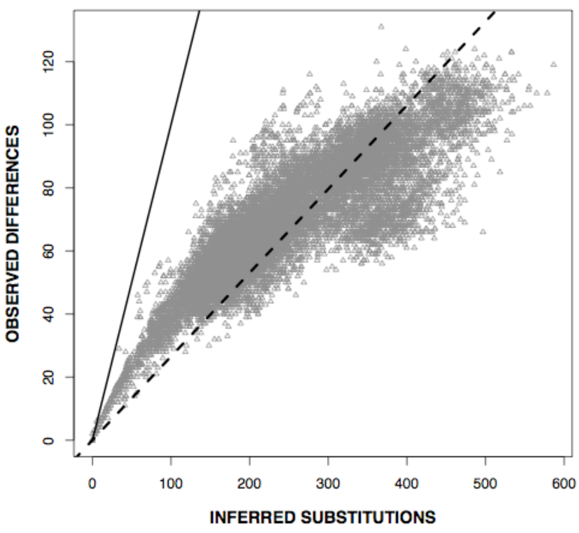
Here we compared saturation of the nucleotide substitutions in the two nuclear recombination activating gene 1 (RAG1) and rhodopsin (RHO) markers and 4 mitochondrial cytochrome b (CYB), 12S rRNA, 16rRNA, and cytochrome c oxidase subunit 1 (COX1) markers when inferring the phylogeny of Mediterranean teleosts.

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.



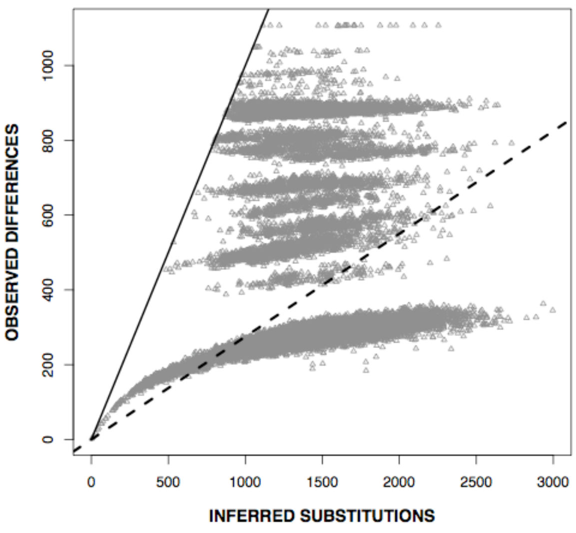
Saturation plot of the RAG1 marker.

Dashes correspond to the regression line through the origin (slope = 0.52).



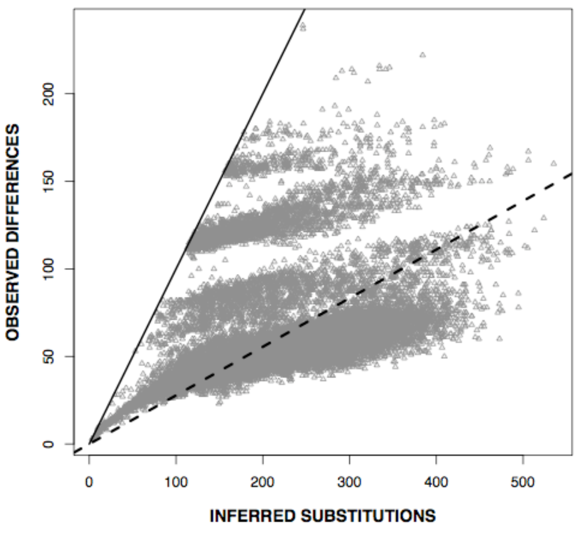
Saturation plot of the RHO marker.

Dashes correspond to the regression line through the origin (slope = 0.26).



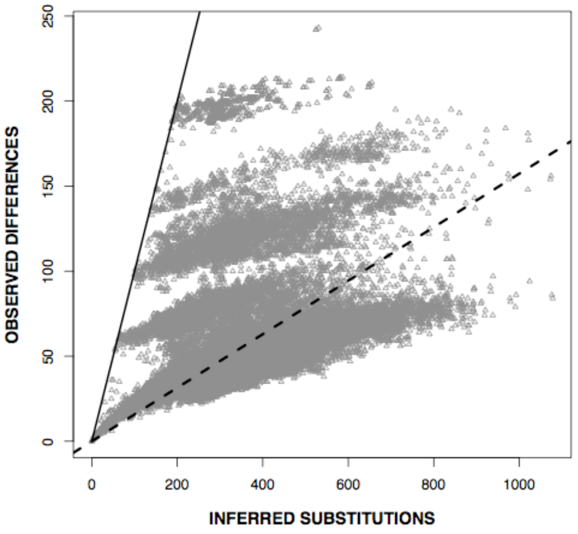
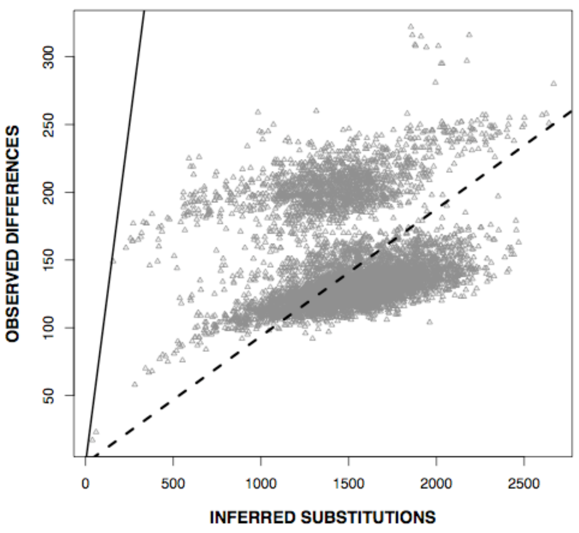
Saturation plot of the CYB marker.

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).

<table>
<tr>
<td>
</td>
<td>
</td>
</tr>
<tr>
<td>Saturation plot of the 16SrRNA marker.
Dashes correspond to the regression line through the origin (slope = 0.16).</td>
<td>Saturation plot of the COX1 marker.
Dashes correspond to the regression line through the origin (slope = 0.09).</td>
</tr>
<tr>
<td colspan="2">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.</td>
</tr>
</table>

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

Table S3.2 List of species attached to the raw chronogram.

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
		Dalophis imberbis (Delaroche 1809)	Native	F
		Ophichthus rufus (Rafinesque 1810)	Endemic	F
		Ophisurus serpens (Linnaeus 1758)	Native	F
		Pisodonophis semicinctus (Richardson 1848)	Exotic	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
	Hemiramphidae	*Hyporhamphus picarti* (Valenciennes 1847)	Native	C1
Clupeiformes	Clupeidae	*Herklotsichthys punctatus* (Rüppell 1837)	Exotic	F
Gadiformes	Macrouridae	*Nezumia sclerorhynchus* (Valenciennes 1838)	Native	F
	Moridae	*Eretmophorus kleinenbergi* Giglioli 1889	Native	F
		Gadella maraldi (Risso 1810)	Native	F
		Lepidion guentheri (Giglioli 1880)	Exotic	F
		Lepidion lepidion (Risso 1810)	Endemic	F
		Physiculus dalwigki Kaup 1858	Native	F

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
Osmeriformes	Alepocephalidae	*Alepocephalus rostratus* Risso 1820	Native	O
	Argentinidae	*Glossanodon leioglossus* (Valenciennes 1848)	Native	F
	Microstomatidae	*Nansenia iberica* Matallanas 1985	Endemic	O
		Nansenia oblita (Facciolà, 1887)	Native	O
		Microstoma microstoma (Risso 1810)	Native	O
Perciformes	Apogonidae	*Apogon pharaonis* (Belloti 1874)	Exotic	C1
	Blenniidae	*Hypleurochilus bananensis* (Poll 1959)	Native	F
		Salaria basilisca (Valenciennes 1836)	Endemic	C1
	Callionymidae	*Callionymus fasciatus* Valenciennes 1837	Native	C2
		Callionymus filamentosus Valenciennes 1837	Exotic	C2
		Callionymus pusillus Delaroche 1809	Native	C2
		Callionymus risso Lesueur 1814	Native	C2
		Synchiropus phaeton (Günther 1861)	Native	F
	Carangidae	*Campogramma glaycos* (Lacepède 1801)	Native	F
		Naucrates 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
		Microichthys coccoi Rüppell 1852	Endemic	F
		Microichthys sanzoi Sparta 1950	Endemic	F
	Gobiidae	*Buenia jeffreysii* (Günther 1867)	Native	C1
		Chromogobius quadrivittatus (Steindachner 1863)	Endemic	F
		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 bentuvii 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
	Upeneus asymmetricus Lachner 1954	Exotic	C1
Nomeidae	*Cubiceps capensis* (Smith 1845)	Native	F

	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 haffara* (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	Bothidae	*Arnoglossus kessleri* Schmidt 1915	Endemic	C2
		Arnoglossus rueppelii (Cocco 1844)	Native	C2
	Cynoglossidae	*Cynoglossus sinusarabici* (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	Liparidae	*Eutelichthys leptochirus* Tortonese 1959	Endemic	O
		Paraliparis murieli Matallanas 1984	Endemic	O
	Peristediidae	*Peristedion cataphractum* (Linnaeus 1758)	Native	O
	Platycephalidae	*Papilloculiceps longiceps* (Cuvier 1829)	Exotic	O
		Platycephalus indicus (Linnaeus 1758)	Exotic	O
		Sorsogona prionota (Sauvage 1873)	Exotic	O
	Scorpaenidae	*Scorpaena loppei* Cadenat 1943	Native	C2
		Scorpaena stephanica Cadenat 1943	Exotic	C2
	Triglidae	*Lepidotrigla dieuzeidei* Blanc & Hureau 1973	Native	C1
Stomiiformes	Phosichthyidae	*Vinciguerria attenuata* (Cocco 1838)	Native	C1
	Sternoptychidae	*Valenciennellus tripunctulatus* (Esmark 1871)	Native	F
	Stomiidae	*Bathophilus nigerrimus* Giglioli 1882	Native	F
Syngnathiformes	Syngnathidae	*Nerophis maculatus* Rafinesque, 1810	Native	C1
		Minyichthys sentus Dawson, 1982	Native	F

		Syngnathus phlegon Risso 1827	Native	C2
		Syngnathus tenuirostris Rathke 1837	Endemic	C2
Tetraodontiformes	Diodontidae	*Diodon hystrix* Linnaeus 1758	Exotic	O
	Monacanthidae	*Stephanolepis diaspros* Fraser-Brunner 1940	Exotic	O
	Ostraciidae	*Tetrosomus gibbosus* (Linnaeus 1758)	Exotic	O
	Tetraodontidae	*Lagocephalus lagocephalus* (Linnaeus 1758)	Exotic	C2
		Lagocephalus suezensis Clark & Gohar 1953	Exotic	C2
		Torquigener flavimaculosus Hardy & Randall 1983	Exotic	F
		Tylerius spinosissimus (Regan 1908)	Exotic	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 sequencially 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/).