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Molecular systematics of the *Capoeta* (Cypriniformes: Cyprinidae) species complex inferred from mitochondrial 16S rDNA sequence data

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Abstract. *Capoeta* species from Anatolia, Turkey were studied using mitochondrial 16S rDNA gene sequencing to determine whether traditionally defined species and subspecies correspond to taxonomic entities. The systematic topology and genetic divergence for *C. antalyensis*, *C. pestai*, *C. tinca*, *C. trutta*, *C. damascina* and *C. barroisi* was enough to classify them as different species. The 16S rDNA data does not corroborate the use of the classic subspecies nomenclature for *C. c. angorae*, *C. c. capoeta* and *C. c. sieboldi*, but supports the use of species nomenclature for *C. angorae*, *C. capoeta* and *C. sieboldi*. On the other hand the genetic evidence does not support the classic subspecies designation for *C.c. umbla* and *C.c. koswigi* because no fixed differences were observed between them. The systematic topology and haplotype differences between these lineages may suggest that these two subspecies are genetically contiguous, and are a member of the species *C. trutta.* Based on combined molecular and morphologic data, the present study suggests that two undescribed *Capoeta* species may exist in Anatolia; one species in the Goksu River, and the second species in the Dalaman stream. The 16S mtDNA gene is a useful genetic marker for species and subspecies identification of the genus *Capoeta* because of its interspecific heterogeneity producing a species specific pattern.

Key words: *Capoeta* species, Anatolia, molecular systematics, sequence, mtDNA, 16S rDNA.

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I. INTRODUCTION

The family *Cyprinidae* is one of the most important families of fish distributed throughout the world (BLANC et al. 1971; HOWES 1991). The cyprinid genus *Capoeta* is distributed from Central Asia to Western Asia including Anatolia, Azerbaijan, Afghanistan, Armenia, Georgia, Iraq, Iran, Israel and Uzbekistan (BANARESCU 1991) and generally occurs in lakes and streams with fast and slow-flowing waters (GELDIAY & BALIK 1996). In Turkish freshwaters, five species (*Capoeta antalyensis*, *Capoeta barroisi*, *Capoeta pestai*, *Capoeta tinca*, *Capoeta trutta*) and seven subspecies (*Capoeta capoeta capoeta*, *Capoeta capoeta angorae*, *Capoeta capoeta bergama*, *Capoeta capoeta damascinus*, *Capoeta capoeta kosswigi*, *Capoeta capoeta sieboldi*, *Capoeta capoeta umbla*) represent the genus *Capoeta* (KURU 1980; KRUPP & SCHNEIDER 1989; GELDIAY & BALIK 1996; DEMIRSOY 1997).

The hitherto the described species of *Capoeta* are based only on morphometric, meristic and limited karyological studies (SLASTENENKO 1956; KARAMAN 1969; KURU 1971, 1975; BALIK 1982; SOLAK 1982; STOUMBOUDI et al. 1993; FISHELSON et al. 1996; DEMIRSOY 1997; YILDIRIM & ARAS 2000; SAHAN & CENGIZLER 2002; GORSHKOVA et al. 2002; TURAN et al. 2004). Despite the plethora of studies, the taxonomic description of these species is still ambiguous. Not enough is known of the genetic relationships and the amount of genetic divergence between these species to support the species and subspecies status of some taxa.

Phylogenetic analysis based on morphology may result in misleading phylogenies since this character type increases the chance of homoplasy in phylogenetic tree reconstruction (KOCHER & STEPIEN 1997). A molecular systematic approach decreases the chance of using homoplasy (NEI & KUMAR 2000). Mitochondrial DNA analysis is a useful tool for molecular systematics because of its unique features (MEYER et al. 1990; NORMARK et al. 1991; MEYER 1992). These include patterns of maternal inheritance and rapid rates of evolutionary change in mtDNA compared to nuclear DNA making it a suitable tool for genetic studies among taxa of several fish groups at multiple taxonomic levels (KOCHER & STEPIEN 1997; ZARDOYA & DOADRIO 1999; DURAND et al. 2002). The mitochondrial 16S rDNA gene has proven a valuable evolutionary marker for fishes because it has produced robust phylogenies at various taxonomic levels (BROWN et al. 1982; KARAISKOU et al. 2003; PEREZ et al. 2005).

The present study aims (1) to genetically classify the species and subspecies of the genus *Capoeta*; (2) to provide information on the mtDNA sequence variability and (3) to examine genetic divergence and taxonomic relationships within the genus *Capoeta*.

II. MATERIALS AND METHODS

Samples

Specimens of *Capoeta* species were collected from their range throughout Turkey (Fig. 1). Six species and six subspecies of *Capoeta* were collected from 16 locations, being representative of the geographic distribution of the genus (GELDIAY & BALIK 1996). The number and location of *Capoeta* species used in the sequence analysis are given in Table I. All samples were fixed in ethanol for

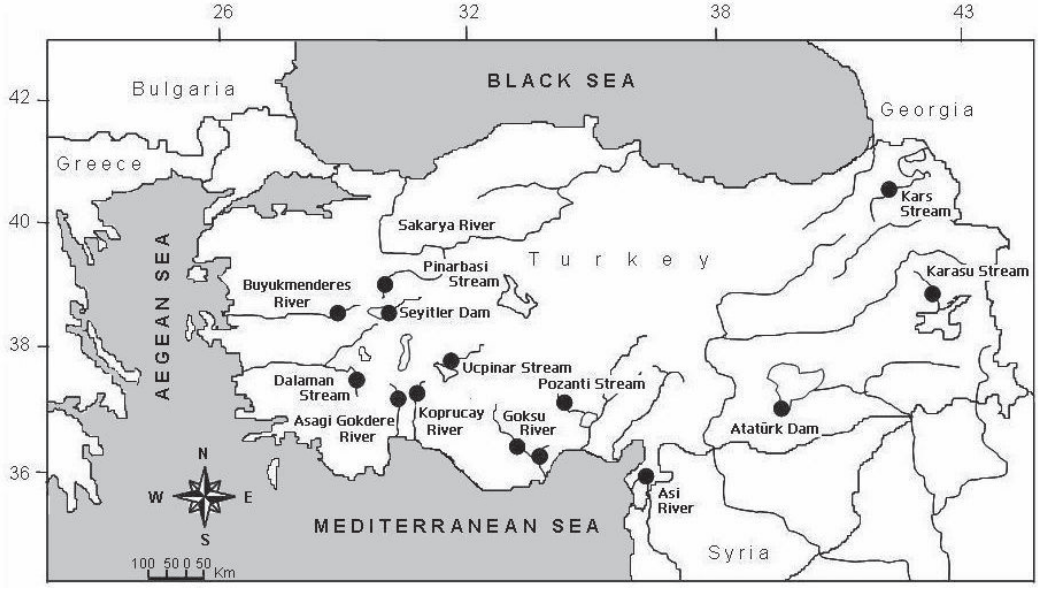

Fig 1. The map of the sampling of *Capoeta* species. Blacks dots indicate sampling locations.

Table I

Sampling details of *Capoeta* species and GenBank accession nos. for 16S rDNA segment; n – sample size sequenced

Species	Sampling location	Latitude	Sampling location abbreviation	n	GenBank Accession No.
L. rutilus				1	AF038484
C. antalyensis	Aşağı Gokdere River	37°32' 02.79'' N 30°56' 40.54'' E	AGR	3	EU707340 EU707341 EU707342
	Koprucay River	37°44' 54.46'' N 31° 01' 45.08'' E	KR	3	EU707343 EU707344 EU707345
C. barroisi	Asi River	36°15' 21.13'' N 36° 14' 23.41'' E	ASI	1	EU707376
C. damascinus	Asi River	36°16' 07.62'' N 36° 17' 05.67'' E	ASI	1	EU707375
C. pestai	Ucpinar Stream	37°49' 22.39'' N 31° 40' 06.08'' E	US	2	EU707368 EU707369
C. tinca	Seyitler Dam	38°47' 51.34'' N 30° 48' 32.16'' E	SD	2	EU707370 EU707371
Capoeta A	Goksu River	36° 25' 10.56'' N 33° 47' 18.26'' E	GR	3	EU707372 EU707373 EU707374
C. trutta	Goksu River-Kurtsuyu Stream	36° 26' 27.79'' N 33° 46' 29.71'' E	GKS	3	EU707351 EU707352 EU707353
C. angorae	Seyhan River- Pozanti Stream	37° 00' 56.42'' N 34° 57' 40.27'' E	PS	3	EU707346 EU707347 EU707348
	Asi River	36° 16' 02.43'' N 36° 18' 01.22'' E	ASI	2	EU707349 EU707350
C. capoeta	Kars Stream	40° 31' 59.91'' N 43° 01' 07.58'' E	KS	3	EU707354 EU707355 EU707356
C. bergama	Buyuk Menderes River	36° 53' 46.84'' N 29° 04' 53.38'' E	BMR	2	EU707357 EU707358
Capoeta B.	Dalaman Stream	37° 08' 45.05'' N 29° 34' 15.04'' E	DS	2	EU707359 EU707360
C. c. koswigi	Karasu Stream	38° 44' 43.33'' N 43° 31' 48.82'' E	KSS	2	EU707361 EU707362
C. sieboldi	Pinarbasi Stream	39° 07' 12.92'' N 31° 23' 41.83'' E	PBS	3	EU707363 EU707364 EU707365
C. c. umbla	Ataturk Dam	37° 24' 40.97'' N 38° 32' 30.14'' E	AD	2	EU707366 EU707367

Table II

Formula of observed meristic characters of *Capoeta* species. n, indicates number of individuals analyzed for the given meristic characters

	Lateral line scales																																		
	n	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	76	77	78	79	80	81	82	83	84	86	87	88
C. antalyensis	6	2	2			2																														
C. angorae	12														3	1	2		1	1		1	2	1												
C. capoeta	12	1		1	2		5	1	1		1																									
C. bergamae	4										1		2		1																					
Capoeta B	4								2	2																										
C. trutta	12																									2	1		3		2	1	1		1	1
C. c. koswigi	6																												2	1	1		1		1	
C. c. umbla	3																																	2	1	
C. sieboldi	4							3	1																											
C. pestai	5																												2		1	2				
C. tinca	5																								1		1	1	1		1					
Capoeta A	5											2	1	2																						
C. damascinus	14														1	1	3	1	3	1	2	1		1												
C. barroisi	14												1	2	2	1	1	1	3	1			1	1												

Transverse Line Scales	Above Lateral Line													Below Lateral Line									
	n	8	9	10	11	12	13	14	15	16	17	18	19	7	8	9	10	11	12	13	14	15	16	17
C. antalyensis	6				3	3									2	4								
C. angorae	12				1	2	6	3							2	4		2	4					
C. capoeta	12	2	8	2										7	4	1								
C. bergamae	4					4																		
Capoeta B	4					4																		
C. trutta	12								5	4	1	1	1						5	3	2	2		
C. c. koswigi	6							1	5										6					
C. c. umbla	3										3							2	1					
C. sieboldi	4		1	2	1									1	1	1	1							
C. pestai	5											2	3								5			
C. tinca	5						2	1	1	1							1		1	3				
Capoeta A	5					3	2																2	3
C. damascinus	14						3	9	2								5	9						
C. barroisi	14				2	3	4	5									8	3	3					

Table II

Continued

Gill Rakers	n	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
C. antalyensis	6					3	3													
C. angorae	12								6	6										
C. capoeta	12										12									
C. bergamae	4													1	2	1				
Capoeta B	4															2	2			
C. trutta	12																	10	1	1
C. c. koswigi	6																5	1		
C. c. umbla	3							3												
C. sieboldi	4					4														
C. pestai	5	5																		
C. tinca	5								3	2										
Capoeta A	5							5												
C. damascinus	14					1	1		1	1	2	8								
C. barroisi	14						1					2	4	7						

	Branched Dorsal Rays						Branched Pectoral Rays								Branched Anal Rays		Barbel	
	n	5	6	7	8	9	12	13	14	15	16	17	18	19	5	6	1	2
C. antalyensis	6		2	4						1	1	4			6			2
C. angorae	12				5	7		1	1	1	1	6	2		6	6	1	
C. capoeta	12				9	3							11	1	12		1	
C. bergamae	4				4				4						3		1	
Capoeta B	4	1	2		1			2	2						3		1	
C. trutta	12			1	10	1	4	1	3	4					12		1	
C. c. koswigi	6				6				1				4	1	6		1	
C. c. umbla	3					3				1	2				3		1	
C. sieboldi	4				4				1	1	1		1		4		1	
C. pestai	5				5					5					5		1	
C. tinca	5			3	2					5					5			2
Capoeta A	5				5								2	3	5			2
C. damascinus	14				10	4						10	3	1	14		1	
C. barroisi	14				9	5					7	7			11	3	1	

molecular analyses and all individuals were identified according to GELDIAY & BALIK (1996) and DEMIRSOY (1997). Meristic characters commonly used to describe *Capoeta* species were also used for species identification and taken as additional taxonomic data in the laboratory. Numbers of unbranched and branched rays in first dorsal fin (DFR), ventral fin (VFR), anal fin (AFR), pectoral fin (PFR), gill rakers (GR), scales in lateral line (LS), scales in transdorsal (LSD), scales in transventral (LSV) and vertebrae numbers (VN) were recorded under a binocular microscope. Observed meristic characters of *Capoeta* species (Table II) were in the range of their description by GELDIAY & BALIK (1996) and DEMIRSOY (1997). The specimens have been deposited at the ichthyological collection of the Faculty of Fisheries, Mustafa Kemal University.

DNA extraction, amplification and sequencing

Total DNA was isolated from a piece of fin tissue (approximately 2 mm^2) soaked in 95% ethanol using the AGOWA mag Midi DNA isolation Kit (AGOWA, Berlin, Germany). The amplification of mitochondrial 16S rDNA by PCR was performed with a profile of 94°C for 4 min, followed by 35 cycles of 94°C/30 s strand denaturation, 52°C/20 s annealing and 72°C/1 min 30 sec primer extension, and a final 7 min elongation at 72°C. The 16S rDNA amplification reactions included: 1.5 μl 10 x polymerase buffer, 0.5 μl dNTP (10 mM), 0.3 μl Teg DNA polymerase (3 U/ μl) equivalent to Taq DNA polymerase, 0.05 μl 16Fi140 primer (100 μM) (5′-CG(CT)AAGGGAA(ACT)GCTGAAA-3′), 0.05 μl 16Fi1524 primer (100 μM) (5′-CCGGTCTGAACTCAGATCACGTAG-3′), 3-5 μl DNA from AGOWA purification, and water for a total reaction volume of 15 μl. Amplified DNA was purified with Exo/Sap enzymes according to the supplier's protocol (Cleveland, Ohio, USA). Finally, all the samples were sequenced in the forward (16Fiseq1463: 5′-TGCACCATTAGGATGTCCRGATCCAAC-3′) and reverse (16sarL: 5′-CGCCTGTTTAACAAAAACAT-3′) directions with an automated sequencing machine (Model ABI3730, Applied Biosystems). The new sequences have been deposited in the GenBank database under the accession numbers shown in Table I.

Sequence alignment and genetic analysis

The partial 16S rDNA nucleotide sequences were aligned using Clustal W (THOMPSON et al. 1994), and the final alignment was done manually with BioEdit (HALL 1999). MtDNA sequence data were analysed to assess levels of pairwise nucleotide variation and to determine the nucleotide composition for each taxon using MEGA 3.1. A molecular phylogenetic tree was constructed using two distinct phylogenetic approaches: a distance-based method using neighbor joining (NJ) (SAITOU & NEI 1987) and a cladistic approach using maximum parsimony (MP). ModelTest (version 3.06; POSADA & CRANDALL 1998) was used to determine the best-fit model of DNA evolution, which was used for the NJ analysis. MP analysis was implemented using heuristic searches, and the analysis was restricted to 10.000 trees in MP. Evaluation of statistical confidence of nodes was based on 1000 non-parametric bootstrap replicates in NJ and MP analyses (FELSENSTEIN 1985). In all models, phylogenetic trees were rooted using the outgroup species *Leuciscus rutilus*, which belongs to the same family as the genus *Capoeta* (Cyprinidae), and its sequence is published in GenBank under accession number AF038484.

III. RESULTS

After alignment, the partial 16S rDNA gene sequences consisted of 898 bp fragments. Examination of the gene reveals anti-bias of thymine (T; 19.9%) and bias of adenine (A; 33.8%). The average base composition of cytosine (C) and guanine (G) were 25.3% and 21.0%, respectively.

The 16S rDNA dataset contained 124 variable sites, 103 of which were parsimony informative. Sequence analysis of 16S rDNA revealed 15 different haplotypes. The variable nucleotide positions and frequencies of haplotypes are given in Figure 2. Haplotype diversity was found to be 0.925.

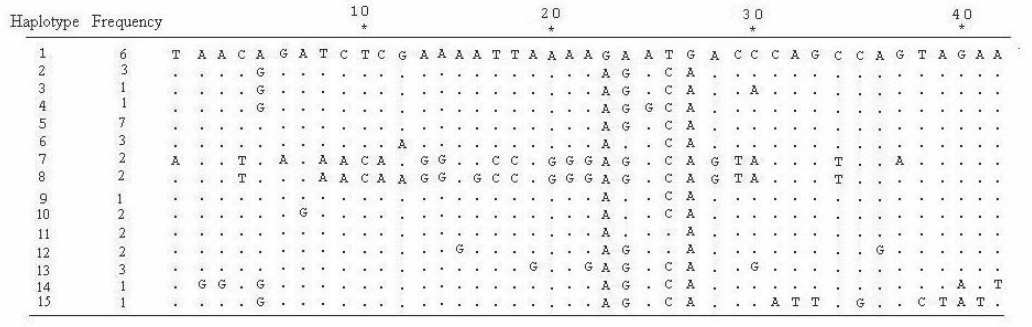

Fig. 2. Variable nucleotide positions and frequencies of 16S rDNA haplotypes in *Capoeta* species. Variable nucleotides are indicated for all haplotypes, while identity is shown by dashes.

Table III

Distribution of 16S rDNA haplotypes of the *Capoeta* species among the studied locations. H, haplotype; first number indicates the frequency of haplotypes.

Location	*C. capoeta*	*C. angorae*	*C. sieboldi*	*C. c. umbla*	*C. bergamae*	*Capoeta B*	*C. c. koswigi*	*C.antalyensis*	*C.barroisi*	*C.damascinus*	*C. pestai*	*C. tinca*	*Capoeta A*	*C. trutta*
Kurtsuyu Stream														3 H5
Pozantı Stream		2 H2 1 H3							1 H15	1 H14				
Asi River		1 H2 1 H4												
Asagi gokdere								3 H1						
Koprucayi River								3 H1						
B.menderes					2 H7									
Dalaman Stream						2 H8								
Pinarbasi Stream			1 H9 2 H10											
Ataturk Dam				2 H5										
Kars Stream	3 H6													
Karasu Stream							2 H5							
Ucpinar Stream											2 H11			
Seyitler Dam												2 H12		
Goksu River													3 H13	

Haplotype 5 was shared by *C. trutta*, *C. c. koswigi* and *C. c. umbla* (Table III). *C. antalyensis* and *C. angorae* had different haplotypes in different populations. The number of mutations was 43 and the mean nucleotide diversity (*Pi*) within the *Capoeta* genera was found to be 0.02218.

ModelTest indicated that the best-fitting model of nucleotide substitution was the HKY model of evolution (YANG 1994) with some sites assumed to be invariable and with variable sites following a discrete gamma distribution (HKY+I+G). For inter-specific differences, no divergence was observed among *C. trutta*, *C. c. umbla* and *C. c. koswigi*. The greatest pairwise genetic divergence between the lineages was found to be 0.095 between *C. barroisi* and *C. bergama* (Table IV). Interestingly, *Capoeta* samples from the Goksu River were clearly divergent from all other species. This finding indicates that a different species (named *Capoeta A*) may exist in the Goksu River. Furthermore *Capoeta* samples from the Dalaman stream were also clearly dissimilar from all other species, also suggestive of a different species (named *Capoeta B*).

Table IV

Pairwise comparisons of mean genetic distances between *Capoeta* lineages

	1	2	3	4	5	6	7	8	9	10	11	12
C. antalyensis (1)	–											
C. angorae (2)	0.017	–										
C. trutta (3)	0.013	0.005	–									
C. capoeta (4)	0.013	0.011	0.006	–								
C. bergama (5)	0.077	0.066	0.063	0.070	–							
Capoeta B (6)	0.074	0.063	0.059	0.059	0.016	–						
C. sieboldi (7)	0.012	0.010	0.005	0.005	0.069	0.065	–					
C. pestai (8)	0.006	0.011	0.006	0.006	0.070	0.066	0.005	–				
C. tinca (9)	0.016	0.014	0.010	0.016	0.073	0.070	0.015	0.010	–			
Capoeta A (10)	0.022	0.013	0.010	0.016	0.063	0.060	0.015	0.016	0.019	–		
C. damascinus (11)	0.029	0.014	0.016	0.022	0.080	0.077	0.021	0.022	0.026	0.026	–	
C. barroisi (12)	0.042	0.027	0.029	0.035	0.094	0.091	0.034	0.035	0.039	0.039	0.032	–

The two different phylogenetic approaches produced similar tree topologies (Fig. 3 and 4). In the NJ tree, *C. antalyensis* from Koprucay and Asagi Gokdere Rivers clustered together in one group, and was a sister group to *C. pestai*. *C. c. capoeta* clustered close to *C. c. sieboldi*. The subspecies *C. c. koswigi* clustered together with the subspecies *C. c. umbla*. *C. damascina* and *C. barroisi* branched together, forming a sister group to the subspecies *C. c. angora*. *C. trutta* was highly separated from these groups. *Capoeta A* was clearly separated from all other species. *C. bergama* and *Capoeta B* clustered close to each other. In the maximum parsimony tree, *C. antalyensis* from Koprucay and Asagi Gokdere Rivers clustered together in one group, being a sister group to *C. pestai*. *C. c. capoeta* clustered close to *C. c. sieboldi*. The species *C. trutta* clustered together with the subspecies *C. c. umbla* and *C. c. koswigi* in one group. *C. damascina* and *C. barroisi* were branched together and comprised the sister group to *C. c. angora*. *C. c. bergama* and *Capoeta B* clustered close to each other and formed a sister group to *Capoeta A*.

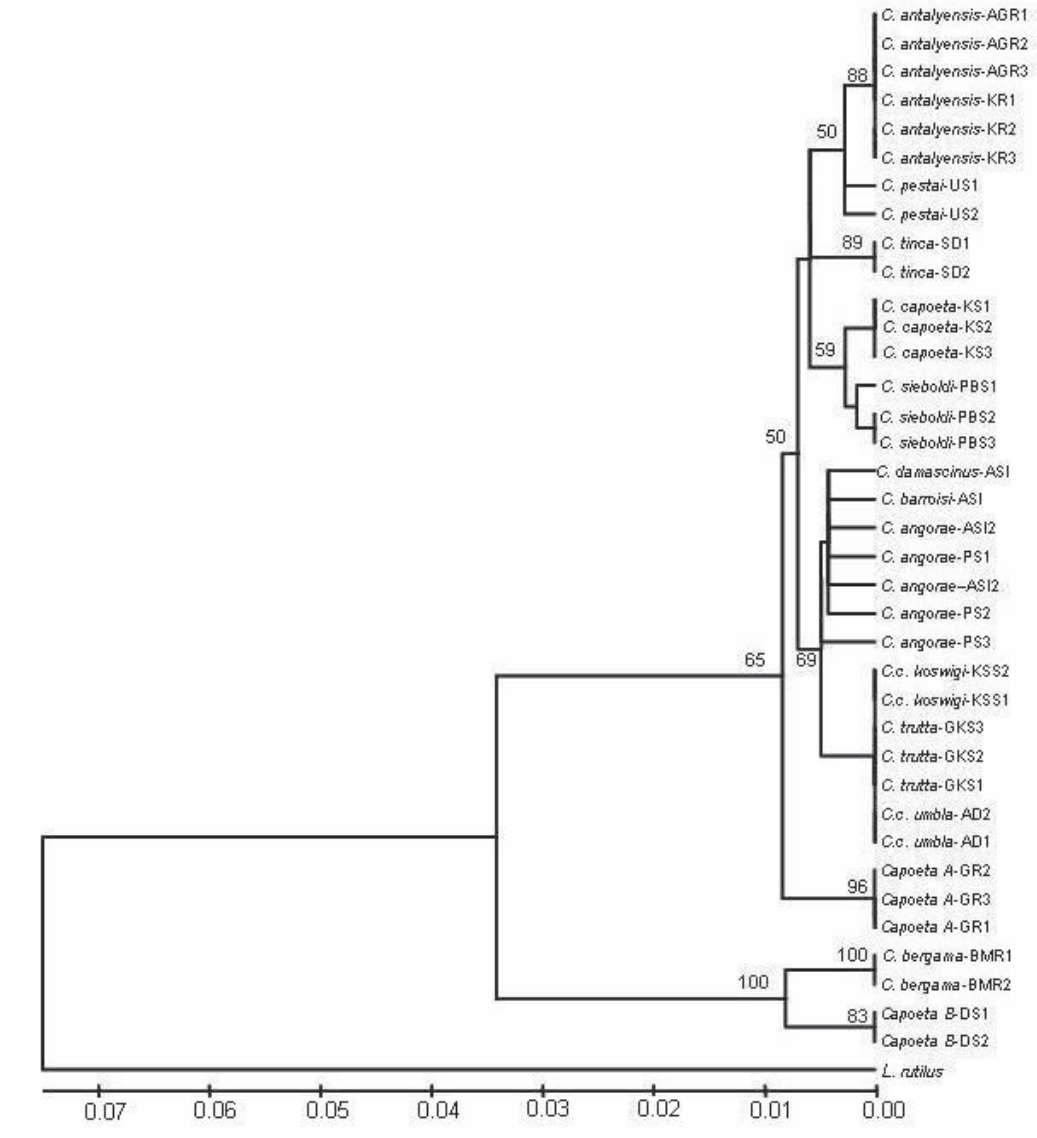

Fig 3. Neighbor joining tree based on the 16S rDNA dataset using multiple individuals of *Capoeta* species and subspecies. Bootstrap values are given above the nodes. Nodes without values indicate less than 50% support.

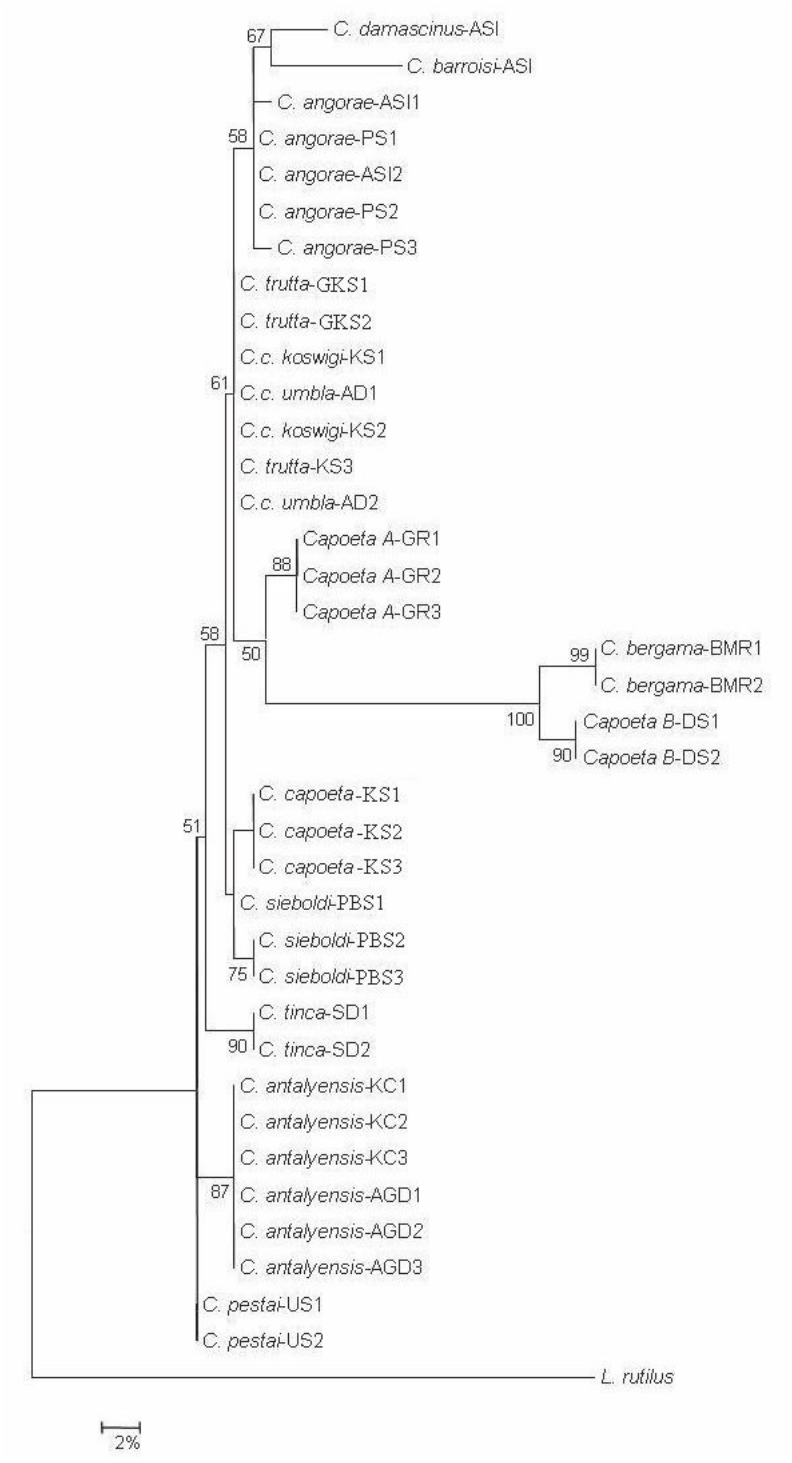

Fig 4. Maximum parsimony tree based on the 16S rDNA dataset using multiple individuals from *Capoeta* species and subspecies. Bootstrap values are given above the nodes. Nodes without values indicate less than 50% support.

IV. DISCUSSION

The molecular systematic relationships of the species and subspecies of the genus *Capoeta* based on genetic data were considered in this study for the first time. The present systematic analyses were not in congruence with the existing taxonomic classification of the *Capoeta* species. The detected pairwise sequence divergence between the species of the genus *Capoeta* ranged from 0.005 to 0.094. The phylogenetic divergence between species of *Capoeta* was comparable to, or greater than that seen between some species of Teleostei (DOUKAKIS et al. 1999; TINTI & PICCINETTI 2000; HRBEK et al. 2004; FARIA et al. 2006). For example, FARIA et al. (2006) studied mtDNA sequence variation in combined cytochrome b and ND1 regions showing that sequence divergence ranged from 0.009 to 0.034 between species of *Alosa*. HRBEK et al. (2004) investigated the phylogenetic relationships of *Pseudophoxinus* species from central Anatolia, Turkey using the complete Cytochrome b mitochondrial gene and found that sequence divergence between individuals within a population ranged from 0.00 to 0.0006, and 0.0506 to 0.1173 among species of *Pseudophoxinus*. TINTI & PICCINETTI (2000) investigated the molecular systematics of *Solea* species, and found that sequence divergences of 16S rDNA between species ranged from 0.0072 to 0.1109.

The species *C. trutta* clustered together with subspecies *C. c. umbla* and *C. c. koswigi* in one group in the maximum parsimony method, and the three lineages shared the same haplotype (H5). The detected genetic differences among the three lineages question the current taxonomic description of these taxons. The parsimony tree does not support the structuring of haplotypes by geography and/or subspecies designation. The phylogenetic topology and genetic differences between these lineages may suggest that the classically designated subspecies *C. c. koswigi* and *C. c. umbla* are genetically contiguous and belong within *C. trutta.* On the other hand, the observed phenotypic differences in morphology between these putative taxons may reflect environmentally induced phenotypic variation. In general, fishes demonstrate greater variance in morphological traits both within and between species than other vertebrates, and are more susceptible to environmentally-induced morphological variation (DUNHAM et al. 1979; ALLENDORF 1988; THOMPSON 1991; WIMBERGER 1992; TURAN 2006), which might reflect different feeding environment, prey types, food availability, temperature or other features. On the other hand**,** there is increasing evidence that differentiation at the nuclear DNA level may not be seen in mitochondrial genes (FERGUSON et al. 1991; WARD & GREWE 1994; TURAN et al. 1998), although there remain many cases to the converse (WARD et al. 1989; REEB & AVISE 1990; HANSEN & LOESCHCKE 1996). Therefore applications of nuclear genes can improve the given taxonomic description of these lineages.

The species *C. damascinus* and *C. barroisi* branched together and the detected genetic divergence was not enough to consider them as species in respect of the 16S rDNA gene sequences. These two species may be considered as subspecies, *C.c. damascinus* and *C.c. barroisi,* but other genetic markers should be used for a more reliable assessment. The species *C. angorae* had three different haplotypes not shared with other *Capoeta* species which may support the structuring of haplotypes by species designation. Therefore the present study corroborates the species status of *C. angorae*. The phylogenetic species concept considers a phylogenetic species as an irreducible cluster of organisms possessing at least one diagnostic character (BAUM 1992). Therefore diagnosable taxa have species-specific genetic characters that discriminate between taxa (HARVEY 1990; BAUM 1992). The three haplotypes were found to be species-specific and could be easily used to distinguish the *C. angorae* species from the others.

The clustering of the *C. antalyensis* lineage with that of *C. pestai* in all methods indicated a close relationship of these two endemic species when compared to the others. Genetic divergence between these species is low, but the present phylogenetic topology and genetic differences between these lineages encourage their current species status due to their endemism.

The present study indicates that a new undescribed species of *Capoeta* may exist in the Goksu River. *C. tinca* from the Goksu River was highly divergent from the Seyitler Dam samples. *Capoeta tinca* is distinguished from other species of the genus by the following meristic characters: two pairs

of barbels; 69-80 lateral line scales; 14-17 scale rows between the lateral line and the dorsal-fin origin, 12-14 between the lateral line and anal-fin origin; 19-23 gill rakers on the first gill arch (BANARESCU 1999; GELDIAY & BALIK 1996; DEMIRSOY 1997). The meristic characters of the Seyitler Dam samples used in this study support the given meristic characteristics of *C. tinca* (Table II). On the other hand, *Capoeta* samples from the Goksu River do not match the meristic characters of *C. tinca* from Seyitler Dam, especially PFR, LS and LSV (Table II). Within *Capoeta* only *C. tinca* and *C. antalyensis* have two pairs of barbels. Therefore *Capoeta* samples from the Goksu River cannot be confused with other *Capoeta* species due to the presence of two pairs of barbells. Moreover the *Capoeta* samples from the Goksu River differed from *C. antalyensis* in genetic and meristic characters (Table II, III, IV, and Fig. 4).

The clustering of *C. c. capoeta* with that of *C. c. sieboldi* in all methods indicated the close relationship between these two subspecies. *C. c. capoeta* had one haplotype which was not shared with *C. c. sieboldi* with two different haplotypes. This supports the structuring of haplotypes by species designation. It is also easy to distinguish these two lineages due to non overlapping meristic characteristics. Therefore the present 16S rDNA data corroborates the historical skepticism regarding the use of subspecific nomenclature within *C. capoeta*, and corroborates the use of species nomenclature for *C. capoeta* and *C. sieboldi*.

The degree of genetic differentiation observed for the endemic species *C. bergama* from the Buyuk Menderes River is high enough to verify its species status. On the other hand, *C. bergama* from the Buyuk Menderes River was highly divergent from the Dalaman stream group. The detected phylogenetic topology, haplotype differences, pairwise genetic divergence and observed meristic characteristics between these two groups indicates a subdivision at the species level, which could be due to recent speciation and/or frequent hybridisation**.** Therefore, *Capoeta* from the Dalaman stream may represent a species new to science.

In conclusion, 16S mtDNA gene is a useful genetic marker for species and subspecies identification in the genus *Capoeta* because of its interspecific heterogeneity capable of producing a species specific pattern. The inferred species and subspecies-level topology of the genus *Capoeta* is not congruent with the existing classic taxonomy of the genus *Capoeta*. The 16S rDNA data corroborates the historical skepticism regarding the use of subspecies nomenclature for *C. c. angorae, C. c. capoeta, C. c. bergama* and *C. c. sieboldi* and instead supports the use of species nomenclature *C. angorae*, *C. capoeta, C. bergama* and *C. sieboldi*, respectively. On the other hand, the subspecies *C. c. umbla* and *C. c. koswigi* are found to be genetically contiguous with *C. trutta*. The present analyses also suggest that two undescribed *Capoeta* species may exist in Anatolia; one species in the Goksu River, and a second undescribed species in the Dalaman stream.

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