Original Article *Capoeta baliki* Turan, Kottelat, Ekmekçi & Imamoglu, 2006 a junior synonym of *Capoeta tinca* (Heckel, 1843) (Teleostei: Cyprinidae)

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Abstract: *Capoeta baliki* was described from Sakarya basin, Turkey. It was distinguished from its nearest congener i.e. *C. tinca* based on a combination of characters, including fewer serrae along posterior margin of last simple dorsal-fin ray, modally fewer scale rows between lateral line and dorsal-fin origin, fewer vertebrae, deeper and shorter head and slenderer caudal peduncle. We examined the synonymy hypothesis of *C. baliki* and *C. tinca* by comparing their morphometric, meristic and molecular characters. Based on the results, their morphometric and meristic characters largely overlapped and no character was found to distinguish them. In addition, a low K2P mean genetic divergence of 0.37% *C. baliki* and *C. tinca* based on cytb gene and clustering in same clad showed that they are identical in molecular characters. As no character could be found to clearly distinguish these species, we treat *C. baliki* as a junior synonym of *C. tinca*.

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Introduction

Capoeta tinca Heckel (1843), described from Nilüfer River, Bursa Province, Susurluk basin of Turkey, is found throughout the Black Sea watersheds, as well as Sakarya and Kizilirmak basins in the central Anatolia (Geldiay and Balık, 2007; Çiçek at al., 2020). Turan et al. (2006) described the *C. tinca* populations of the central Anatolian basins i.e. Sakarya and Kizilirmak as a distinct species of *C. baliki* based on some morphological characters. However, further works (Özdemir, 2013, 2015; Kaya, 2019) rejected distinguishing characters of *C. baliki* from *C. tinca* suggesting *C. baliki* as a junior synonym of *C. tinca*. Additionally, pronounced discriminative differences was not found in the osteological characteristics of *C. baliki* and *C. tinca* (Küçük et al., 2008).

In recent years, molecular studies (Özdemir, 2013; Bektaş et al., 2017, 2019; Ghanavi et al., 2016; Levin, 2012; Zareian and Esmaeili, 2017; Zareian et al., 2018) based on both COI and *cytb* genes of mitochondrion indicated clustering of *C. tinca* and *C. baliki* in the same clade supporting *C. baliki* as junior synonym of *C. tinca.* Hence in the present study, we decided to examine the synonymy hypothesis of *C. baliki* and *C. tinca* by comparing their morphometric, meristic and molecular (mtDNA *cytb* gene) characters.

Materials and Methods

Specimens of *C. tinca* were collected from its type locality (Susurluk basin) and *C. baliki* from Sakarya basins. All specimens caught by electrofishing, and after anaesthesia by MS222, they were fixed into 5% buffered formaldehyde and stored in 70% ethanol after two weeks. Methods for counts and measurements followed Armbruster (2012). Measurements were made with a dial calliper to the nearest 0.1 mm. Gill rakers were counted on the outer margin of the anterior gill arch. The posterior pair of the branched rays articulating on a single pterygiophore in the dorsal and anal fins were noted as "1½".

Twenty morphometric characters were measured (Table 1) and then standardized using an allometric method to remove size-dependent variation using M_{adj}

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Species	Drainage	Country	Published by	Genbank Acc. No.
Capoeta baliki	Black Sea basin, Sakarya	Turkey	Bektas et al. 2017	GQ424019
Capoeta baliki	Black Sea basin, Sakarya	Turkey	Bektas et al. 2017	GQ424016
Capoeta baliki	Black Sea basin, Sakarya	Turkey	Bektas et al. 2017	GQ424015
Capoeta baliki	Black Sea basin, Sakarya	Turkey	Bektas et al. 2017	GQ424014
Capoeta baliki	Black Sea Basin, Sakarya	Turkey	Bektas et al. 2017	GQ424013
Capoeta baliki	Black Sea basin, Sakarya	Turkey	Bektas et al. 2017	GQ424012
Capoeta baliki	Black Sea basin, Sakarya	Turkey	Bektas et al. 2017	GQ424011
Capoeta baliki	Lake Iznik basin, Çakirca Stream	Turkey	Levin et al. 2012	JF798275
Capoeta baliki	Sakarya basin, Kurtbogazi Dam Lake	Turkey	Levin et al. 2012	JF798274
Capoeta baliki	Sakarya basin, Kurtbogazi Dam Lake	Turkey	Levin et al. 2012	JF798273
Capoeta baliki	Black Sea Basin, Kelkit River	Turkey	Levin et al. 2012 Levin et al. 2012	JF798272
Capoeta baliki	Black Sea Basin, Kızılırmak River	Turkey		JF798271
Capoeta tinca	Eber Lake Basin, Afyon	Turkey	Bektas et al. 2017	GQ424010
Capoeta tinca	Eber Lake Basin, Afyon	Turkey	Bektas et al. 2017	GQ424009
Capoeta tinca	Marmara Basin, Susurluk	Turkey	Bektas et al. 2017	GQ424008
Capoeta tinca	Marmara Basin, Susurluk	Turkey	Bektas et al. 2017	GQ424007
Capoeta tinca	Marmara Basin, Susurluk	Turkey	Bektas et al. 2017	GQ424006
Capoeta tinca	Marmara Basin, Susurluk	Turkey	Bektas et al. 2017	GQ424005
Capoeta tinca	Marmara Basin, Susurluk	Turkey	Bektas et al. 2017	GQ424004
Capoeta damascina	Mediterranean Sea basin, Orontes River	Turkey	Levin et al. 2012	JF798306
Capoeta damascina	Mediterranean Sea basin, Orontes River	Turkey	Levin et al. 2012	JF798305
Capoeta aydinensis	Aegean Sea Basin, B. Menderes	Turkey	Bektas et al. 2017	KY065274
Capoeta aydinensis	Aegean Sea Basin, B. Menderes	Turkey	Bektas et al. 2017	KY065275
Capoeta caelestis	Mediterranean Sea basin, Kargi Stream	Turkey	Levin et al. 2012	JF798287
Capoeta caelestis	Mediterranean Sea basin, Kargi Stream	Turkey	Levin et al. 2012	JF798288
Capoeta antalyensis	Mediterranean Sea Basin, Aksu at Gokdere	Turkey	Bektas et al. 2017	GQ424021
Capoeta antalyensis	Mediterranean Sea Basin, Aksu at Gokdere	Turkey	Bektas et al. 2017	GQ424023
Capoeta sieboldii	Black Sea Basin, Kelkit River	Turkey	Levin et al. 2012	JF798330
Capoeta sieboldii	Black Sea Basin, Kızılırmak River	Turkey	Levin et al. 2012	JF798329
Capoeta sieboldii	Black Sea Basin, Kizilirmak	Turkey	Bektas et al. 2017	KY065259
Capoeta sieboldii	Black Sea Basin, Kizilirmak	Turkey	Bektas et al. 2017	KY065258
Capoeta sieboldii	Black Sea Basin, Yesilirmak	Turkey	Bektas et al. 2017	KY065256
Capoeta bergamae	Marmara Basin, Bakacak stream	Turkey	Levin et al. 2012	JF798282
Capoeta bergamae	Bakırçay River	Turkey	Levin et al. 2012	JF798280
Capoeta banarescui	Black Sea Basin, Coruh	Turkey	Bektas et al. 2017	GQ423988
Capoeta banarescui	Black Sea Basin, Coruh	Turkey	Bektas et al. 2017	GQ423984
Capoeta banarescui	Black Sea Basin, Coruh	Turkey	Bektas et al. 2017	GQ423992
Capoeta banarescui	Black Sea Basin, Coruh	Turkey	Bektas et al. 2017	GQ423991
Capoeta banarescui	Black Sea Basin, Coruh	Turkey	Bektas et al. 2017	GQ423990
Capoeta banarescui	Black Sea Basin, Coruh	Turkey	Bektas et al. 2017	GQ423989
Capoeta capoeta	Caspian Sea basin, Aras River	Iran	Ghanavi et al. 2016	KU167938
Capoeta trutta	Karoun River Drainage, Lordegan	Iran	Ghanavi et al. 2016	KM459673
Luciobarbus esocinus	Tigris River	Turkey	Yang et al. 2015	KP712264

= M (L_s / L_0)^{*b*}, where M is the original measurement, M_{adj} the size adjusted measurement, L₀ the standard length of the fish, L_s the overall mean of the standard length for all fish from all samples in each analysis, and *b* was estimated for each character from the observed data as the slope of the regression of log M on log L_0 using all fish in any group (Elliot, 1999). The results derived from the allometric method were confirmed by testing significance of the correlation between transformed variables and standard length (Buj et al., 2008). The morphometric data of the two species were analyzed using multivariate analyzes of



Figure 1. Lateral view of (A) *Capoeta baliki*, NUIC-1816, 106.3 mm SL; Derecik Stream, Ankara province, and (B) *C. tinca*, NUIC-1717, 119.2 mm SL Değirmen Stream, Balıkesir province (all from Turkey).

principal component analysis (PCA) and *P*-value obtained from permutation test of one-way NPMANOVA. All outliers were removed from further analysis. All analyses were performed using PAST software.

Molecular data analysis: For this study, we retrieved 43 cytb sequences of the published Capoeta from GenBank using the (BLASTn) basic local alignment search tool (Altschul et al., 1990) (Table 1). For phylogenetic reconstruction, the datasets were analysed by Bayesian Inference (BI) using MrBayes 3.1.2 (Ronquist et al., 2011) and the maximum likelihood (ML) method in IQ-TREE 1.6.0 (Nguyen et al., 2015). We determined the best-fit model of molecular evolution for the genomic dataset using the Bayesian information criterion (BIC) in IQTREE 1.6.0 (Kalyaanamoorthy et al., 2017). MrBayes was run with 6 substitution types (nst=6) and considered the gamma-distributed rate variation across sites plus a proportion of invariable sites (GTR) for the COI datasets. For BI, Bayesian inference was calculated with MrBayes v.3.2.6 (Ronquist et al., 2011). Two simultaneous analyses were run with each 2,000,000 generations and four MCMC chains sampling every 10,000 generations. Convergence was checked on Tracer 1.6 (Rambaut and Drummond, 2013). After discarding the first 10% of generations as burn-in, we obtained the 50% majority rule consensus tree and the posterior probabilities. For ML analyses, we conducted heuristic searches (1,000 runs) under a TN+F+G4 model. Uncorrected pairwise genetic distances (p-distances) were investigated based on Kimura two-parameter (K2P) distances (Tamura et al., 2013). *Capoeta capoeta* (KU167938), *Capoeta trutta* (KM459673) and *Luciobarbus esocinus* (KP712264) were used as outgroups.

Abbreviations used. HL, Head length; SL, standard length; K2P, Kimura 2-parameter. Collection codes: NUIC, Ichthyological Collection of the Nevsehir Haci Bektas Veli University.

Results

General appearances of *C. tinca* and *C. baliki* are presented in Figure 1 showing their body shapes and colour patterns similarity. Tables 2 and 3 represent their morphometric measurements and meristic counts, respectively. All morphometric and meristic features of *C. baliki* are largely overlapped with those of *C. tinca*. We failed to find any non-overlapping morphological differences between the *C. tinca* and Table 2. Morphometric data of *Capoeta tinca* (n=20) and *C. baliki* populations (n=20).

	Capoet		Capoeta baliki		
Morphometric characters	(NUIC	/	(NUIC-1816)		
	min-max	mean±SD	min-max	mean±SD	
Standard length (mm)	90.6-140.7	117.0±15.2	90.8-227.3	127.4±37.2	
In percent of standard length					
Head length	23.4-24.9	24.3±0.5	21.4-26.4	24.2±1.3	
Body depth at dorsal fin origin	22.9-26.3	24.5±1.1	20.2-24.8	23.1±1.4	
Predorsal length	47.0-53.2	49.6±2.1	46.7-53.8	50.9±1.9	
Prepelvic length	51.2-54.4	52.5±1.1	49.8-54.9	52.8±1.5	
Preanal length	72.9-76.0	74.7±1.1	73.5-78.1	75.0±1.6	
Distance between pectoral-fin origin to anal fin	51.1-55.7	53.9±1.5	43.3-58.1	52.7±3.5	
Distance between pectoral-fin origin to pelvic fin	29.1-32.9	31.1±1.2	29.7-34.2	31.1±1.3	
Distance between pelvic-fin origin to anal fin	21.1-23.9	22.6±0.9	20.2-24.2	22.3±1.0	
Dorsal-fin height	19.3-22.8	21.4±1.1	18.1-22.7	20.4±1.5	
Anal-fin length	16.0-18.4	16.9±0.9	16.0-22.2	18.3 ± 2.1	
Pectoral-fin length	17.6-19.7	18.7 ± 0.7	17.3-19.6	18.6 ± 0.8	
Pelvic-fin length	15.0-16.6	15.8±0.6	14.7-17.1	15.7±0.7	
Upper caudal-fin lobe	16.9-25.3	22.7±2.2	18.1-23.6	21.6±1.4	
Length of caudal peduncle	17.6-19.3	18.6 ± 0.7	16.3-20.3	18.0 ± 1.4	
Depth of caudal peduncle	11.5-12.8	12.1±0.4	10.9-12.9	12.2±0.6	
In percent of Head length					
Head depth at eye	45.2-51.0	48.4±1.7	48.0-55.2	50.9 ± 2.2	
Snout length	32.4-38.2	35.2±1.4	35.3-40.4	37.5±1.6	
Eye horizontal diameter	17.1-22.5	19.8±1.5	16.6-22.1	19.0 ± 1.8	
Interorbital width	37.5-42.0	39.9±1.7	37.4-44.2	41.1±2.0	
Postorbital distance	46.2-51.2	48.8±1.8	46.2-52.3	49.1±1.7	
Maximum head width	60.2-68.3	62.9±2.5	60.5-69.2	63.7±2.8	

Table 3. Meristic data of Capoeta tinca (NUIC-1717) and C. baliki (NUIC-1816) (n=20 in each populations).

		Gill raker							
Examined materials	19		20	21	22	2	23		
Capoeta tinca	3		6		10				
Capoeta baliki	2		5	3	8		1	1	
	Lateral Line Scales								
Examined materials	75		76	77	78	7	79	80	
Capoeta tinca	8			4				3	
Capoeta baliki	12		5	5			3		
		Scale	Scales below lateral line						
Examined materials	14	15	16	17	18	7	8	9	
Capoeta tinca	1	15	4			1	14	5	
Capoeta baliki	1	12	6	1			18	2	
	Branched dorsal-fin rays								
Examined materials	71/	/2		81/2	9	1/2	mode		
Capoeta tinca				4		16		9	
Capoeta baliki				2		18	9		
				Branched a	anal-fin rays				
Examined materials	5 6			6		7	n	mode	
Capoeta tinca	2			18				6	
Capoeta baliki	4			16			6		
	Pelvic-fin rays								
Examined materials	7			8	8		n	mode	
Capoeta tinca	2			18			8		
Capoeta baliki	20 8						8		
	Pectoral-fin rays								
Examined materials	17		18		19	20		mode	
Capoeta tinca	2		16			2		18	
Capoeta baliki			18					18	

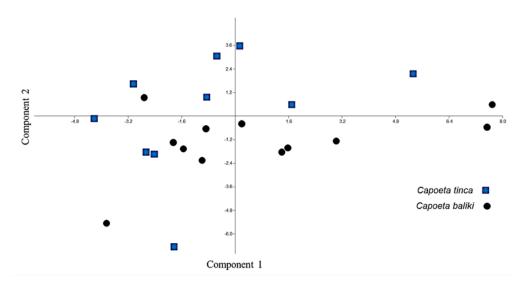


Figure 2. The PCA graph of morphometric characters in Capoeta tinca (NUIC-1717, n=10) and C. baliki (NUIC-1816, n=12).

C. baliki.

In PCA, the first three PCs accounted a total of 84.49% of the variances (PC1=41.02, PC2=20.90 and PC3=12.857) (Jolliffe cut-off=1.119). By plotting the first two PCs, the distribution of the studied species based on their morphometrics are presented in Figure 2, showing overlapping the specimens of both *C. tinca* and *C. baliki*. The result of multivariate one-way NPMANOVA showed no different between two species (P=0.1003, F=1.913).

Based on Figure 3, *Capoeta* species, including *C. tinca*, *C. baliki*, *C. banarescui* and *C. antalyensis* were clustered in the same clade with those *C. baliki* and *C. tinca* merged in the same clade. A low K2P mean genetic divergence of 0.37% was calculated between *C. baliki* and *C. tinca* (Özdemir, 2013; Bektaş et al., 2019) (Table 4).

Discussions

According to Turan et al. (2006), *C. baliki* is distinguished from *C. tinca* by having fewer serrae along posterior margin of last simple dorsal-fin ray (17-23 vs. 24-28), modally fewer scale rows between lateral line and dorsal-fin origin (14 vs. 16), fewer vertebrae (43-44 vs. 44-46), shorter head (21.8-24.5 vs. 23.3-26.7% SL), deeper head (55.6-63.5 vs. 49.3-56.5% HL) and lower caudal peduncle (9.5-12.2 vs. 10.8-13.4% SL). Based on the examined materials, our data in line with pervious findings (Özdemir, 2013, 2015; Kaya, 2019) showed overlapping of all above-mentioned distinguishing characters as well as others (Tables 2 and 3). Therefore, there is no morphological diagnostic characters to distinguish *C. baliki* from *C. tinca*.

Capoeta tinca and C. baliki were clustered in the

Table 4. Estimates of average K2P genetic divergence over sequence pairs between the studied Capoeta species.

Species	No	1	2	3	4	5	6	7	8
C. baliki	1								
C. tinca	2	<u>0.37</u>							
C. damascina	3	3.63	4.19						
C. aydinensis	3	4.29	4.84	3.19					
C. caelestis	4	4.11	4.47	1.43	3.93				
C. antalyensis	5	1.81	2.02	3.77	4.71	4.04			
C. sieboldi	6	4.49	5.07	2.99	4.15	3.58	4.74		
C. bergamae	7	4.74	5.31	3.74	2.61	4.39	5.18	4.83	
C. banarescu	8	4.30	4.33	3.51	5.05	4.18	3.56	5.40	5.53

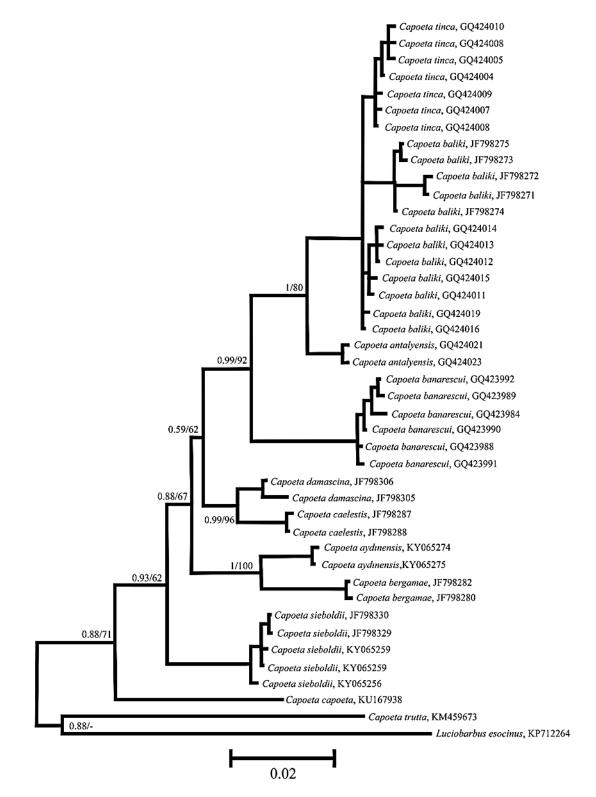


Figure 3. Bayesian Inference of the phylogenetic relationships based on the mitochondrial *cytb* barcode region (values at nodes correspond to BI posterior probability/ ML bootstrap).

same clade and cannot be considered as distinct species based on phylogenetic species concept (PSC). In addition, mean K2P genetic divergence of 0.37% between *C. tinca* and *C. baliki* is low for species

delimitation criteria suggested by Geiger et al. (2014) for freshwater fishes of the Mediterranean region and also this genetic distance stands within intraspecific range in the genus *Capoeta* based on the pervious

studies (Bektaş et al., 2017, 2019; Ghanavi et al., 2016; Levin, 2012; Zareian and Esmaeili, 2017; Zareian et al., 2018). Furthermore, Bektaş et al. (2017) reported that the haplotypes of *C. baliki* (from Sakarya river drainage and Lake Eber) and *C. tinca* (from Susurluk drainage) are closely related. The basins drain to Black Sea and the Sea of Marmara are not fully isolate from each other and exchanges routes are still available e.g. via river capture (Yıldırım and Emre, 2004). Therefore, *C. tinca* and *C. baliki* have perhaps recently isolated. Moreover, even existence of minor molecular and morphological differences between populations of widespread freshwater fish species, is a well-studied phenomenon (Marcil et al., 2006).

Since no morphological diagnostic characters to distinguish *C. baliki* from *C. tinca* is available and they are identical in molecular characters i.e. *cytb* gene, therefore we treat *C. baliki* as a junior synonym of *C. tinca*.

Material examined. All from Turkey.

Capoeta baliki, NUIC-1816, 20, 90.8-227.3 mm SL; Ankara prov.: Derecik Stream, Sakarya basin, 40°30'44"N 32°19'30"E; 15 May 2018. — ESFM-PISI/2004-74 (Holotype), 202 mm SL; Ankara prov.: Sakarya River: Kizilcahamam Stream, 60 km west of Ankara, 40°29'N 32°39'E; 15 April 2004. — ESFM-PISI/2004-75, 4, 140-190 mm SL; same data as holotype.

Capoeta tinca, NUIC-1717, 20, 90.6-140.7 mm SL; Balıkesir prov.: Değirmen Stream, Susurluk basin, 39°54'50"N 27°33'50"E.

Zoobank Registration: urn:lsid:zoobank.org:pub:72 9C2166-8359-401D-B3CA-102A9CBACB4B

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