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Barbus ida, a new barbel species from the Southern Marmara Sea basin (Teleostei: Cyprinidae)

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Barbus ida, a new species, is described from the streams Gönen and Biga, in western Anatolia. It is distinguished from other *Barbus* species in the adjacent basins by a less thickened last unbranched dorsal-fin ray, serrated along the proximal half of its posterior margin; larger irregular black blotches on the back and the flanks, and small black spots on the head, extending downwards to the cheeks; 56–61 total lateral line scales, 11–13 scale rows between dorsal-fin origin and lateral line and 7–8 scale rows between anal-fin origin and lateral line. *Barbus ida* also differs from its most closely related species, *B. niluferensis*, by 8 nucleotide substitution sites in the mtDNA cytochrome oxidase I barcode region.

Introduction

A total of 11 species of the cyprinid genus *Barbus* have been reported from Turkish inland waters (Güçlü et al., 2020). Eight of which have been reported from Western Anatolia and the Thrace region, including: *B. anatolicus* Turan, Kaya, Geiger & Freyhof 2018 from Yeşilırmak and Kızılırmak rivers, *B. cyclolepis* Heckel, 1837 from Ergene River and Istranca stream, *B. escherichii* Steindachner, 1897 from Sakarya River, *B. niluferensis* Turan, Kottelat & Ekmekci, 2009 from Susurluk River, *B. oligolepis* Battalgil, 1941 from the streams and rivers in Biga Peninsula, *B. xanthos* Güçlü, Kalaycı, Küçük & Turan, 2020 and *B. pergamonensis* Karaman, 1971 from the streams and rivers in the Aegean Sea basin, and *B. tauricus* Kessler, 1877 from small streams in southeastern and western Black Sea (Kottelat & Freyhof, 2007; Geiger et al., 2014; Turan et al., 2018; Levin et al., 2019; Güçlü et al., 2020).

Geiger et al. (2014) recorded *Barbus* (as *Barbus* sp.) from Gönen Stream. Later on, we examined additional specimens of *Barbus* sp. from both Gönen and Biga streams and compared these with the morphologically similar (and putatively genetically similar) species, *B. niluferensis*, and also species in adjacent basin. Based on morphological and genetic characters, we consider the specimens of

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Barbus sp. from Gönen and Biga streams to belong to an unnamed species, which is described herein.

Material and methods

Morphological analyses. Approval to conduct work on live animals was obtained from the Recep Tayyip Erdogan University Local Ethics Committee for Animal Experiments (Permit reference number 2011/04), at the beginning of the field study, in accordance with animal welfare laws, guidelines and policies of Turkish Republic. An electrofishing device was used for sampling. Photos of live individuals were taken in an aquarium immediately after capture so as to record aspects of color pattern in life. Specimens were anesthetized using a lethal dose of MS222. Fin clips were obtained from euthanized specimens and stored in 96 % ethanol. Euthanized specimens were fixed in 5 % formaldehyde.

All measurements and counts follow Turan et al. (2009). The last two branched rays articulating on a single pterygiophore in the anal and dorsal fins are counted as "1½". Thirty measurements and five counts of new species (n = 15) and *B. niluferensis* (n = 20) were analyzed with a free-size principal components analysis (PCA) using the software package PAST version 1.8 (Hammer et al., 2001).

DNA extraction, PCR and sequencing. Total DNA was isolated from fin tissue with Qiacube automated purification system using Qiagen DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany). The quality and the quantity of the DNA extracts were assessed on agarose gel, ensuring intact DNA bands and on Nanodrop. The mitochondrial barcoding cytochrome c oxidase subunit 1 (COI) region was amplified using the FishF1 and FishR1 primer pair designed by Ward et al. (2005). PCR reactions were performed in a 50 µl reaction volume containing 100 ng

Table 1. List of COI sequences downloaded from NCBI GenBank with information on species, GenBank accession numbers, drainage and their references.

Species	Accession N.	Drainage	Reference
Barbus ida	KJ553004	Gönen	Geiger et al., 2014
B. ida	KJ552894	Gönen	Geiger et al., 2014
B. niluferensis	MK716236	Nilüfer	Güçlü et al., 2020
B. niluferensis	KJ552904	Nilüfer	Geiger et al., 2014
B. xanthos	MK716232	Akçay	Güçlü et al., 2020
B. xanthos	MK716233	Eşen	Güçlü et al., 2020
B. xanthos	MK716234	Akçay	Güçlü et al., 2020
B. xanthos	MF106152	Büyük Menderes	Khaefi et al., 2017
B. xanthos	MF106153	Büyük Menderes	Khaefi et al., 2017
B. xanthos	KJ552972	Büyük Menderes	Geiger et al., 2014
B. pergamonensis	KJ552853	Bakir	Geiger et al., 2014
B. pergamonensis	KJ552792	Bakir	Geiger et al., 2014
B. pergamonensis	KJ552883	Bakir	Geiger et al., 2014
B. pergamonensis	KJ552991	Kum	Geiger et al., 2014
B. pergamonensis	MK716235	Gediz	Güçlü et al., 2020
B. cyclolepis	MK716237	Ergene	Güçlü et al., 2020
B. cyclolepis	KJ553100	İstanbul	Geiger et al., 2014
B. anatolicus	MH407648	Yeşilırmak	Turan et al., 2018
B. anatolicus	MH407645	Yeşilırmak	Turan et al., 2018
B. anatolicus	MH407629	Yeşilırmak	Turan et al., 2018
B. tauricus	MH407630	Kavukkavla	Turan et al., 2018
B. tauricus	MK716238	İyidere	Güçlü et al., 2020
B. escherichii	MH407634	Sakarya	Turan et al., 2018
B. escherichii	MH407639	Sakarya	Turan et al., 2018
B. oligolepis	KJ552990	Simav	Geiger et al.,2014
B. oligolepis	MK716240	Karpuz	Güçlü ett al., 2020

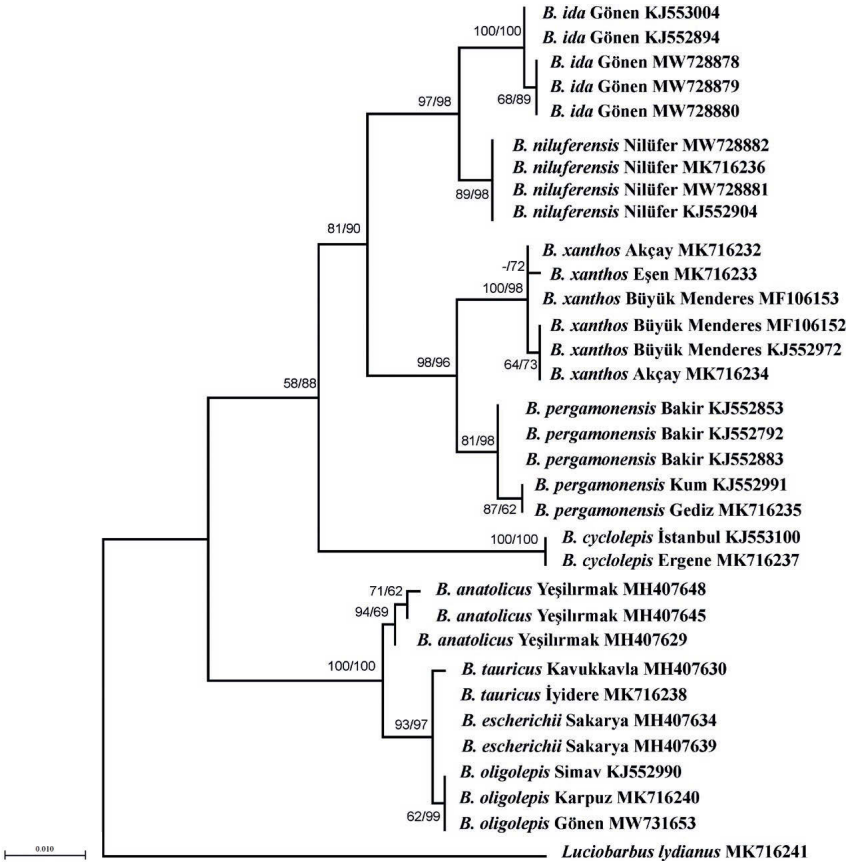

Fig. 1. Maximum likelihood tree based on mitochondrial COI gene sequences of *Barbus* species in the western Anatolia and Thrace region. Maximum likelihood and Neighbor Joining analyses resulted in congruent trees. Numbers associated with nodes represent bootstrap/posterior probability values higher than 50 %.

template DNA, 5 µl 10 x PCR buffer, 0.5 mM of each primer, 0.5 mM dNTPs mix, 5 mM $MgCl_2$ and 1 unit Taq DNA polymerase (New England Biolabs). The PCR amplifications were carried out with a BioRad T100TM (Bio–Rad, Hercules, CA, USA) thermal cycler under the following conditions: initial denaturation at 95 °C for 30 seconds, denaturation at 95 °C for 30 seconds, annealing at 58 °C for 45 seconds, extension at 68 °C for 45 seconds through 35 cycles followed up with a final extension at 68 °C for 5 minutes. The PCR products were displayed under UV Quantum–Capt ST4 system (Vilber Lourmat, France).

Molecular analyses. Molecular analyses were conducted with six newly generated COI sequences from the new species, *B. niluferensis*, *B. oligolepis* and published COI sequences of six *Barbus* species obtained from NCBI GenBank indicated in Table 1. Clustal W algorithm (Thompson et al., 1994) in Bioedit v. 7.2.5 (Hall, 1999) was used to align COI barcode sequences and the sequences were submitted to NCBI GenBank with accession numbers MW728878–MW728882 and MW731653. Phylogenetic assignment among species was carried out using both maximum likelihood (ML) and Neighbor Joining (NJ) analysis using MEGA X (Kumar et al., 2018). The TrN + G model (Kimura, 1980) was chosen as the best nucleotide substitution model according to the Bayesian information criterion (BIC) and the Akaike information criterion (AIC) in jModeltest v. 0.0.1 (Posada, 2008). In all phylogenetic analyses *Luciobarbus lydianus* (Boulenger, 1896) (MK 716241) was included as the outgroup taxon. The K2P (Kimura 2 parameter) distance model (Kimura, 1980) in MEGA X

Fig. 2. *Barbus ida*, IFC-ESUF 03-0518, holotype, 123 mm SL; Turkey: Çanakkale Prov.: Gönen Stream.

Fig. 3. *Barbus ida*, IFC-ESUF 03-0520, paratypes, **a,** 100 mm SL, **b,** 97 mm SL, **c,** 82 mm SL; Turkey: Çanakkale Prov.: Gönen Stream.

	B. ida	*B. niluferensis*	*B. pergamonensis*	*B. xanthos*	*B. cyclolepis*	*B. anatolicus*
B. ida		0.004	0.008	0.007	0.010	0.009
B. niluferensis	0.014		0.007	0.007	0.010	0.009
B. pergamonensis	0.033	0.028		0.005	0.011	0.008
B. xanthos	0.036	0.032	0.014		0.010	0.008
B. cyclolepis	0.045	0.045	0.049	0.047		0.010
B. anatolicus	0.059	0.055	0.055	0.059	0.062	
B. escherichii	0.062	0.058	0.058	0.062	0.069	0.007
B. tauricus	0.062	0.058	0.058	0.062	0.069	0.007
B. oligolepis	0.064	0.060	0.060	0.064	0.071	0.009

Fig. 4. *Barbus ida*, IFC-ESUF 03-0519, paratype, (in live), 103 mm SL (top) and 114 mm SL (bottom); Turkey: Çanakkale Prov.: Gönen Stream.

(Kumar et al., 2018) was used to estimate pairwise genetic distances among species. We also used two different species delimitation methods (one tree-based and one distance-based): Poisson Tree Processes (PTP) with Maximum Likelihood Solution (Zhang et al., 2013), via web server (http://mptp.h-its.org/#/tree) [accessed March 12 2021) to test candidate species and Refined Single Link-age (RESL) analysis for determining the operational taxonomic units (OTU) of species.

Table 2. Pairwise distance Kimura's two parameters (K2P) values based on cytochrome oxidase sequences of Barbus species (below the diagonal); Standard error of pairwise distance (above the diagonal).

B. escherichii	*B. tauricus*	*B. oligolepis*
0.010	0.010	0.010
0.009	0.009	0.009
0.008	0.008	0.008
0.009	0.009	0.009
0.011	0.011	0.011
0.003	0.003	0.004
	0.000	0.002
0.000		0.002
0.002	0.002	

Collection codes. IFC-ESUF, Inland Fishes Collection, Faculty of Eğirdir Fisheries, Isparta University of Applied Sciences, Isparta; and FFR, Zoology Museum, Faculty of Fisheries, Recep Tayyip Erdoğan University, Rize.

Results

COI sequences were analyzed in nine species of *Barbus* from Western Anatolia and the Thrace region. Species of *Barbus* were divided into two main clades in the tree topologies resulting from the analyses. *Barbus* new species constituted a highly supported clade sister to *B. niluferensis* with high ML and NJ bootstrap value as 97/98

(Fig. 1). Intrageneric K2P distances between species ranged from 0.000 (*B. escherichii* and *B. tauricus*) to 0.071 (*B. cyclolepis* and *B. oligolepis*). K2P distance is 0.014 between *Barbus* new species and the putative closest relative *B. niluferensis*, and 0.033 between *Barbus* new species and *B. pergamonensis* (Table 2). *Barbus* new species differs from its most closely related congener, *B. niluferensis*, by 8 nucleotide substitution sites and 1.4 % K2P distance in the mtDNA COI barcoding region. Seventy-two variable nucleotide positions in the COI barcoding region were determined between the species of *Barbus* included in the dataset. *Barbus* new species was differentiated from all other *Barbus* species in southwestern Anatolia by three diagnostic and unique nucleotide substitution sites in the COI barcoding region (Table 3). In addition, The PTP model-based species delimitation approaches using the ML topology estimated that *Barbus* new species is a candidate species, with maximum likelihood partition support value of 0.307. According to the results of the RESL analysis, *Barbus* new species is determined to represent a distinct OTU.

Barbus ida, new species
(Figs. 2–4)

Holotype. IFC-ESUF 03-0518, male, 123 mm SL; Turkey: Çanakkale Prov.: Kocaçay at Kalkım, Gönen Stream drainage, 39°48'52"N 27°13'47"E; S. S. Güçlü & H. Güçlü, 25 Aug 2010.

Paratypes. All from Turkey, Çanakkale Province: IFC-ESUF 03-0520, 5, 82–100 mm SL; collected with the holotype. – IFC-ESUF 03-0519, 7, 86–114 mm SL; Kocaçay at Kalabakbaşı Village, Kalkım, Gönen Stream drainage, 39°46'09"N 27°13'33"E; S. S. Güçlü & Z. Güçlü, 14 Jun 2019. – FFR 08822, 6, 75–114 mm SL; Kocaçay at İnova Village, Yenice, Gönen Stream drainage 40°06'16"N 27°19'10"E; D. Turan, C. Kaya & E. Bayçelebi, 31 Aug 2014. – FFR 008823, 4, 107–116 mm SL; Zeytinli Stream at Akçakoyun Village, Kalkım, Gönen Stream drainage, 39°46'12"N 27°06'18"E; D. Turan, C. Kaya & E. Bayçelebi, 1 Sep 2014. – FFR 08824, 2, 80–96 mm SL; Kocaçay at Aşağıçavuş Village, Kalkım, Gönen Stream drainage 39°49'36"N 27°08'48"E; D. Turan, C. Kaya & E. Bayçelebi, 1 Sep 2014.

Material used in molecular genetic analysis. FFR DNA Bar335–337, 3; Turkey: Çanakkale Province: Kocaçay at Kalkım, Gönen Stream drainage, 39°48'52"N 27°13'47"E; S. S. Güçlü & H. Güçlü, 25 Aug 2010 (GenBank accession numbers: MW728878–MW728880).

Diagnosis. *Barbus ida* is distinguished from all species of *Barbus* in adjacent waters in having a combination of the following characters: a weakly thickened last unbranched dorsal-fin ray, body with large (greater than scale) and irregular shaped black blotches on back and flanks, 56–61 total lateral line scales, 11–13 scale rows between dorsal-fin origin and lateral line, 7–8 scale rows between anal-fin origin and lateral line, 9–10 gill rakers on first gill arch and 39–40 total vertebrae (Fig. 4)

Description. General appearance in Figures 2 and 4 and morphometric data in Table 4. Body slender and wide, not compressed laterally. Dorsal profile slightly arched and ventral profile straight. Predorsal profile convex and postdorsal profile straight. Head short, its upper profile strongly convex in interorbital area and on snout, slightly concave in front of nostrils. Mouth small, sub-inferior, with very slightly developed lips. Lips with papillae, lower lip thicker than upper lip. Lower lip with produced lateral lobes and median pad. Median lobe more developed than lateral lobes (Fig. 5a). Rostral barbels reaching imaginary vertical line through nostril. Maxillary barbel almost reaching to imaginary vertical line through posterior margin of pupil in most individuals. Snout short and pointed.

Table 3. List of the variable nucleotide substitutions in the 652 base pairs long mt DNA COI barcode region.

Species, Locality, GenBank number	Variable nucleotides positions
	1 2 2 2 3 4 5 5 5
	6 6 7 8 6 2 0 2 7
	0 5 7 3 7 4 5 6 1
Barbus ida Gönen MW728878	A A T G G T T T G
Barbus ida Gönen MW728879	. . . . .
Barbus ida Gönen MW728880	. . . . .
Barbus ida Gönen KJ553004	. . . . . C .
Barbus ida Gönen KJ552894	. . . . . C .
Barbus niluferensis Nilüfer MK716236	G G C A A C C C A
Barbus niluferensis Nilüfer KJ552904	G G C A A C C C A
Barbus niluferensis Bolot MW728881	G G C A A C C C A
Barbus niluferensis Bolot MW728882	G G C A A C C C A

Dorsal-fin with 3 unbranched and 8½ branched rays. Posterior margin of dorsal fin straight or slightly convex. Dorsal-fin origin slightly in front of vertical of pelvic-fin origin. Last unbranched dorsal-fin ray thickened, approximately 41–55 % of its length (Fig. 5b). Pectoral fin with 1 unbranched and 16 branched rays, outer margin convex. Pelvic fin with 1 unbranched and 8 branched rays; its outer margin convex. Anal fin with 3 unbranched and 5½ branched rays, outer margin straight or slightly convex anteriorly, straight posteriorly, reaching to base of caudal fin. Caudal fin moderately forked, lobes slightly pointed. Lateral line complete with 56 (3), 57 (2), 58 (2), 59 (1), 60 (2) or 61 (5) scales; 11 (2), 12 (8) or 13 (5) scale rows between dorsal-fin origin and lateral line, 7 (7) or 8 (8) scale rows between anal-fin origin and lateral line. Nine (9) or 10 (6) gill rakers on anterior edge of first gill arch. Total vertebrae 39 (1) or 40 (4). Pharyngeal teeth 5.3.2–2.3.5.

Coloration. Formalin preserved adults and juveniles grayish or brown on back and flank, yellowish on belly. Dorsal, anal and pelvic fins yellowish, caudal and dorsal fin greyish. Numerous very large and irregular shaped black blotches on back and flanks. Small black spots on head, extending downwards to cheek. Fine black spots on rays of

Table 4. Morphometric data of *Barbus ida* (holotype, IFC-ESUF 03-0518; paratypes, IFC-ESUF 03-0520, n = 5; FFR 08822, n = 6; and FFR 008823, n = 4) and *Barbus niluferensis* (FFR 218, n = 20; from Turan et al., 2008). Ranges and means of *B. ida* include the holotype. SD, standard deviation.

	B. ida			*B. niluferensis*	
	holotype	holotype & paratypes			
		range	mean ± SD	range	mean ± SD
Standard length (mm)	122.6	75.3–122.6		93.1–143.3	
In percent of standard length					
Head length	25.8	24.3–26.8	25.8 ± 0.6	23.3–25.7	24.7 ± 0.7
Body depth at dorsal-fin origin	21.7	18.6–21.9	21.1 ± 0.9	17.2–22.4	20.9 ± 1.2
Predorsal length	52.7	51.9–54.4	53.0 ± 0.9	49.5–53.3	51.4 ± 1.1
Preventral length	53.9	51.3–53.9	52.9 ± 1.0	49.6–52.3	51.3 ± 0.6
Preanal length	77.8	73.3–80.6	76.8 ± 1.6	74.4–77.2	75.7 ± 0.8
Distance pectoral to anal-fin origins	55.3	49.1–56.9	53.5 ± 2.0	52.2–55.8	53.6 ± 0.9
Distance pectoral to pelvic-fin origins	31.4	26.8–31.4	29.4 ± 1.2	27.4–31.8	29.3 ± 0.9
Distance pelvic to anal-fin origins	25.3	22.7–26.6	24.9 ± 1.1	23.3–26.3	25.0 ± 0.9
Length of caudal peduncle	18.4	14.9–18.4	16.4 ± 1.0	14.9–18.5	17.1 ± 1.1
Depth of caudal peduncle	9.8	8.6–11.1	9.8 ± 0.6	9.8–10.9	10.4 ± 0.3
Dorsal-fin height	17.9	17.9–21.9	19.8 ± 1.2	15.1–18.8	17.1 ± 1.0
Pectoral-fin length	18.6	18.1–21.4	19.8 ± 1.0	15.7–19.3	17.7 ± 1.0
Anal-fin height	22.5	17.7–24.5	21.2 ± 2.3	18.1–23.0	21.1 ± 1.3
Ventral-fin length	15.2	14.7–17.3	15.9 ± 0.8	13.7–16.5	15.3 ± 0.8
Caudal-fin length	18.6	18.6–23.5	21.1 ± 1.5	15.7–20.4	18.3 ± 1.8
Length of lower caudal-fin lobe	11.7	11.7–14.9	13.5 ± 0.8	10.4–12.9	11.7 ± 0.8
In percent of head length					
Head width at anterior margin of eye	49	35–49	44 ± 3.5	40–44	42 ± 1.1
Head width at posterior margin of eye	59	46–60	56 ± 3.7	51–56	53 ± 1.2
Head width at occiput	63	56–69	61 ± 3.3	60–68	63 ± 1.7
Head depth at eye	54	47–62	52 ± 3.6	46–50	48 ± 1.3
Head depth at nape	66	62–72	65 ± 4.3	60–68	63 ± 1.7
Eye diameter	16	16–21	19 ± 1.6	15–17	16 ± 0.8
Nostril length	41	34–41	37 ± 1.6	38–42	41 ± 1.1
Interorbital distance	35	30–35	33 ± 1.3	30–34	32 ± 1.1
Width of snout at nostrils	43	35–43	39 ± 2.5	34–41	38 ± 1.9
Depth of snout at nostrils	36	29–36	33 ± 2.3	30–35	33 ± 1.6
Distance between rostral barbels	24	18–26	22 ± 2.5	18–23	22 ± 1.3
Distance between maxillary barbels	29	23–29	27 ± 1.8	23–28	26 ± 1.0
Rostral barbel length	19	15–21	18 ± 2.0	13–19	16 ± 1.5
Maxillary barbel length	25	20–27	24 ± 2.1	22–25	23 ± 1.1

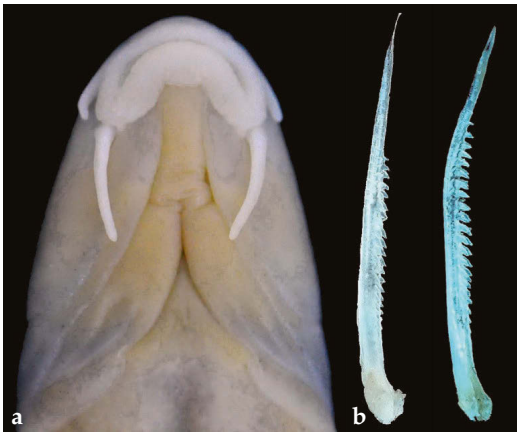

Fig. 5. Ventral view of head: **a,** *Barbus ida*, IFC-ESUF 03-0518, holotype, 123 mm SL and **b,** last unbranched dorsal fin ray (left side): *Barbus ida*, IFC-ESUF 03-0520, paratypes, 100 and 97 mm SL.

all fins. Live adults and juveniles greenish-khaki on back and flank, yellowish on belly. Dorsal, anal, pelvic, caudal and dorsal fins light brownish, pectoral fin dark brownish-soft orange. Numerous small to large irregular shaped black or dark brown blotches on back and flanks.

Sexual dimorphism. Females with a longer anal fin (19.2–28.1 % SL, mean 23.4 % SL) than males (15.3–21.0 % SL, mean 16.2%-SL).

Distribution and notes on habitat. *Barbus ida* is known from Gönen and Biga streams (southern Marmara drainages, Fig. 6). It inhabits swift flowing water, with cobble and pebble substrate. *Barbus oligolepis* has been collected together with *B. ida*.

Fig. 6. Distribution of *Barbus ida* (T: Type locality)

Fig. 7. *B. niluferensis*, IFC-ESUF 03-0495, 131 mm SL; Turkey: Bursa Prov.: Nilüfer Stream-Karaköprü creek.

Etymology. Gönen Stream, the type locality, is located in the Kaz Mountains. The new species is given the name "*ida*", which is an ancient mythological name for the Kaz Mountains. A noun in apposition.

Key to the species of *Barbus* of the western Anatolia and Thrace region (modified from Güçlü et al., 2020)

1a – Thirty-nine to 44 total vertebrae; small-sized fish reaching about 200 mm SL 2

1b – Forty-five to 49 total vertebrae; large-sized fish reaching usually 300–500 mm SL 6

2a – Forty-three to 44 total vertebrae; 62–72 total lateral line scales .... *B. niluferensis*

2b – Thirty-nine to 42 total vertebrae; 50–61 total lateral line scales 3

3a – Last simple dorsal-fin ray more thickened, thickened part more than 67 % of its length; interorbital distance 24.2–29.1 % SL..... .....*B. cyclolepis*

3b – Last simple dorsal-fin ray less thickened, thickened part less than 67 % of its length; interorbital distance 29.5–34.6 % SL..... 4

4a – Thirty-nine or 40 total vertebrae; large irregular black blotches (greater than size of scale) on dorsum and flanks ....*B. ida*

4b – Forty-one or 42 total vertebrae; a few or numerous small blackish spots (smaller than size of scale) on dorsum, flank and fins 5

5a – Last simple dorsal-fin ray thickened 52–72 % of its length; the posterior margin of dorsal-fin slightly concave..... ..... *B. pergamonensis*

5b – Last simple dorsal-fin ray thickened 33-50 % of its length; the posterior margin of dorsal-fin straight or slightly convex ....*B. xanthos*

Fig. 8. Ventral view of head: **a,** *B. niluferensis*, IFC-ESUF 03-0495, 131 mm SL; Turkey: Bursa Prov.: Nilüfer Stream-Karaköprü creek and **b,** last unbranched dorsal fin ray (left side): *B. niluferensis*, IFC-ESUF 03-0495, 131 mm SL.

6a – Length of the anal fin equal in both sexes; outer margin of the dorsal fin markedly concave ....*B. anatolicus*

6b – Length of the anal fin in female longer than that of male; the outer margin of the dorsal fin straight or slightly concave 7

7a – Lower lip with median lobe; flank, back and head with many irregularly shaped black or brown spots, often also with large, dark-brown blotches in juveniles and adults .....*B. escherichii*

7b – Lower lip with a median pad except for some individuals larger than about 200 mm SL; flank plain-brown or with many minute dark-brown spots in adults 8

8a – Head length 1.2–1.5 times in body depth; snout length 1.5–1.7 times interorbital distance .... *B. oligolepis*

8b – Head length 1.0–1.2 times body depth; snout length 1.4–1.5 times interorbital distance ..... *B. tauricus*

Discussion

Genetics and morphology sometimes are incompatible in some *Barbus* species, such as *B. anatolicus*, *B. tauricus* or *B. escherichii*. Although the genetic distance between the aforementioned species is relatively low, they are considered as distinct species on the basis of clear morphological differences. Based on our data set, genetic distance between *Barbus ida* and the putative closest species *B. niluferensis* is relatively low (1.4 %). A similar situation was documented by Turan et al. (2018), who showed a low genetic distance of only 0.9 % between *B. anatolicus* with the group containing *B. escherichii*, *B. oligolepis* and *B. tauricus*. These two examples serve to show that genetic distance can be relatively low between morphologically distinguishable species of *Barbus*. Additionally, two different genetic species delimitation methods (PTP and RESL) also assigned *B. ida* as a distinct OTU, corroborating the results of the ML analysis, and the morphological differences between *B. ida* and *B. niluferensis*.

Barbus ida is distinguished from the morphologically and genetically most closely related species, *B. niluferensis* (Fig. 7), in having fewer lateral line scales (56–61, vs. 62–71), fewer scale rows between dorsal-fin origin and lateral line (11–13, mode 12, vs. 13–15, mode 14), fewer vertebrae (39–40, 43–44), fewer scale rows between anal-fin origin and lateral line (7–8, mode 7 and 8, vs. 8–10, mode 9), more gill rakers on first gill arch (9–10, vs. 6-8) and (Fig. 8a) and longer serrae on the last unbranched dorsal-fin ray (Fig. 8b). The results of the PCA further confirm the differences between the new species and *B. niluferensis*, which are clearly separated in the multivariate plot space (Table 5, Fig. 9). The most important loadings on the first principal component (PC I) include three metric (caudal-fin length, length of lower caudal-fin lobe, rostral barbel length) and five meristic characters (lateral line scales, scale rows between lateral line and dorsal-fin origin, scale rows between lateral line and anal-fin origin, branched pectoral-fin rays, gill rakers) (see Table 5, highlighted in bold). *Barbus ida* is distinguished from *B. pergamonensis* in having a less ossified last unbranched dorsal-fin ray (41–55 % of its length, vs. 52–72 %) and fewer vertebrae (39–40 vs. 41–42). *Barbus ida* further differs from *B. pergamonensis* by having numerous large irregular black spots on the back, fins and flank (Fig. 2–3) (vs. a few small blackish spots on the back, flanks and fins (Fig. 10). *Barbus ida* is distinguished from *B. xanthos* by having a fewer vertebrae (39–40, vs. 41–43) and the upper head profile strongly convex (Figs. 2–3) vs. straight or slightly convex (Fig. 11). It also differs from *B. xanthos* by the body color and pattern. In *B. ida*,

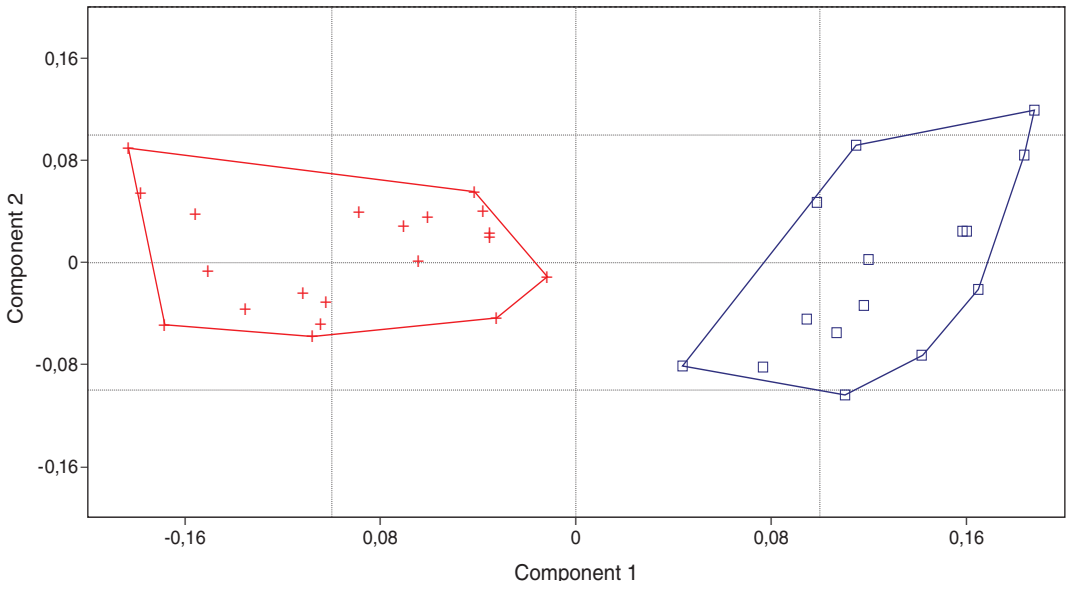

Fig. 9. A scatter plot of the scores of the first two principal components (PC I, PC II) for 35 specimens of two *Barbus* species, *B. ida* (□) and *B. niluferensis* (+), based on 35 morphometric and meristics characters.

the body has very larger irregular black blotches on the back and the flanks (vs. body with numerous small irregular shaped black or dark brown spots).

Barbus ida is distinguished from *B. cyclolepis* by having fewer lateral line scales (56–61, vs. 63–76), fewer scale rows between dorsal-fin origin and lateral line (11–13, mode 12, vs. 12–17, mode 15), fewer scale rows between anal-fin origin and lateral line (7–8, vs. 9–12) and a less thickened last unbranched dorsal-fin ray (thickened 41–55 % of its length, vs. 63–75). It further differs from *B. cyclolepis* by having large and numerous irregular black spots on the back, as well as on the fins and flank, and small black spots on the head, extending downwards to the cheek (vs. body plain brownish, or with few dark brownish spot on flank, smaller than scales and there is no black spot on top of head and cheek).

Barbus ida is distinguished from *B. anatolicus, B. escherichii, B. oligolepis* and *B. tauricus*, by reaching a smaller size (maximum size about 112 mm SL, vs. usually 300–500 mm SL), fewer total vertebrae (39–40, vs. 45–49) and the height of anal-fin approximately equal to the caudal-fin length (vs. the height of anal-fin usually smaller than caudal-fin length). *Barbus ida* is further distinguished from *Barbus oligolepis* in having a less thickened last simple dorsal-fin ray (thickened 41–55 % of its length, vs. 71–73), a smaller head (24.3–26.8 % SL, vs. 27.3–30.1) and fewer gill rakers on first gill arch (9–10, vs. 11–14). The two species are also easily distinguished from each other by body color and pattern in adult specimens. In *B. ida*, the body has very large irregular shaped black blotches on the back and the flanks, and small black spots on the head, extending downwards to the cheeks (vs. the body with a few small irregular shaped black or dark brown spots, smaller than scales, often forming dark-brown blotches on the back and flank). *Barbus ida* is further distinguished from *Barbus escherichii* by having a less thickened last simple dorsal-fin ray (thickened 41–55 % of its length, vs. 73–77), the lower lip with median pad (vs. the lower lip with median lobe), the dorsal-fin origin slightly in front of the vertical of the pelvic-fin origin (vs. dorsal-fin origin above the pelvic-fin origin) and the body is with very larger irregular black blotches on the back and the flanks (vs. numerous black spots on back and flanks, smaller than scales). *Barbus ida* is further distinguished from *B. tauricus* by having fewer gill rakers on first gill arch (9–10, vs. 11–13) and less developed lips (slightly developed, vs. well developed, especially specimens larger than about 200 mm SL). Two species are also easily distinguished each other by body color and pattern in adult specimens. In *B. ida*, the body has very large irregular black blotches on the back and the flanks, and small black spots on the head, extending downwards to the cheeks (vs. the body with a few small irregular shaped black or dark brown spots, smaller than scales, not forming dark-brown blotches on back and flank in adult specimens).

Table 5. Character loadings on principal components I and II (PC I and PC II) for 35 measurements taken on 35 specimens of *B. ida* and *B. niluferensis*.

Morphometric featurest	PC I	PC II
In percent of standard length		
Head length	0.0905	−0.0649
Body depth at dorsal-fin origin	0.0645	0.0482
Predorsal length	0.0659	0.0359
Preventral length	0.0522	−0.0461
Preanal length	0.0214	−0.0390
Distance pectoral to anal-fin origins	−0.0204	−0.1201
Distance pectoral to pelvic-fin origins	0.0068	−0.1038
Distance pelvic to anal-fin origins	−0.0243	−0.0485
Length of caudal peduncle	−0.0850	−0.2492
Depth of caudal peduncle	−0.0693	0.0572
Dorsal-fin height	0.3175	0.0176
Pectoral-fin length	0.2479	0.0848
Anal-fin height	0.0402	−0.2905
Pelvic-fin length	0.1353	0.0687
Caudal-fin length	**0.3191**	0.2075
Length of lower caudal-fin lobe	**0.3082**	0.0723
In percent of head length		
Head width at anterior margin of eye	0.0544	−0.2480
Head width at posterior margin of eye	0.1331	−0.0932
Head width at occiput	0.0793	−0.1129
Head depth throughout eye	0.1195	−0.1261
Head depth at nape	0.0830	−0.0082
Eye diameter	0.3622	0.1294
Snout length	−0.1487	−0.0314
Interorbital distance	0.0654	−0.1355
Width of snout at nostrils	0.0125	−0.1471
Depth of snout at nostrils	−0.0182	0.0245
Distance between rostral barbels	0.0431	−0.3358
Distance between maxillary barbels	0.0522	0.0033
Rostral barbel length	**0.1970**	0.5433
Maxillary barbel length	0.0522	0.1868
Meristics		
Lateral Line scales	**−0.1784**	0.0785
Scale rows lateral line to dorsal-fin origin	**−0.2099**	0.1286
Scale rows lateral line to anal-fin origin	**−0.3048**	0.2033
Branched pectoral fin rays	**−0.1301**	0.0455
Gill rakers	**0.3874**	−0.2907

Fig. 10. *Barbus pergamonensis*, IFC-ESUF 03-0451, 132 mm SL; Turkey: Manisa Prov.: Gediz River-Derbent

Fig. 11. *Barbus xanthos*, IFC-ESUF 03-0482, 146 mm SL; Turkey: Burdur Prov.: Dalaman River-Köke

Comparative material. Morphometric and meristic data for *B. cyclolepis*, *Barbus escherichii*, *B. niluferensis*, *B. oligolepis*, *B. tauricus* and *B. xanthos* are from Turan et al. (2009) and Güçlü et al. (2020**).**

Material used in molecular genetic analysis. *Barbus niluferensis*: FFR DNA BB15–16, 2, Turkey: Bursa Province.: Nilüfer Stream at Osmangazi (GenBank accession number MW728881–MW728882).

B. oligolepis: FFR DNA Bar338, 1, Turkey: Çanakkale Province.: Kocaçay at Kalkım, Gönen Stream drainage (GenBank accession number: MW731653).

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