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## Short Communication

Molecular systematics, phylogeny and biogeography of roaches (*Rutilus*, Teleostei, Cyprinidae)V. Ketmaier<sup>a,\*</sup>, P.G. Bianco<sup>b</sup>, J.-D. Durand<sup>c</sup><sup>a</sup> Unit of Evolutionary Biology/Systematic Zoology, Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Strasse 24-25, Haus 25, D-14476 Potsdam, Germany<sup>b</sup> Dipartimento di Zoologia, Università di Napoli "Federico II", V. Mezzocannone 8, I-80134 Naples, Italy<sup>c</sup> IRD UR 070 RAP route des hydrocarbures, BP 1386, Bel Air, Dakar, Sénégal

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## 1. Introduction

Cyprinid species diversity is not evenly distributed across Europe. Central Europe hosts a homogeneous ichthyofauna, while many taxa are endemic to relatively narrow Southern European areas. This led to the recognition of 13 ichthyogeographic districts around the Mediterranean Sea (Fig. 1), each of which characterized by its own assemblage of endemic species (Bianco, 1990). Two biogeographic hypotheses have been proposed to explain the evolution of cyprinids in Southern Europe. Banarescu (1960) suggested that cyprinids reached the area in the early Miocene from Siberia and Central Europe via several independent waves of colonization through river captures. This scenario foresees a long and gradual process of dispersal influenced by the hydrological and geotectonic evolution of European landmasses. In contrast, Bianco (1990) proposed that the major diversification was rapid and took place in the late Messinian. At that time the Mediterranean Sea was an evaporative basin; it almost completely dried up following the closure of the connection with the Atlantic Ocean (Messinian salinity crisis, 5.5 MYA; Hsü, 1987). The desiccated Mediterranean was then flooded with freshwaters drained from the Paratethys, an oligosaline internal sea covering large parts of Central-Western Europe. This freshwater phase of the Mediterranean Sea (termed "Lago Mare") represented a main route of dispersal for a variety of strictly freshwater taxa and ended with the re-opening of the Strait of Gibraltar when the invasion of Atlantic waters re-established normal salinity conditions in the Mediterranean Sea. This promoted multiple vicariant events by forcing isolation of strictly freshwater taxa in the peri-Mediterranean river drainages and led to the present high level of endemism in the area (Bianco, 1990). So far, several cyprinid lineages have been used to test the

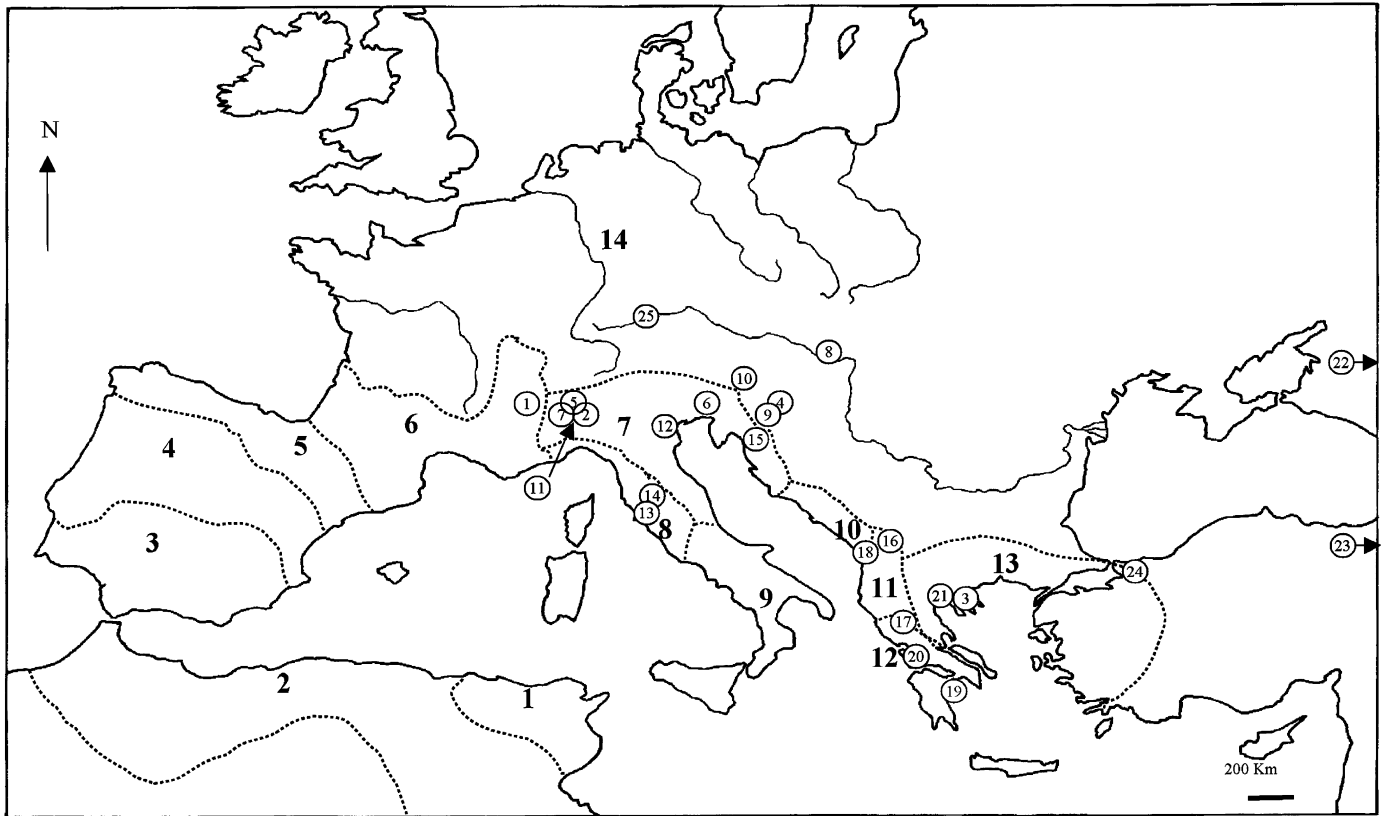
two alternative hypotheses (Zardoya and Doadrio, 1999; Durand et al., 2000, 2002a,b, 2003; Ketmaier et al., 1998, 2003, 2004; Tsigenopoulos et al., 2003 and references therein). Although many of these studies have invoked the Lago Mare phase to explain patterns of divergence, evidence supporting it as the most likely scenario is not always unambiguous.

Here, we used roaches (genus *Rutilus*) to infer speciation patterns of primary freshwater fishes in the Eastern peri-Mediterranean area. The genus is widespread in the Central and Western Palearctic region (Bianco et al., 2004). It shows a clear preference for riverine and lacustrine warm waters. Only three species are reported for Central Europe, *R. meidingerii*, *R. rutilus* and *R. pigus*, the latter with a dubious disjunct distribution in the Danube and Po (Northern Italy) river basins. Conversely, at least eight species are endemic to Southern Europe, although their exact number is difficult to establish because the genus systematics has been revised to a great extent (Bianco et al., 2004). Recently, Bogutskaya and Iliadou (2006) split the genus in two subgenera (*Rutilus* and *Pararutilus*). *Rutilus* would include all the recognized species but *R. frisii* and *R. pigus* that should belong to *Pararutilus*. The nomenclature of the group has been plagued by an extensive usage of subspecies. Here, according to Kottelat (1997), we have adopted the binomial nomenclature considered valid in the FishBase database (<http://www.fishbase.org>).

The aims of the present study are: (1) to produce a phylogenetic hypothesis for the genus *Rutilus* by using sequence variation of the entire mitochondrial (mt) cytochrome *b* (*cyt-b*) gene. To accomplish this we had sampled a total of 22 populations from the Eastern peri-Mediterranean area and Central Europe. These include 12 species (five of which with multiple populations; Fig. 1 and Table 1) out of the 14 reported in FishBase; (2) to test whether molecular data support the current systematics of the genus; and (3) to produce a biogeographic hypothesis for its diversification based on estimates of divergence times. Results will be discussed in light

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**Fig. 1.** Schematic map of the peri-Mediterranean areas with the ichthyogeographic districts identified by Bianco (1990) and the localities from which the different populations and species included in the study have been collected. Dotted lines represent district boundaries; bold numbers identify the different districts; circled numbers are sampled localities. Bold and circled numbers correspond to the bracketed numbers in the second column of Table 1 (first and second value, respectively).

of the two contrasting biogeographic scenarios available for Southern European primary freshwater fishes.

## 2. Methods

### 2.1. Sampling and DNA sequencing

Fishes were caught by hand-net or portable electro-shocker. Scales or fragments of fins were dissected and fixed directly in ethanol 98%. Most fishes were released alive. The geographic origins of individuals used for genetic analyses are listed in Table 1 and shown in Fig. 1; the same figure depicts the different peri-Mediterranean ichthyogeographic districts (Bianco, 1990). Samples that were not released are deposited in the Fish Collection IZA, at the Department of Biological Sciences, University of Naples "Federico II", Italy.

Total DNA was extracted from 98% ethanol-preserved fins and/or scales of 59 individuals (Table 1) and the entire mt *cyt-b* gene was PCR amplified and directly sequenced. Experimental conditions were as in Ketmaier et al. (2004). Each PCR product was sequenced in either direction. Sequences have been submitted to GenBank (Accession No. FJ025061–FJ025085).

### 2.2. Phylogenetic analyses

Sequences were edited and aligned using Sequencher 4.6 (Gene Code Corporation, Ann Arbor, MI). Alignment was straightforward; it was further checked by eye with the aid of the reading frame. No ambiguities were found and there was no need to postulate any gap.

Aligned sequences were analyzed by the Maximum Parsimony (MP), Maximum Likelihood (ML), Neighbor-Joining (NJ) and Bayesian methods using PAUP\* 4.0β10 (Swofford, 2002) and MrBayes (Huelsenbeck, 2000). We ran the ML analyses with the best-fit model estimated using ModelTest (Posada and Crandall, 1998), which was the GTR +  $\Gamma$  model (variable rates, shape parameter  $\alpha = 0.1563$ ; parameters selected with the Akaike Information Criterion-AIC). NJ analyses were carried out on ML distances ( $D_{ML}$ ) calculated with the same parameters used for ML analyses. The same model of sequence evolution was also employed in the Bayesian searches. Site-specific rate variation was partitioned by codon positions. MrBayes was run for 2 million generations (one cold and three heated Markov chains; two independent runs) with a sampling frequency of 100 generations. We discarded the first 25% of the trees found before constructing a majority rule consensus tree. Statistical significance was evaluated by bootstrapping (1000 replicates for MP and NJ and 100 replicates for ML) and posterior probabilities. As outgroups we used *Leuciscus cephalus*, *Telestes souffia* (Accession No. AY509827 and AY509859, respectively), *Leuciscus pyrenaicus* (Accession No. AF421827), *Leuciscus smyrnaeus* (Accession No. AJ252814), and *Chondrostoma nasus* (Accession No. Z75109). Given the availability of a recent phylogeographic study of *R. frisii* (Kotlík et al., 2008), we included in our data set three haplotypes of this species (Table 1). These are representatives of the three main haplogroups found across the species' range.

Trees yielded by different phylogenetic methods as well as competing phylogenetic hypotheses were tested using the ML-based Approximately Unbiased tree selection test (AU) in Consel

**Table 1**

Sample sizes (N), sampled rivers/lakes and ichthyogeographic districts (second column; first bracketed value; bold numbers in Fig. 1), number of scored haplotypes, haplotype codes (only for multiple haplotypes within each population) and GenBank Accession Numbers for the *Rutilus* populations and species included in the study

Taxon (with authority)	N	River basin/Lake (Ichth. district/Map)	Country	No. of haplotypes (haplotype code)	Accession No./Reference
<i>R. rutilus</i> (Linnaeus, 1758)	–	Rhone R. (6/1)	France	1 (-)	FJ025072
<i>R. rutilus</i>	3	Ticino R. (7/2)	Italy	3 (T-1; T-2; T-3)	FJ025077–FJ025079
<i>R. rutilus</i>	3	Volvi L. (13/3)	Greece	1 (-)	FJ025074
<i>R. rutilus</i>	3	Danube R. (14/4)	Slovenia	1 (-)	FJ025068
<i>R. pigus</i> (Lacepède, 1803)	3	Naviglio Langosco (7/5)	Italy	1 (-)	FJ025084
<i>R. pigus</i>	3	Livenza R. (7/6)	Italy	1 (-)	FJ025063
<i>R. pigus</i>	3	Can. Quintino Sella (7/7)	Italy	2 (QS-1; QS-2)	FJ025081–FJ025082
<i>R. pigus</i>	3	Danube R. (14/8)	Slovakia	1 (-)	FJ025064
<i>R. pigus</i>	3	Danube R. (14/9)	Slovenia	1 (-)	FJ025065
<i>R. pigus</i>	3	Drava R. (14/10)	Slovenia	2 (D-1; D-2)	FJ025075–FJ025076
<i>R. aula</i> (Bonaparte, 1841)	3	Ticino R. (7/11)	Italy	1 (-)	FJ025069
<i>R. aula</i>	3	Brenta R. (7/12)	Italy	1 (-)	FJ025080
<i>R. rubilio</i> (Bonaparte, 1837)	3	Farma R. (8/13)	Italy	1 (-)	FJ025067
<i>R. rubilio</i>	3	Tiber R. (8/14)	Italy	1 (-)	FJ025061
<i>R. basak</i> (Heckel, 1843)	3	Neretva R. (10/15)	Croatia	1 (-)	FJ025083
<i>R. ohridanus</i> (Karaman, 1924)	3	Moraca R. (11/16)	Montenegro	1 (-)	FJ025085
<i>R. prespensis</i> (Karaman, 1924)	3	Micraprespa L. (11/17)	Greece	1 (-)	FJ025062
<i>R. prespensis</i>	2	Skadar L. (11/18)	Montenegro	1 (-)	FJ025066
<i>R. ylikensis</i> Economidis, 1991	3	Kifissios R. (12/19)	Greece	1 (-)	FJ025070
<i>R. panosi</i> Bogutskaya and Iliadou, 2006	3	Trichonis L. (12/20)	Greece	1 (-)	FJ025071
<i>R. vegariticus</i> Stephanidis, 1950	3	Vegoritiss L. (13/21)	Greece	1 (-)	FJ025073
<i>R. caspicus</i> (Yakovlev, 1870)	–	Samur R. (14/22)	Caucasus	1 (-)	AF095610
<i>R. frisii</i> Nordmann, 1840	–	Caspian S. (14/23)	Iran	1 (-)	EU285048
<i>R. frisii</i>	–	Iznik L. (14/24)	Turkey	1 (-)	EU285042
<i>R. frisii</i>	–	Mondsee L. (14/25)	Austria	1 (-)	EU285053

Map numbers (second column; second bracketed value) label sampling localities and correspond to circled numbers in Fig. 1. Each species authority is also given.

(Shimodaira and Hasegawa, 2001). Tree topologies were always compared simultaneously.

### 2.3. Biogeographic analysis and divergence times

We used TreeMap (Page, 1994) to test for concordance between the *cyt-b* and the ichthyogeographic district trees. The district area tree was drawn on the Banareescu hypothesis following Durand et al. (2003). We used the heuristic search option to reconcile the fish and the area trees and to find a single optimal reconstruction. We then tested the significance of the fit between the two trees by comparing the distribution of the same measure of fit for the random trees (Page, 1994). Our null hypothesis is that the ichthyogeographic district area is independent of the *cyt-b* tree. To test this hypothesis we generated 10,000 random trees in TreeMap. These trees had the same number of taxa as the actual *cyt-b* phylogeny. We then measured how the random trees fit the observed *cyt-b* tree in comparison with the district area tree. The proportion of random gene trees that have the same (or greater) number of speciation-separation events as the observed tree is the probability of obtaining the observed value by chance alone. Divergence times were calculated in a Bayesian MCMC framework by using Beast 1.4.6 (Drummond and Rambaut, 2007). We adopted a model of uncorrelated but log-normally distributed rates of molecular evolution (Drummond et al., 2006). We took advantage of a recent study on fossil utricular otoliths of cyprinids that assigns the first appearance of such records for *Rutilus* to the Middle Miocene (14.5–15.5 MYA) (Schulz-Mirbach and Reichenbacher, 2006). We used priors with soft upper bounds in the analysis; this implies that the fossils provided a hard minimum age for the origin of the genus but no hard maximum age. We adopted lognormal prior distributions for the calibration in the analysis so that 95% of the prior weight fell on the specified interval. We also used a Yule prior on rates of evolution because this more accurately resembles phylogenetic processes at the species level. We adopted the same GTR+model as in the ML and Bayesian searches. We ran five independent analyses of 50,000,000 generations each; the corresponding

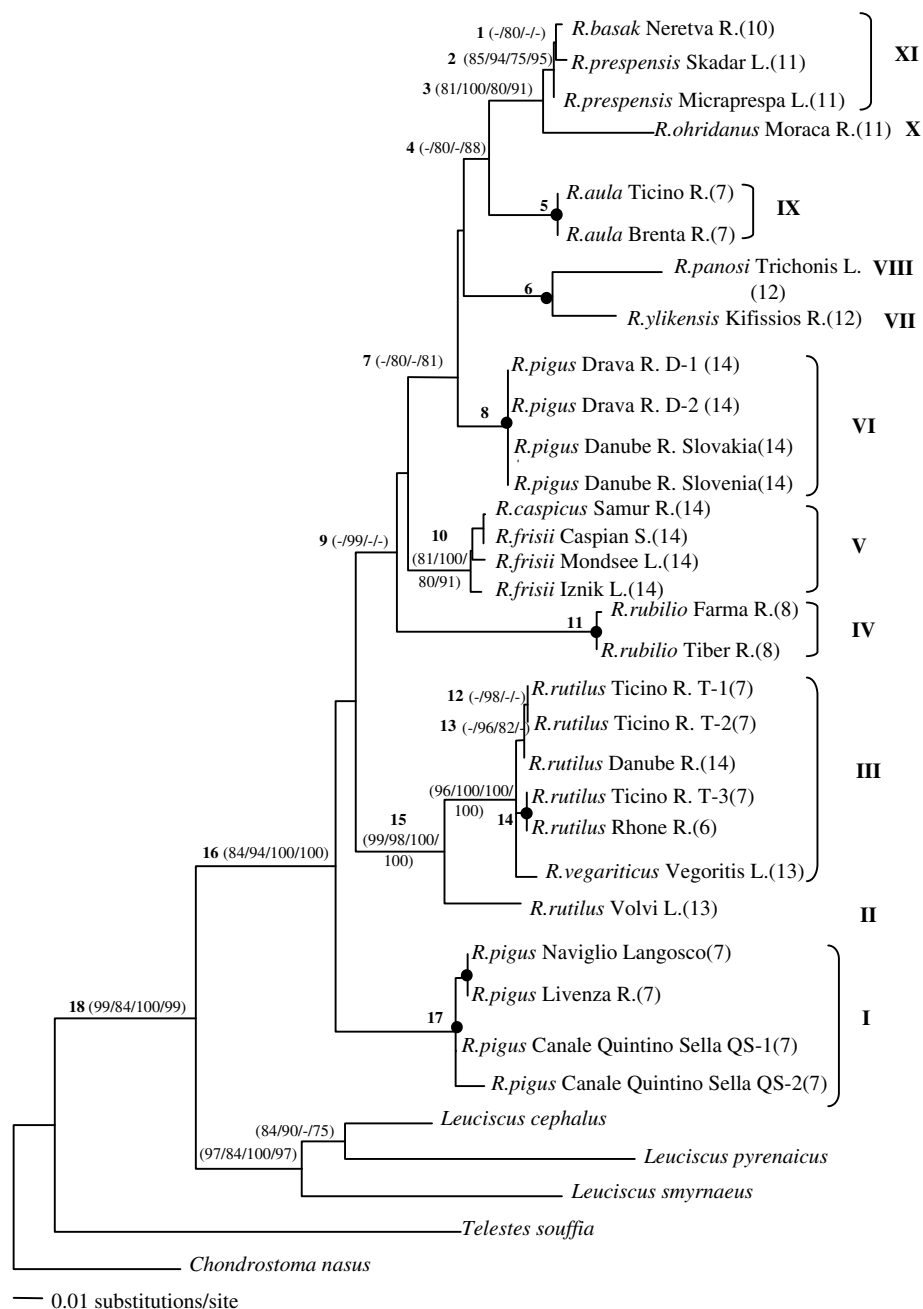
outputs were analyzed using Tracer 1.4, TreeAnnotator 1.4.6 and FigTree 1.0 (Drummond and Rambaut, 2007).

## 3. Results and discussion

### 3.1. Phylogeny and taxonomic implications

Fig. 2 shows the single topology produced by our ML analysis (-ln likelihood = 5410.49) and summarizes the results of the other phylogenetic methods employed in the study. The ML, Bayesian, NJ and MP trees were statistically indistinguishable with the AU test ( $0.99 \geq p \geq 0.083$ ).

Our results are at odds with the morphological revision of Bogutskaya and Iliadou (2006) as they do not support reciprocal monophyly for the two putative subgenera *Rutilus* and *Pararutilus*. *R. frisii* and *R. pigus*, which should both belong to the subgenus *Pararutilus*, do not cluster together. Instead *Pararutilus* is embedded within *Rutilus*. We recognize eleven statistically supported lineages (I–XI in Fig. 2). The vast majority of them correspond to nominal taxa, with three noticeable exceptions. These are (1) lineages I and VI (both identified morphologically as *R. pigus*); (2) lineages II and III, with *R. vegariticus* embedded within *R. rutilus* and with a very divergent haplotype found in the Lake Volvi; and (3) lineage XI, which includes the genetically very similar *R. prespensis* and *R. basak*. Average ML distances within *R. pigus* ( $0.128 \pm 0.01$ ) and *R. rutilus* ( $0.102 \pm 0.04$ ) are only slightly lower than those obtained comparing *R. aula*, *R. rubilio*, *R. panosi* and *R. ylikensis* ( $0.140 \pm 0.04$ ). Moreover, results of the AU tests show that alternatively enforcing monophyly of *R. pigus* and *R. rutilus* produced trees statistically worse than the unconstrained topology ( $p \leq 0.002$ ); the AU test could not reject *R. prespensis* as monophyletic ( $p = 0.102$ ). *R. pigus* haplotypes are clearly split into two allopatric groups, one from the Padano-Venetian district (7) and one from the Danubian district (14). Kottelat (1997) already postulated the existence of two different endemic species in the districts (*R. pigus* and *R. virgo*, respectively); our data support such a taxonomic arrangement. It's worth noting that the Padano-Venetian *R. pigus* (lineage I) is



**Fig. 2.** ML estimate of the phylogenetic relationships among the populations and species included in the study using the GTR +  $\Gamma$  model ( $-\ln$  likelihood = 5410.49; shape parameter  $\alpha = 0.1563$ ). Progressive bold numbers at nodes (from 1 to 18) refer to time estimates and the associated mean age (MYA) and 95% HPD are shown in Table 2. Node 18 is the calibration node. Bracketed numbers at nodes are bootstrap support for ML, NJ and MP searches (first, third and fourth values) and posterior probability for the Bayesian searches (2 million generations). Only nodes with a support  $\geq 75\%$  for at least one phylogenetic method are labeled. Dots identify nodes with maximum support for all phylogenetic methods. Numbers after population/species names are ichthyogeographic districts and correspond to those in Table 1 and Fig. 1. Roman numbers identify different lineages (see text).

currently threatened by the artificial introduction of *R. rutilus* in that district from Central Europe (Bianco, 1995). The close genetic affinity among *R. rutilus* haplotypes from Southern France, Danubian and Padano-Venetian districts may be taken as an indirect indication of these human-induced changes (Bianco and Ketmaier, 2001). Our data also suggest the existence of a new species in Lake Volvi; this lineage is currently attributed to *R. rutilus*. The formal morphological description of this new entity, which has to be coupled with a thorough taxonomic revision of the entire genus, is out of the scopes of this study and will be the subject of another contribution. Molecular data support neither *R. basak/R. prespensis*

nor *R. vegariticus/R. rutilus* as different species. For priority reasons *R. prespensis* should therefore be synonymized into *R. basak* and *R. vegariticus* into *R. rutilus* (see Table 1 for species authorities). Lineages IV (*R. rubilio*), VII (*R. ylikensis*), IX (*R. aula*) and XI (*R. prespensis/R. basak*) also have distinct karyological profiles (Bianco et al., 2004). *R. prespensis* and *R. basak* share the same chromosomal organization (Bianco et al., 2004). The low genetic divergence between *R. caspicus* and *R. frisii* is quite puzzling, as the two species have clearly different morphologies. We cannot draw any firm conclusion on this point, as our results are based on published data only and just on a single sequence for *R. caspicus*. It is evident that



further comparative molecular work on the two species coupled with an accurate morphological examination is much needed to test whether they represent different lineages or not. Our phylogenetic results, although robust, are based on a single, exclusively maternally inherited marker. Therefore we cannot exclude that screening nuclear loci might change the scenario proposed here. On the other hand, we are confident that more extensive sequencing would not challenge our major conclusions regarding allopatric and reciprocally monophyletic lineages carrying profoundly divergent haplotypes (i.e. I, III, IV, VI, VII, VIII, and IX). Mesquita et al. (2005) showed that it took a minimum of 0.1 MYA for allopatric lineages of the cyprinid *Squalius aradensis* to acquire reciprocal monophyly at both nuclear and mitochondrial loci. The species is endemic to a few Portuguese drainages. Divergence times among our allopatric lineages are from 7 to 145 times wider than those reported for *Squalius aradensis* (see next paragraph and Table 2), not to mention the much ampler geographical scale.

### 3.2. Divergence times and biogeographic scenario

Our TreeMap test for congruence between the *cyt-b* tree and the area cladogram based on the Banarescu hypothesis found 7 speciations by district, 19 speciations inside a district, 1 migratory event and 23 sorting events. Sorting events identify sister species present in the area in the past but absent nowadays. There is no statistical support for the association between the gene and the area trees; the *p* value inferred from the percentage of random trees with the same (or greater) number of speciation-by-district events as the actual *cyt-b* tree indicates that such an association may be due to chance alone ( $p = 1.000$ ). This implies that the cladogenesis of the genus cannot be explained under the assumption of a long, ancient (started around 35 MYA) and gradual colonization of the peri-Mediterranean area (Banarescu, 1960). Divergence times further support this view, although they have ample confidence intervals and should thus be considered cautiously (Table 2). Based on our phylogenetic and divergence time results we propose the fol-

lowing scenario for the diversification of the genus *Rutilus*. The origin of the group most likely dates back to the middle Miocene (Zardoya and Doadrio, 1999; Schulz-Mirbach and Reichenbacher, 2006). We do not have the proper taxon sampling to directly infer neither the tempo nor the geographic origin of the genus. However, there is a considerable amount of evidence suggesting that present European fish-fauna is mostly of Eastern Asian origin (Bianco, 1990; Durand et al., 2002a,b). This ancestral lineage(s) might have reached the Mediterranean area through the Paratethys and the water captures around that basin in the middle Miocene. The Paratethys was a dilution oligosaline sea at that time and therefore a route of dispersal for freshwater taxa (Bianco, 1990; Kotlík et al., 2008). In our phylogeny there are two nearly coeval nodes (4, and 7; Fig. 2 and Table 2). This implies a common vicariant event behind them (Ketmaier et al., 2006). Our time estimates for those nodes are centered on the Messinian salinity crisis of the Mediterranean Sea (Krijgsman et al., 1999). During this phase the desiccated Mediterranean Sea was flooded with fresh or slightly brackish waters from the Paratethys and the whole basin was reduced to a network of freshwater lakes (Lago Mare phase) (Bianco, 1990; Clauzon et al., 2005). This phase, although short, allowed a major penetration of *Rutilus* (and other freshwater taxa) in the area. The resettlement of normal salinity conditions that followed the re-opening of the Strait of Gibraltar isolated the different peri-Mediterranean river drainages from one another and led to the main splits within the genus. Later on, at every Pleistocene glacial maximum, marine regressions determined river confluences in epicontinental seas. Many previously isolated river basins came in contact at that time (Bianco, 1990). The affinity between Greek and Danubian lineages of *R. rutilus* and their Pleistocene origin are in line with this scenario and closely resembles the findings of Durand et al. (1999, 2003) for the genera *Leuciscus* and *Chondrostoma*. According to Zardoya et al. (1999) cyprinids colonized Greece at least two times independently, in the middle Miocene and in the Plio-Pleistocene. All Greek taxa included in the present study are Plio-Pleistocene in origin (nodes 6 and 14; Fig. 2 and Table 2). They therefore should belong to the second, most recent wave of colonization, which involved other warm-water adapted and/or eurythermal genera such as *Barbus*, *Alburnus* and *Leuciscus*. In contrast, the most ancient Miocene wave of colonization included mainly cold-water adapted taxa with the notable exception of the dace *Leuciscus leuciscus* (Costedoat et al., 2007). We obtained similar time estimates for the diversifications of the cold-adapted riverine *Telestes* and of the warm-adapted riverine/lacustrine *Scardinius* at the scale of the East peri-Mediterranean area (Ketmaier et al., 2004).

We found general support to the Lago Mare hypothesis, although, since the genus is not entirely Messinian in origin, we cannot fully embrace it. More realistically, the Banarescu and Lago Mare scenarios are not mutually exclusive but target different moments in the evolution of the family in Southern Europe (Robalo et al., 2007). The earlier phases of colonization can be brought back to the Banarescu's hypothesis of dispersal through river captures from the Paratethys and its surroundings. The rapid Messinian changes in the ecological conditions of the Mediterranean Sea determined a dramatic increase in the diversity of the group by a combination of dispersal through the Lago Mare and subsequent vicariance when the freshwater phase of the Mediterranean Sea came to an abrupt end.

The results presented here provide further support for Southern Europe as a major area of regionalization for the freshwater fauna. Furthermore, they are in line with the criteria recently proposed by Abell et al. (2008) to delineate freshwater ecoregions at a global scale. A question still open is whether the low Central European diversity (in terms of number of endemic species) testifies a pre-Quaternary evolution or rather is the resultant of the effects of

**Table 2**  
Mean and 95% HPD of *Rutilus* divergence times

Node	Mean (MYA)	95% HPD (MYA)
1	0.73	0.08–1.75
2	1.28	0.19–2.75
3	2.72	1.05–4.83
4	4.93	2.21–8.04
5	0.71	0.02–1.98
6	3.32	1.12–5.91
7	7.17	3.49–11.34
8	0.86	0.05–2.31
9	10.47	5.40–16.45
10	1.99	0.29–4.75
11	0.61	0.006–1.96
12	0.12	0.00–0.43
13	0.49	0.01–1.29
14	0.17	0.00–0.63
15	1.75	0.35–3.71
16	10.5	8.13–12.69
17	1.90	0.25–4.68
18 <sup>a</sup>	14.90	14.60–15.30

Node refers to labels in Fig. 2. In all cases 95% credible intervals for node age estimates overlap. The mean evolutionary rate is 0.0027 substitutions per site per million years (95% High Posterior Density, HPD: 0.0011–0.0090; Effective Sample Size, ESS: 1227.03). The Yule process birth rate is 0.215 (95% HPD: 0.124–0.316; ESS: 2181.41). The data slightly deviate from a strict clock-like behavior, the coefficient of variation being 0.004 (HPD: 0.001–0.016; ESS: 1380.87). The mean covariance value between parent and daughter branches spans zero (0.144; HPD: –0.133 to 0.398; ESS: 5918.81). This implies that branches with fast and slow rates are next to each other in the phylogenetic tree. There is thus no evidence of autocorrelation of rates in the tree.

<sup>a</sup> Calibration node.

the Quaternary glaciations that extirpated most of the original fauna. If so, the lineages currently found in Central Europe would represent recent immigrants of Asian origin (Durand et al., 2002a,b). Should this hypothesis be true, this would imply that Central and Southern European species are phylogenetically less related than previously thought. This scenario obviously requires further testing with an appropriate sampling scheme. Nonetheless, it opens interesting perspectives for additional research aimed at a more global understanding of the phylogeny and evolution of this successful family of freshwater fishes.

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