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Short Communication

Phylogenetic relationships of the algae scraping cyprinid genus *Capoeta* (Teleostei: Cyprinidae)

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ABSTRACT

We reconstructed the matrilineal phylogeny of Asian algae-eating fishes of the genus *Capoeta* based on complete mitochondrial gene for cytochrome *b* sequences obtained from 20 species sampled from the majority of the range and 44 species of closely related barbs of the genera *Barbus* s. str. and *Luciobarbus*. The results of this study show that *Capoeta* forms a strongly supported monophyletic subclade nested within the *Luciobarbus* clade, suggesting that specialized scraping morphology appeared once in the evolutionary history of the genus. We detected three main groups of *Capoeta*: the Mesopotamian group, which includes three species from the Tigris–Euphrates system and adjacent water bodies, the Anatolian–Iranian group, which has the most diversified structure and encompasses many species distributed throughout Anatolian and Iranian inland waters, and the Aralo-Caspian group, which consists of species distributed in basins of the Caspian and Aral Seas, including many dead-end rivers in Central Asia and Northern Iran. The most probable origination pathway of the genus *Capoeta* is hypothesized to occur as a result of allopolyploidization. The origin of *Capoeta* was found around the Langhian–Serravallian boundary according to our molecular clock. The diversification within the genus occurred along Middle Miocene–Late Pliocene periods.

1. Introduction

The Cyprininae, which represents the largest and most complex subfamily within Cyprinidae (Bănărescu and Coad, 1991; Berrebi et al., 1996), encompasses several specialized trophic groups characterized by their singular morphology. One of these groups are algae scrapers, which feed predominantly on periphyton scraped from rocks and stones using a horny cutting edge on the lower jaw. Several genera (*Capoeta*, *Cyprinion*, *Onychostoma*, *Scaphiodonichthys, Semiplotus, Varicorhinus*) are algae scrapers exclusively, whereas only some species or intraspecies trophic morphs (or populations) in other genera (*Diptychus*, *Schizocypris*, *Schizopygopsis*, *Schizothorax*, *Poropuntius* and *Labeobarbus*) are specialized algae scrapers (Berg, 1949; Groenewald, 1958; Roberts, 1998). The phylogenetic position of various algae scrapers within the Cyprininae suggests replicated origins of algae scraping as a foraging strategy.

Cyprinines of the genus *Capoeta* are widely distributed throughout Western Asia from Anatolia to the Levant, Transcaucasia, the Tigris and Euphrates basins, most of Iran, Turkmenistan, Northern Afghanistan, and the upper reaches of the Amu-Darya and Syr-Darya drainages (Bănărescu, 1999). The phylogenetic relationships of the genus *Capoeta* remain poorly studied until now. Out of about 20 species currently recognized within *Capoeta* (Turan et al., 2008), only few have been included in previous phylogenetic analyses (Durand et al., 2002; Tsigenopoulos et al., 2003; Turan, 2008). These studies showed that *Capoeta* are closely related to the Euro-Mediterranean barbs of the genus *Luciobarbus* (Berrebi and Tsigenopoulos, 2003; Tsigenopoulos et al., 2010), which are known to be evolutionarily tetraploid (Bănărescu and Bogutskaya, 2003). However, all karyotyped *Capoeta* species are hexaploid (Krysanov,

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1999 and others, see http://www.briancoad.com in detail), thereby suggesting that hexaploidy is evolutionarily fixed within the *Capoeta* lineage.

Our main goals are to contribute to the understanding of the inner phylogeny of the genus *Capoeta* and to find whether this genus constitutes a monophyletic mitochondrial lineage nested within the *Luciobarbus* clade (which suggests that scraping morphology appeared once in the evolutionary history of the group) or it demonstrates polyphyly (whereby the specialized scraping morphology has been derived independently several times). For this purpose, we used the complete cytochrome *b* gene sequence polymorphism, which has shown its utility in previous mtDNA genetic studies on barbs (Zardoya and Doadrio, 1999; Tsigenopoulos et al., 2003, 2010), on a large set of *Capoeta* and *Luciobarbus* species with a broad geographic coverage.

2. Material and methods

2.1. Sample collection

DNA samples were collected from 20 described taxa of *Capoeta* from 57 populations inhabiting basins of the Mediterranean, Aegean, Marmara, Black, Caspian and Aral Seas, the Gulfs of Persia and Oman in the Arabian Sea, and inland basins from Central Asia, Iran and Turkey; three samples of *Capoeta* were included from Genbank. In order to perform a comparison with closely related barbs, 44 species of both *Barbus s.* str. and *Luciobarbus* lineages were also included in the analysis. *Cyprinion macrostomus* and *Cyprinus carpio* were used as most external outgroups. *Aulopyge huegelii* was used as outgroup based on a previous published phylogeny, which demonstrates this species as the closest relative to our ingroup (*Barbus*/*Luciobarbus*/*Capoeta*) (Machordom and Doadrio, 2001; Tsigenopoulos et al., 2003). All these samples as well as the new GenBank accession numbers are listed in Table 1.

2.2. DNA extraction, PCR amplification and sequencing

DNA was extracted from a fin-clip or muscle using the DNeasy® Blood & Tissue Kit (QIAGEN). The entire cytochrome b (*cyt b*) gene (1140 bp) was amplified by PCR using those primers mentioned in Perdices and Doadrio (2001). In most cases, each PCR product was sequenced using the two amplification primers and sometimes using two internal primers, namely Cygmf (5′-GTYCAATGAAT TTGRGGTGGNTT-3′, designed by C. Pedraza-Lara, unpublished) and Cytbcap1 (5′-AANAGGAGGTGNAGAATGGTTG-3′; designed by C. Pedraza-Lara and B.A. Levin, unpublished). Double-stranded DNA was amplified in 25–50 μl reactions [1× buffer, 1.5 μM $MgCl_2$, 0.5 mM of each primer, 0.2 μM dNTP of each nucleotide, 17.55 μl ddH_2O, 1 μl template DNA, and 1U Taq polymerase (BioTools)]. PCR was performed at 94 °C (2 min), followed by 30 cycles at 94 °C (45 s), 46 °C (1 min), 72 °C (1 min 30 s), and a final extension at 72 °C (5 min). PCR products were visualized on 0.8% agarose gels and later purified by ethanol. Both strands were sequenced on an Applied Biosystems 3700 DNA sequencer following the manufacturer's instructions.

2.3. Sequence alignment and phylogenetic reconstructions

Homologous regions were aligned manually against previously published cytochrome *b* sequences of cyprinids (Zardoya and Doadrio, 1999) and visually checked. The transition (ti)/transversion (tv) rate was estimated using a maximum-likelihood approach (*cyt b* ti/tv = 12.37). The nucleotide composition was examined and the χ^2 homogeneity test of base frequencies for *cyt b* was carried out in Paup *4.0b10 (Swofford, 2002). This test indicated that base frequency distributions were always homogeneous across all sites (base frequencies: $A = 0.285$, $C = 0.292$, $G = 0.151$, $T = 0.272$). Saturation of transition and transversion changes was checked by plotting the absolute number of changes for transitions and transversions independently against uncorrected-p pairwise distances at each codon position. No evidence of saturation was found (Supplementary material). The Akaike Information Criterion (AIC) implemented in jModelTest 0.1.1 (Posada, 2008) was used to determine which evolutionary model best fitted the data set (GTR + G + I; rate matrix: $R(a)[A-C] = 0.51$, $R(b)[A-G] = 30.33$, $R(c)[A-T] = 0.46$, $R(d)[C-G] = 0.88$, $R(e)[C-T] = 9.97$, $R(f)[G-T] = 1.00$; $\alpha = 1.167$; $I = 0.566$). The model selected was used for subsequent analyses. Bayesian inference (BI) was performed with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) by simulating two simultaneous Markov chain analyses (MCMC) for 2,500,000 generations each to estimate the posterior probabilities distribution. The topologies were sampled every 100 generations and a majority-rule consensus tree was estimated after eliminating the first 10^5 generations in each analysis. The first 4000 trees were discarded as burn-in. Maximum Parsimony (MP) analysis was performed with the package Paup *4.0b10 (Swofford, 2002) with the TBR branch swapping and 10 random stepwise addition using the heuristic search algorithm. Maximum Likelihood (ML) analysis was carried out with PhyML package (Guindon and Gascuel, 2003). Confidence for these analyses was estimated by bootstrapping (500 replicates) (Felsenstein, 1985). MP and ML trees are represented as Supplementary material.

2.4. Molecular clocks and divergence time

Divergence times and their credibility intervals (highest posterior density: HPD) were estimated using a relaxed clock model in BEAST v1.4.8 (Drummond and Rambaut, 2007), which employs a Bayesian Markov chain Monte Carlo method to co-estimate tree topology, substitution rates and node ages. The branch rates were drawn following an uncorrelated lognormal distribution and a Yule speciation prior (Drummond et al., 2006). To carry out the molecular clock we set several calibration points based on fossil evidences for the Barbini: *Barbus* sp. and *Luciobarbus* sp. from central Europe dated as 17–19 mya and 16–17 mya respectively (Böhme and Ilg, 2003), and *Luciobarbus* sp. from the Iberian Peninsula dated as 4.9–7 mya (Doadrio and Casado, 1989). In the molecular clock estimation two independent analyses were performed and then combined using the LogCombiner v1.4.8 software within the Beast package (Drummond and Rambaut, 2007). Each final MCMC chain was run for 20,000,000 generations (10% burn-in), with parameters sampled every 1000 steps. Tracer v1.4 (Drummond and Rambaut, 2007) was used to plot the log-likelihood scores against generation time to evaluate run convergence and the burn-in needed before reconstructing the 50% majority-rule consensus. The effective sample sizes for all parameters of interest were greater than 200. Finally, the trees were summarized with the software TreeAnnotator v1.4.8 to obtain a maximum clade credibility tree (Drummond and Rambaut, 2007) with the estimated divergence times.

3. Results

3.1. Phylogenetic relationships and origin of Capoeta

Based on mitochondrial marker *cyt b*, BI, ML, and MP analyses highly supported the monophyly of all species of *Capoeta* included in this study (Fig. 1 and Supplementary material) and confirms that *Capoeta* is nested within the *Luciobarbus* lineage, which largely agree with opinions based on three species of the genus *Capoeta* included in previous mitochondrial phylogenetic studies (Berrebi

Table 1
Species names, sampling localities, and GenBank Accession Numbers. Sequences with * have been obtained in this study.

Species/subspecies	*n*	River, drainage, country	Accession nos.
Aulopyge huegelii	1	Sevarova jaruga, Cetina bas., Bosnia-Herzegovina	AF287415
Barbus balcanicus	1	Aliakmon River, Greece	AF287439
Barbus barbus	1	Durance River, France	Y10450
Barbus caninus	1	Judrio River, Po bas., Italy	AF287424
Barbus ciscaucasicus		Kuma River, Russia	AF095604
Barbus cyclolepis	1	Erithropotamos River, Evros bas., Greece	AF237579
Barbus cyri	1	Aras River, Armenia	AF145936
Barbus bergi	1	Kamchia River, Bulgaria	AY331035
Barbus kubanicus	1	Kuban River, Russia	AF095605
Barbus lacerta	1	Karakopru River, Tigris bas., Diyarbakir, Turkey	AF145935
Barbus macedonicus	1	Axios River, Greece	AY004753
Barbus meridionalis	1	Tordera River, Spain	JF798256*
	1	Besos River, Spain	JF798257*
Barbus peloponnesius	1	Thyamis River, Greece	AF287438
Barbus pergamonensis	1	Turkey	AF112434
Barbus sperchiensis	1	Sperchios River, Greece	AF090783
Barbus thessalus	1	Pinios River, Greece	AF090781
Luciobarbus amguidensis	1	Imirhou Riverl, Algeria	AY004724
Luciobarbus antinorii	1	Spring in Fartnassa, Tunicia	AY004692
Luciobarbus biscarensis	1	El Abiod, Arris, Algeria	AY004726
Luciobarbus bocagei	1	Huso River, Spain	AF334053
Luciobarbus brachycephalus	1	Terek River, Russia	AY004729
Luciobarbus callensis	1	Kebir River, Algeria	AF045974
Luciobarbus capito	1	Terek River, Russia	AF045975
Luciobarbus comizo	1	Tietar River, Tagus basin, Spain	AF334042
	1	Quejigares River, Guadiana bas., Spain	AF045968
Luciobarbus esocinus	1	Tigris River, Diyarbakir, Turkey	AF145934
Luciobarbus graecus	1	Kiffisos River, Greece	AF090786
Luciobarbus graellsii	1	Cadagua River, Spain	JF798258*
Luciobarbus guiraonis	1	Bullent River, Spain	AF045972
Luciobarbus issenensis	1	Souss River, Morocco	AF145928
Luciobarbus ksibi	1	Kasab River, Essaouira, Morocco	AY004738
Luciobarbus labiosa	1	Loukos River, Morocco	JF798259*
	1	Ifrane River, Sebou basin, Morocco	AY004733
	1	Hajera River, Morocco	JF798260*
Luciobarbus lepineyi	1	Dra River, Morocco	JF798261*
Luciobarbus longiceps	1	Tiberias Lake, Israel	AF145942
Luciobarbus magniatlantis	1	Oum er-Rbia River, Morocco	AY004734
Luciobarbus massaensis	1	Zag Mouzen River, Morocco	AY004737
Luciobarbus microcephalus	1	Estena River, Spain	AF334085
Luciobarbus moulouyensis	1	Moulouya River, Morocco	AF145925
Luciobarbus mursa	3	Arax River, Armenia	AF145943
			JF798262*
			JF798263*
Luciobarbus mystaceous	1	Kebam dam lake, Euphrates river basin, Turkey	AF145938
Luciobarbus nasus	1	Oum er-Rbia River, Morocco	AF145924
Luciobarbus pallaryi	1	Guir River, Morocco	AY004736
Luciobarbus sclateri	1	Manilva River, Spain	AF334076
Luciobarbus setivimensis	1	Soumman and Aissi Rivers, Algeria	AY004748
Luciobarbus subquincunciatus	1	Kebam dam lake, Euphrates river basin, Turkey	AF145937
Luciobarbus xanthopterus	1	Tigris River, Diyarbakir, Turkey	AF145939
Capoeta aculeata	1	Stream Sangan, Kārūn River bas., Persian Gulf, Iran	JF798267*
	1	Beshar River, Kārūn bas., Persian Gulf, Iran	JF798266*
	3	Sevah River, Daryacheh-ye-Tashk bas., inland waters, Iran	JF798264*
			JF798265*
Capoeta angorae	1	Pozanti River, Mediterranean Sea bas., Turkey	JF798268*
	1	Seyhan River, Turkey	AF145950
Capoeta antalyensis	2	Boga Cayi River, Mediterranean Sea bas., Turkey	JF798269*
			JF798270*
Capoeta baliki	2	Kizilirmak River, Black Sea bas., Turkey	JF798271*
	1	Kelkit Cayi River, Black Sea bas., Turkey	JF798272*
	2	Biggest tributary of Kurtboğazi dam lake, Sakarya River bas., Turkey	JF798273*
			JF798274*
	1	Stream Çakirca, Lake Iznik basin, Turkey	JF798275*
Capoeta cf. *banarescui*	1	Kelkit Cayi River, Black Sea bas., Turkey	JF798276*
	2	Harsit River, Black Sea bas., Turkey	JF798277*
			JF798278*
Capoeta barroisi	1	Karasu River, Orontes bas., Turkey	JF798279*
Capoeta bergamae	1	Bakircay River, Turkey	JF798280*
	1	Stream Güzelhisar, Aegean Sea bas., Turkey	JF798281*
	1	Bakacak stream, Marmara Sea bas., Turkey	JF798282*
Capoeta buhsei	1	Stream Taghra-Rud, inland bas., Iran	JF798283*
Capoeta cf. *buhsei*	1	Stream Morghab below dam, north-west of Tondaran	JF798284*
	1	Stream Sangan at Sangan	JF798285*

Table 1 (*continued*)

Species/subspecies	*n*	River, drainage, country	Accession nos.
Capoeta caelestis	1	Göksu River, Mediterranean Sea bas., Turkey	JF798286*
	2	Kargi Cayi River, Mediterranean Sea bas., Turkey	JF798287*
			JF798288*
	1	Ilica stream, Gulf of Antalya, Mediterranean Sea bas., Turkey	JF798336*
Capoeta capoeta	1	Agstev River, Kura tributary, Caspian Sea bas., Armenia	JF798289*
Capoeta damascina	1	Stream Arsuz, Iskenderun Gulf bas., Mediterranean Sea, Turkey	JF798303*
	1	Stream Yildirim, Orontes bas., Mediterranean Sea bas., Turkey	JF798304*
	1	Orontes River, Mediterranean Sea bas., Turkey	JF798305*
	1	Spring Incesu, Orontes bas., Mediterranean Sea, Turkey	JF798306*
	2	Yolçati River, Mediterranean Sea bas., Turkey	JF798307*
			JF798308*
	2	Karadut River, Euphrates bas., Turkey	JF798309*
			JF798310*
Capoeta heratensis	5	Murgab River, inland bas., Turkmenistan	JF798316*
	2	Yanbash River, dead-end river, Central Kopet Dagh Mountains, Turkmenistan	JF798317*
			JF798318*
	4	Keltechinar River, dead-end river, Central Kopet Dagh Mountains, Turkmenistan	JF798319*
Capoeta kosswigi	4	Deli Cayi River, Van Lake bas., Turkey	JF798320*
			JF798321*
			JF798322*
			JF798323*
Capoeta mauricii	1	Stream Sariöz, Beysehir Lake bas., Turkey	JF798324*
	1	Spring Eflatun, Beysehir Lake bas., Turkey	JF798325*
Capoeta saadi	1	Kor River, inland bas., Iran	JF798326*
	1	Rodan River, Oman Gulf basin, Iran	JF798327*
Capoeta cf. *saadi*	1	Spring Golabii, 35 km north from Darab, Iran	JF798328*
Capoeta sevangi	5	Sevan Lake, Armenia	JF798290*
			JF798291*
			JF798292*
			JF798293*
			JF798294*
	2	Mezamor River, Aras tributary, Caspian Sea bas., Armenia	JF798295*
			JF798296*
	2	Lake Arpi, source of Akhuryan River, Aras tributary, Caspian Sea bas., Armenia	JF798297*
			JF798298*
	2	Uraget River, Hrazdan-Aras tributary, Caspian Sea bas., Armenia	JF798299*
			JF798300*
	2	Arpa River, Aras tributary, Caspian Sea bas., Armenia	JF798301*
			JF798302*
	1	Lake Sevan, Armenia	AF145951
Capoeta sieboldi	1	Kizilirmak River, Black Sea bas., Turkey	JF798329*
	1	Kelkit Cayi River, Black Sea bas., Turkey	JF798330*
Capoeta steindachneri	4	Kugitangdarya River, former tributary of Amudarya, Aral Sea bas., Turkmenistan	JF798331*
Capoeta trutta	1	Gelal River, Ab-e-Seymareh bas. (Persian Gulf), Iran	JF798332*
	1	Sultansuyu River, Euphrates bas., Turkey	JF798333*
	1	Dez River, Rud-e-Karun bas. (Persian Gulf), Iran	JF798334*
	1	Tigris River, Turkey	AF145949
Capoeta turani	1	Çatkıt River, Mediterranean Sea bas., Turkey	JF798335*
Capoeta sp.1[a]	2	Sumbar River, tributary of Atrek, Caspian Sea bas., Turkmenistan	JF798311*
			JF798312*
	1	Beurme River, dead-end river, West Kopet Dagh Mountains, Turkmenistan	JF798313*
	2	Adjidere River, dead-end river, northwestern Kopet Dagh Mountains, Turkmenistan	JF798314*
			JF798315*
Capoeta sp.2	1	Dalaman River, Aegean Sea bas., Turkey	JF798337*
	1	Stream Yenicay, a tributary of Büyük Menderes River, Aegean Sea bas., Turkey	JF798338*
Capoeta sp.3	2	Gelal River, Ab-e-Seymareh bas. (Persian Gulf), Iran	JF798339*
			JF798340*
Cyprinion macrostomus	1	Tigris River, Diyarbakir, Turkey	AF180826
Cyprinus carpio	1		AY347287

[a] Name for this species in literature is used as *Capoeta gracilis* (Keyserling, 1861), however this species must have another name, as initially *Capoeta gracilis* was described originally by Temminck and Schlegel in 1846 from Japan and today it is a synonym of *Squalidus gracilis*. *Scaphiodon gracilis* Keyserling, 1861 is a homonym described from Esfahan, Iran.

and Tsigenopoulos, 2003; Tsigenopoulos et al., 2010). We focus our discussion on the more resolved Bayesian tree.

According to our molecular clock based on a fossil calibration we obtained an evolutionary rate of 0.52% per lineage per million year, which differs from previous estimates for *cyt b* (0.76–1.31% among Zardoya and Doadrio, 1999; Machordom and Doadrio, 2001; Durand et al., 2002; Mesquita et al., 2007; Tsigenopoulos et al., 2003, 2010). The closest estimates of 0.76% for *cyt b* and 0.82% for combined ND + tRNAs were obtained by Zardoya and Doadrio (1999), and Gante et al. (2009) respectively. The fossil

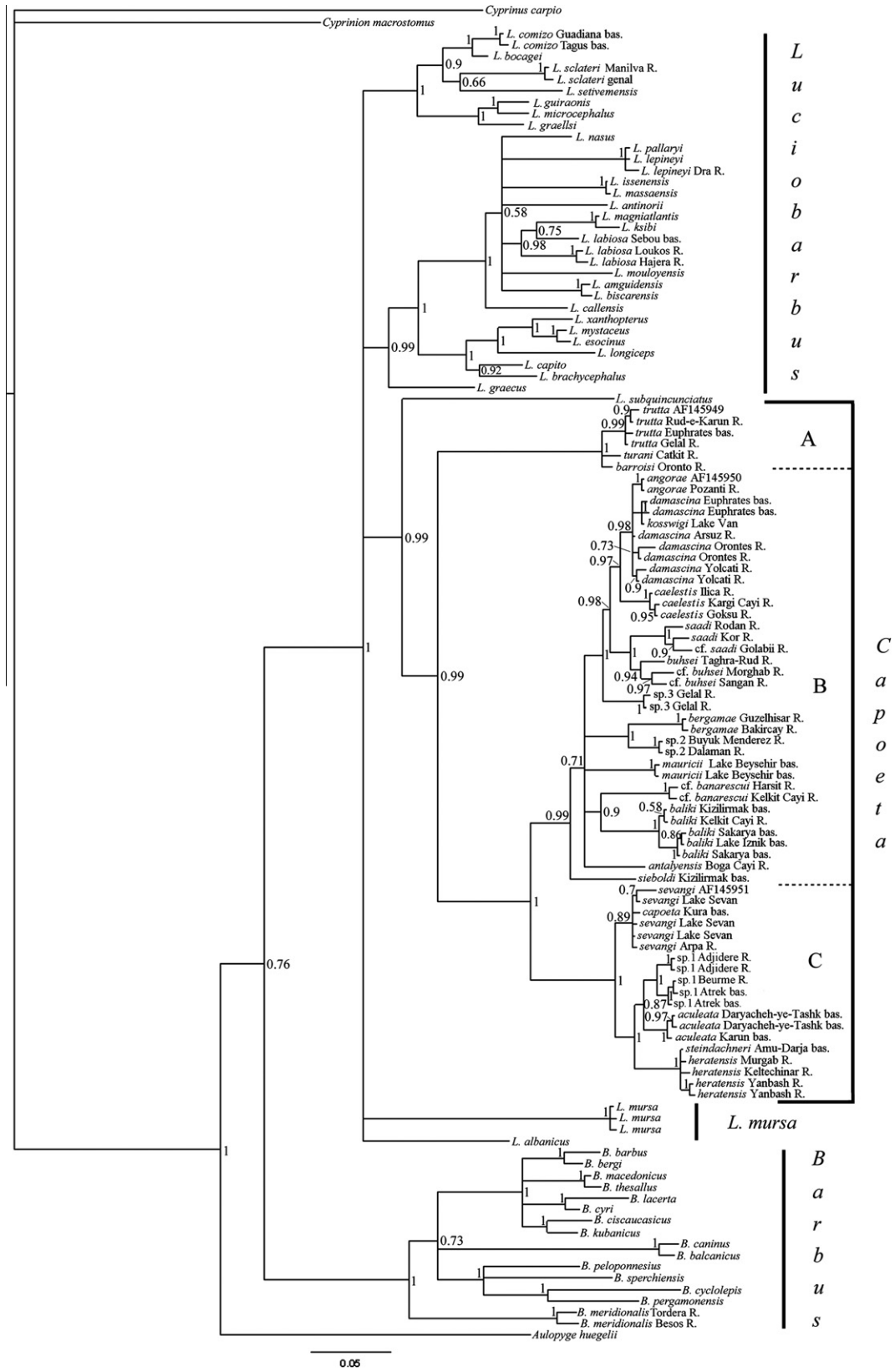

Fig. 1. Phylogenetic tree rendered by Bayesian analysis of the mitochondrial cytochrome *b* data set. Numbers above branches means posterior probabilities of BI.

calibration has been also applied in the latter study for an estimation of *Luciobarbus* divergence in Iberian peninsula.

The well-established divergence between *Barbus* s. str. and *Luciobarbus* clades (Doadrio, 1990; Zardoya and Doadrio, 1999) occurred, by our estimates, approximately 25.1 MYA (95% CI: 20.9–30.8; see Fig. 2) in the Late Oligocene–Early Miocene, in an older period than it was proposed by other authors (e.g. Zardoya and Doadrio, 1999; Machordom and Doadrio, 2001). However, this cladogenetic event showed a very low support (post. prob. = 60), so this divergence time estimate has to be taken with caution.

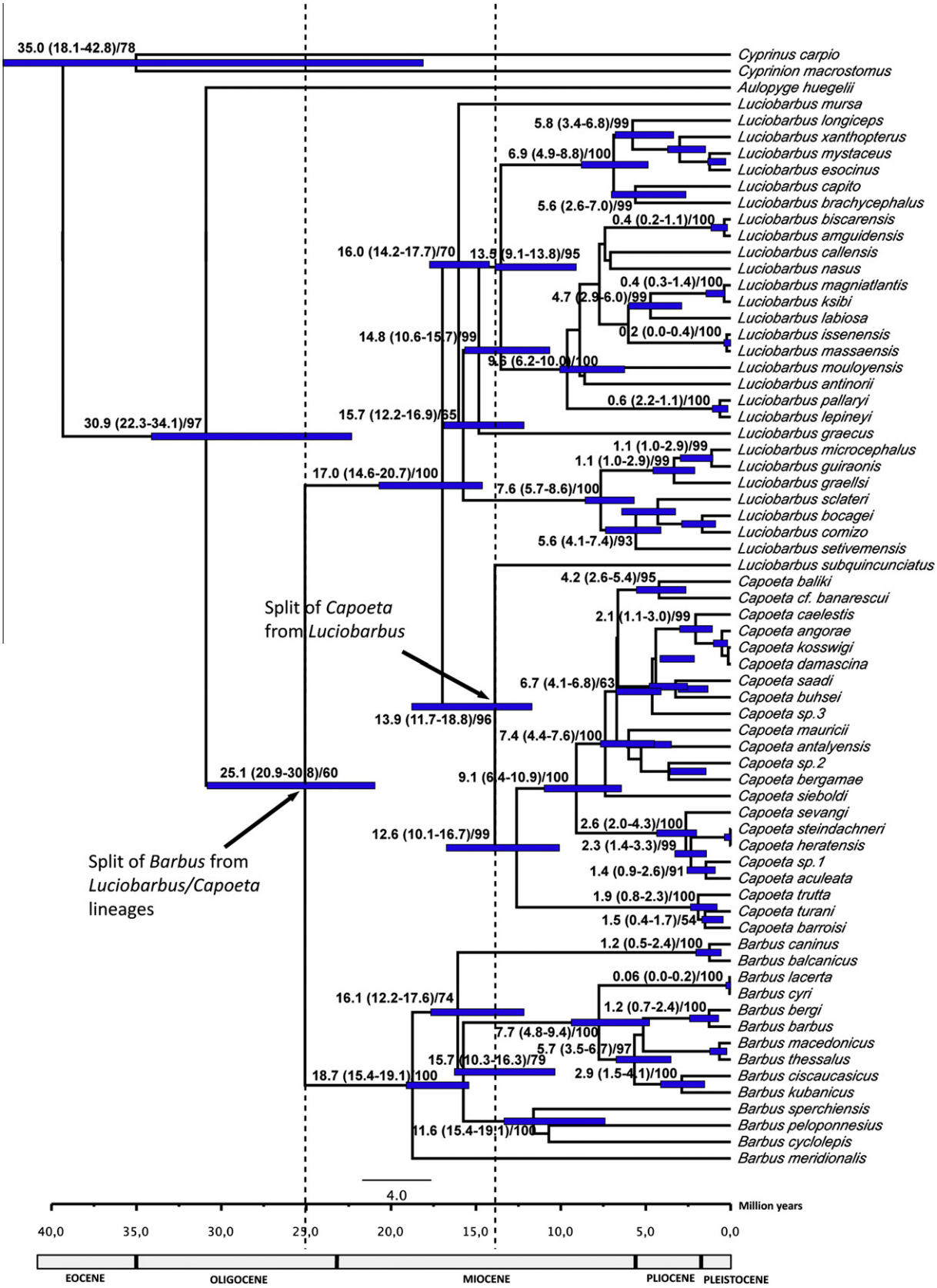

Fig. 2. Divergence time estimates of the major cladogenetic events within the *Luciobarbus*/*Capoeta* lineages. Numbers before slash represent divergence age estimation and their HPD 95% confidence intervals. Numbers after slash mean posterior probability values for Bayesian Inference.

Subsequent divergence in *Luciobarbus* between the Middle East, North Africa, the Caucasus and Southern-Central Asia and the Iberian Peninsula on one hand and the *Capoeta*/*Luciobarbus subquincunciatus* cluster on the other hand, was estimated to have

occurred in the Early Miocene approximately 17.0 MYA (95% CI: 14.6–20.7). The separation of *Capoeta* clade from *L. subquincunciatus* occurred approximately 13.9 MYA (95% CI: 11.7–18.8), in the Middle Miocene, possibly close to the Langhian–Serravallian boundary.

3.2. Relationships and divergence within the genus Capoeta

Phylogenetic analyses recovered three main groups inside the genus *Capoeta*: the Mesopotamian group (A), the Anatolian–Iranian group (B) and the Aralo-Caspian group (C) on the basis of their geographic distribution despite the fact that groups A and B partially overlapped (Fig. 1). The most diverged clade, the Mesopotamian group, included closely related taxa such as *Capoeta trutta*, *Capoeta turani* and *Capoeta barroisi* (group A). This clade was the sister group to all other *Capoeta* species and its separation occurred in the Middle Miocene approximately 12.6 MYA (95% CI: 10.1–16.7; posterior prob. = 0.99; Fig. 2).

The next divergence event, which divided the major part of the *Capoeta* lineage into two groups of species (posterior prob. = 1) occurred significantly later (about 9.1 MYA; 95% CI: 6.4–10.9) in the Tortonian period. The first of these groups (Anatolian–Iranian group, B) is the most diversified and encompasses many species occupying the majority of *Capoeta*'s range, including Anatolia, the Zagros Mountains, Mesopotamia and the Iranian plateau. The second group (Aralo-Caspian group, C) is formed by species inhabiting the northeastern part of the range of this genus, namely the Caspian and Aral Sea drainages.

The Anatolian–Iranian group (B) constitutes a widespread and diversified group of species. Within this clade, the first species to diverge was *Capoeta sieboldi* (inhabiting the Kizilirmak River, Black Sea drainage), which split off approximately 7.4 MYA (95% CI: 4.4–7.6). Several other subgroups subsequently diverged inside this group after the *C. sieboldi* separation event. One of these divergences involved the subgroup of species from Southwestern Turkey (*Capoeta antalyensis* and *Capoeta mauricii* from the Mediterranean drainage and Beysehir Lake respectively) and from West Turkey (Aegean and Marmara Seas, *Capoeta bergamae* and *Capoeta* sp.2), at approximately 6.7 MYA (95% CI: 4.1–6.8) from its sister subgroup, which includes the remaining *Capoeta* species belonging to the Anatolian–Iranian group. Soon afterwards, during Pliocene, the separation of the Black Sea clade (*Capoeta baliki* and *Capoeta banarescui*) and other species (*Capoeta buhsei*,*Capoeta saadi, Capoeta caelestis, Capoeta damascina*, *Capoeta angorae* and *Capoeta kosswigi*) occupying the Mediterranean drainage of Southeastern Turkey, the Tigris–Euphrates system, and small rivers which drain into the Gulfs of Persia and Oman, as well as inland waters in Iran took place (Fig. 2).

The Aralo-Caspian group (C) was formed by two subgroups. The *Capoeta capoeta* subgroup (including *Capoeta sevangi*), is widespread in the Kura and Aras rivers and Lake Sevan drainages (Caspian Sea) and diverged early (approx. 2.6 MYA; 95% CI: 2.0–4.3) from its sister subgroup (*Capoeta aculeata, Capoeta steindachneri, Capoeta heratensis,* and *Capoeta* sp.1), which occupies a wide area in the Aral and Caspian Sea drainages. The main diversification events of the species belonging to these two groups occurred during the Pliocene (Fig. 2).

4. Discussion

4.1. Phylogenetic relationships and origin of Capoeta

The most interesting result of the present study is that *Capoeta* is monophyletic and nested within *Luciobarbus*. This hypothesis was previously formulated for three *Capoeta* species only (Berrebi and Tsigenopoulos, 2003; Tsigenopoulos et al., 2010) and here is corroborated for more than 20 *Capoeta* species. Nevertheless, this finding makes the genus *Luciobarbus* a paraphyletic entity.

Capoeta probably originated in the Middle Miocene, as shown by our molecular data. We can reasonably assume that this event took place in the palaeo-drainage of the Tigris–Euphrates system or adjacent water bodies in light of the present restricted distribution of *L. subquincunciatus* (the closest mitochondrial relative). Some authors have considered the Tigris–Euphrates system to be one of important centers of speciation for inland fauna as well as a basin of exchange for fish fauna during the Late Miocene (Por and Dimentman, 1989; Coad, 1996; Durand et al., 2002). Indeed, according to Por and Dimentman (1989) a Proto-Euphrates collected water from the Levant and had contact with the Black and Caspian Sea drainages before the Pliocene orogeny. The present-day location of the upper stream of the Tigris River is close to the upper reaches of the Kura-Aras system (Caspian Sea drainage) and to some rivers belonging to the Black Sea drainage system. Since the main phylogenetic relationships amongst *Capoeta* (Anatolian–Iranian and Aralo-Caspian groups) agree with a geographic distribution, it seems likely that the tree topology displays the dispersal of *Capoeta*.

Since all karyotyped species of *Capoeta*, especially *C. capoeta*, *C. damascina, C. trutta, Capoeta umbla*, and *Capoeta* sp.1 are evolutionary hexaploids ($2n = 150$; see http://www.briancoad.com in detail) and four of these five species were analyzed in the present study, we can propose the hypothesis that *Capoeta* originated as a result of a polyploidization event. Around half of the *Barbus s.* str. and *Luciobarbus* genera have been karyotyped, and all of them were found to be evolutionary tetraploids ($2n = 100$) (Bănărescu and Bogutskaya, 2003). The ploidy level of *L. subquincunciatus*, the sister group of the genus *Capoeta*, is unknown. The evolutionary allotetraploid state *Luciobarbus* has been already discovered by Chenuil et al. (1999). The morphology of *Luciobarbus* is not close to that of *Capoeta*. *Capoeta* displays several significant evolutionary novelties, which suggests that the hexaploid state of the *Capoeta* genome could be a result of hybridization, in other words allopolyploidy again. *Capoeta* species have very distinct morphological features, with some having spoon-shaped pharyngeal teeth and a horny sheath on the lower jaw, none of which are shared by any species of *Luciobarbus*. It seems likely that *Capoeta* originated from a hybridization event and the matrilineal ancestral species belonged to *Luciobarbus*, while the second ancestral species of *Capoeta* is unknown. We suggest one species of the genus *Hemigrammocapoeta* as a putative candidate to be the father species for *Capoeta*. Small-sized fishes of the genus *Hemigrammocapoeta* inhabit water bodies of Levant, including the Tigris–Euphrates system, and share morphological characters as spoon-shaped pharyngeal teeth and a horny covering on the lower jaw. However this hypothesis needs further demonstration.

4.2. Correspondence between molecular and morphological relationships

The phylogenetic relationships inside the genus *Capoeta*, as determined from the cytochrome *b* analysis, differ somewhat from their morphological interpretation. The main disagreement involves the proposal that species of *Capoeta* with four barbels are more primitive than species with only two as all species of *Luciobarbus* have four barbels (Karaman, 1969). A reduction in the length and number of barbels is considered to be associated with the specialization required to scrape algae from stones. Indeed, although taxa in both main groups in *Capoeta*, namely the Anatolian–Iranian (*C. antalyensis*, *C. baliki*, *C.* cf. *banarescui*, *Capoeta tinca*) and Aralo-Caspian (*C. heratensis*), have four barbels, the position of these taxa inside their clades tends to be more basal than

other taxa. However, species of the *C. trutta* group, the earlier diverged lineage of the genus, does not share primitive character states such as "two pairs of barbels" and a "horseshoe-shaped lower jaw" (Karaman, 1969). Moreover, one taxon, assigned here as *C. steindachneri*, which inhabits the Aral Sea basin, shows intrapopulational variability in terms of the number of barbels (two, three or four; Nikol'skii, 1938; Levin et al., 2005). It therefore appears that the number of barbels may be retained in some taxa, whereas other species could rapidly lose them independently of their branch. It is also likely that the number of barbels is an evolutionarily reversible state of character in *Capoeta*.

Some degree of correspondence between the molecular and phenetic relationships is evident at the level of the smaller branches. For instance, Levin et al. (2005) recently suggested that the aggregation of taxa in the *C. capoeta* complex could be split into two groups (multi- and oligovertebrate) on the basis of osteological characteristics. This subdivision agrees well with the molecular one for the group assigned as the Aralo-Caspian group.

5. Conclusions

According with mitochondrial data *Capoeta* forms a well-supported monophyletic genus which is nested inside the *Luciobarbus*, suggesting that specialized scraping morphology appeared once in the evolutionary history of the genus. The most probable mechanism for *Capoeta* origination is an allopolyploidization. The phylogenetic organization of *Capoeta* is composed of three main groups: the Mesopotamian group, which includes three species from the Tigris–Euphrates system and adjacent water bodies, the Anatolian–Iranian group, which has the most diversified structure and encompasses many species distributed throughout Anatolia and Iranian inland waters, and the Aralo-Caspian group, which consists of species distributed in basins of the Caspian and Aral Seas, including many dead-end rivers in Central Asia and Northern Iran. The genus *Capoeta* originated around the Langhian–Serravalian boundary according to our molecular clock and the diversification of the group occurred along Middle Miocene–Late Pliocene periods, being more intense in the Anatolian–Iranian subclade.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.09.004.

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