


Barbus xanthos, a new barbel from the Southern Aegean basin (Teleostei: Cyprinidae)

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Abstract

Barbus xanthos, a new species, is described from the Eşen, Dalaman, Tersakan and Büyük Menderes rivers in south-western Anatolia. It differs from other *Barbus* species in the adjacent basins by having 53–60 lateral line scales, a weakly ossified last unbranched dorsal-fin ray (about 33–50%), numerous small irregular-shaped black or dark-brown spots smaller than scales, often forming large, black or dark-brown blotches on back and flank in juveniles and adults, and a straight or slightly convex posterior dorsal-fin margin. *B. xanthos* differs from its most closely related congener, *B. pergamonensis*, by nine nucleotide substitution sites in the mitochondrial DNA cytochrome oxidase I barcode region.

KEYWORDS

cytochrome oxidase I, freshwater fish, taxonomy, Turkey

1 | INTRODUCTION

The genus *Barbus* Daudin, 1805 comprises 33 fishes species widely distributed from the south-eastern Caspian Sea basin to eastern Spain. The species diversity of *Barbus* in Europe has been well known since publication of a report by Kottelat and Freyhof (2007). Recently, two species, *B. anatolicus* and *B. biharicus*, have been discovered (Antal *et al.*, 2016; Turan *et al.*, 2018a).

A total of 10 species has been reported from Turkish inland waters: *B. anatolicus* Turan, Kaya, Geiger & Freyhof, 2018 from the Yeşilirmak and Kızılırmak rivers, *B. cyclolepis* Heckel, 1837 from the Ergene River, *B. cyri* De, 1865 from the Kura and Aras rivers, *B. escherichii* Steindachner, 1897 from the Sakarya River, *B. lacerta* Heckel, 1843 from the Tigris, Euphrates and Van Lake basin, *B. rionicus* Kamensky, 1899 from the Çoruh River, *B. niluferensis* Turan *et al.*, 2009 from the Susurluk River, *B. oligolepis* Battalgil, 1941 from the streams and rivers in the Biga Peninsula, *B. pergamonensis* Karaman, 1971 from the streams and rivers in the Aegean Sea basin, and *B. tauricus* Kessler, 1877 from small streams in the south-eastern and western Black Sea (Geiger *et al.*, 2014; Kottelat & Freyhof, 2007; Levin *et al.*, 2019; Turan *et al.*, 2018a).

We examined materials from the Gediz, Bakır, Büyük Menderes, Dalaman, Tersakan and Eşen Rivers, and concluded that *B. pergamonensis* is restricted in the Bakır, Gediz and Madra rivers (Freyhof, pers. comm). The purpose of this study was to review the taxonomy of barbels of the south-western Anatolia region and to describe the new species from the Dalaman, Tersakan and Büyük Menderes River drainages.

2 | MATERIALS AND METHODS

The care of experimental animals was consistent with Republic of Turkey animal welfare laws, guidelines and policies approved by the Rize University Local Ethics Committee for Animal Experiments (Permit reference number 2011/04). Fishes were collected for faunal surveys and preserved in 5% formaldehyde or 96% ethanol and stored in 70% ethanol after anaesthesia using tricaine methanesulfonate. Surgical procedures were only performed for excision of fin clips. The experimental conditions did not distress any fishes involved in our experiments.

2.1 | Morphological analyses

All measurements were done point to point (never by projections) as described in Turan *et al.* (2018a) with a dial calliper calibrated to

ABBREVIATIONS: COI, cytochrome oxidase subunit I; K2P, Kimura 2-parameter; SL, standard length; HL, head length.

1 mm. The lateral line scale count, standard length and the length of the caudal peduncle were recorded according to Turan *et al.* (2018a). The last two branched rays articulating on a single pterygiophore in the anal and dorsal fins were counted as "1½". Vertebrae counts from radiographs included the hypural complex and the four Weberian vertebrae.

2.2 | DNA extraction, PCR and sequencing

Total DNA was isolated from fin tissue with the Qiacube automated purification system using Qiagen DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany). The mitochondrial barcoding cytochrome *c* oxidase subunit 1 (COI) region was amplified using the mostly used FishF1 and R1 primer pairs as described in Ward *et al.* (2005). PCR reactions were performed in a 50 µl reaction volume containing 100 ng of template DNA, 5 µl of 10× PCR buffer, 0.5 mM of each primer, 0.5 mM dNTPs mix, 5 mM MgCl₂ and 1 u Taq DNA polymerase (Bio Basic, Toronto, Canada). The PCR amplifications were carried out with a Biorad T100TM (Bio-Rad, Hercules, CA, USA) thermal cycler under the conditions as given in Turan *et al.* (2018b). The PCR products were displayed under the UV Quantum-Capt ST4 system (Vilber Lourmat, Marne-La-Vallee, France) as described in Turan *et al.* (2018b).

2.3 | Molecular analysis

Clustal W algorithm (Thompson *et al.*, 1994) in Bioedit v7.2.5 (Hall, 1999) was used to align COI barcode sequences (652 bp). DnaSP v5.1 (Librado & Rozas, 2009) software was used to identify haplotypes and sequences were submitted to GenBank number MK716232-MK716240. The Network 5.0.0.3 software (Bandelt *et al.*, 1999) was used to illustrate nucleotide differences among species. Phylogenetic assignment among haplotypes and species was carried out using both maximum likelihood (ML) and Bayesian inference (BI) analysis with MEGA X (Kumar *et al.*, 2018) and MrBayes 3.2.6 software (Ronquist *et al.*, 2012). The K2 + G model (Kimura, 1980) was chosen as the best nucleotide substitution model according to Bayesian information criterion (BIC) and Akaike information criterion (AIC) in jModeltest v. 0.0.1 (Posada, 2008). In all phylogenetic analyses *Luciobarbus lydianus* (Boulenger, 1896) (MK716241) was selected as outgroup taxa. The Kimura 2-parameter (K2P) distance model (Kimura, 1980) in MEGA X (Kumar *et al.*, 2018) was used to estimate pairwise genetic distances among species.

2.4 | Collection codes

IFC-ESUF, Inland Fishes Collection, Isparta University of Applied Sciences, Isparta-Turkey; FFR, Zoology Museum, Faculty of Fisheries, Recep Tayyip Erdogan University, Rize; FFR DNA, Faculty of Fisheries, Genetic Lab., DNA Collection, Recep Tayyip Erdogan University, Rize.

3 | RESULTS

3.1 | *Barbus xanthos* sp. nov.

(Figures 1–3)



FIGURE 1 *B. xanthos*, IFC-ESUF 03–0512; holotype, 190 mm SL; Turkey: Eşen River



FIGURE 2 *B. xanthos*, paratypes, from top: IFC-ESUF 03–0471, 175 mm SL, Tersakan Stream; IFC-ESUF 03–0474, 160 mm SL, the River Büyük Menderes drainage; IFC-ESUF 03–0482, 135 mm SL, the Dalaman River drainage



FIGURE 3 *B. xanthos* (in live), IFC-ESUF 03–0487, 146 mm SL, the River Büyük Menderes drainage

3.2 | Holotype

IFC-ESUF 03-0512, 190 mm SL; male; Turkey: Muğla prov.: Eşen Stream at Ören-Seydikemer, 36°44'51"N, 29°23'15"E; Güçlü, S.S. & Güçlü, Z.; 27 July 2010.

3.3 | Paratypes

IFC-ESUF 03-0470, 16, 109–178 mm SL; same data as holotype. — FFR 266, 32, 88–176 mm SL; Turkey: Muğla prov.: Eşen Stream at Sahilceylan, Seydikemer, 36°42'47"N, 29°21'51"E; Turan, D., Kaya, C. & Baycelebi, E.; 24 August 2004. — FFR 268, 18, 44–140 mm SL; Turkey: Aydın prov.: Akçay Stream at Beğlerli, Büyük Menderes drainage at Boğazyurt Stream, 37°45'34"N, 28°20'07"E; Turan, D., Kaya, C. & Baycelebi, E.; 25 August 2014. — IFC-ESUF 03-0474, 45, 86–166 mm SL; Turkey: Aydın prov.: Dandalas Stream at Karacasu County, Büyük Menderes drainage, 37°44'57"N, 28°37'19"E; Güçlü, S.S. & Güçlü, H.; 14 July 2010. — IFC-ESUF 03-0482, 20, 74–132 mm SL; Turkey: Denizli prov.: Dalaman River at Köke-Acıpayam, 37°20'16"N, 29°23'09"E; Güçlü, S.S. & Güçlü, Z.; 27 July 2010. — IFC-ESUF 03-0483, 30, 87–131 mm SL; Turkey, Burdur Prov.: outlet of Belkaya Dam Lake at Çavdır, Dalaman River drainage, 37°16'41"N, 29°35'10"E; Güçlü, S.S. & Güçlü, Z.; 27 July 2010. — IFC-ESUF 03-0471, 32, 97–185 mm SL; Turkey: Muğla prov.: Tersakan Stream at Dalaman, 36°47'29"N, 28°49'44"E; Güçlü, S.S. & Güçlü, Z.; 27 July 2010.

3.4 | Diagnosis

B. xanthos differs from *B. pergamonensis* in having a less ossified last unbranched dorsal-fin ray (33–50% vs. 52–72% ossified), less developed lateral lobes (the lateral lobes less developed than median swollen pad vs. lateral lobes approximately equal to median swollen pad) and a straight or slightly convex posterior margin of dorsal-fin (vs. slightly concave). *B. xanthos* is distinguished from *B. niluferensis* and *B. cyclolepis* by having fewer lateral line scales (53–60 vs. 62–71 in *B. niluferensis*, 63–76 in *B. cyclolepis*), fewer scale rows between the dorsal-fin origin and the lateral line (11–13, mode 12 vs. 13–15, mode 14 in *B. niluferensis* and 12–17, mode 15 in *B. cyclolepis*) and fewer scale rows between the anal-fin origin and the lateral line (7–8, mode 7 vs. 8–10, mode 9 in *B. niluferensis* and 9–12 in *B. cyclolepis*). It is further distinguished from *B. cyclolepis* in having a less ossified last unbranched dorsal-fin ray (33–50% vs. 63–75% ossified).

3.5 | Description

General appearance is shown Figures 1–3 and morphometric and meristic data are given Tables 1 and 2. A small species with a moderately deep and slightly compressed body. Dorsal profile arched and ventral profile straight or slightly arched. Predorsal profile convex and postdorsal profile straight. Head short, its upper profile slightly convex

in interorbital area, straight or slightly convex at nostrils and slightly concave in front of nostrils. Mouth subinferior, with slightly developed lips. Lips with papillae, lower lip thicker than upper lip. Lower lip with produced lateral lobes and median pad. Lateral lobes less developed than median swollen pad (Figure 4). Rostral barbels reaching vertical of nostril. Maxillary barbels not reaching to vertical of posterior margin of pupil in most individuals. In a few specimens, maxillary barbels reaching to vertical of posterior margin of pupil. Snout slightly pointed. Largest known specimen about 180 mm SL.

Lateral line totally with 53–60 scales, 11–13 scale rows between dorsal-fin origin and lateral line, seven or eight scale rows between the anal-fin origin and the lateral line. Seven to 10 gill rakers on outer side of first gill arch. Dorsal-fin with four unbranched and 8½ branched rays. Posterior margin of dorsal fin slightly convex. Dorsal-fin origin slightly in front of vertical of pelvic-fin origin. The last unbranched dorsal-fin ray weakly ossified, approximately 33–50% of its length (Figure 5). Pectoral fin slightly convex, with one unbranched and 16–17 branched rays. Pelvic fin slightly convex, with one unbranched and seven or eight branched rays. Anal fin with three unbranched and 5½ branched rays, outer margin straight or slightly convex, not reaching to base of caudal-fin in the stream Eşen specimens but reaching to base of caudal-fin in other populations. Caudal-fin moderately forked, lobes slightly pointed. Total vertebrae 41–43 (modally 41). Pharyngeal teeth 5.3.2–2.3.5.

3.6 | Coloration

Formalin-preserved juveniles and adults with brown background colour on back and flank, yellowish on belly. Many small black or dark-brown spots on head in some individuals, absent in others. Numerous small irregular shaped black or dark brown spots, smaller than scales, often forming large, black or dark-brown blotches on back and flank in juveniles and adults. Dorsal and caudal fins greyish. Anal, pelvic and pectoral fins yellowish. Numerous black or dark-brown spots on rays of all fins.

3.7 | Sexual dimorphism

Females possess longer anal fin than males.

3.8 | Distribution and notes on habitat

B. xanthos is known from the drainages of the Büyük Menderes, Dalaman, Tersakan and Eşen rivers (Figure 6). It inhabits swift flowing water with a rocky and pebble bottom.

3.9 | Etymology

The name of the species originates from Xanthos River as Xanthos is the ancient name of the Eşen River. An adjective.

TABLE 1 Pairwise distance K2P values based on cytochrome oxidase sequences of *Barbus* species (below the diagonal; S.E. above the diagonal)

	<i>B. xanthos</i>	<i>B. pergamonensis</i>	<i>B. niluferensis</i>	<i>B. cyclolepis</i>	<i>B. anatolicus</i>	<i>B. escherichii</i>	<i>B. tauricus</i>	<i>B. oligolepis</i>
<i>B. xanthos</i>		0.005	0.008	0.010	0.011	0.011	0.011	0.011
<i>B. pergamonensis</i>	0.016		0.007	0.010	0.011	0.011	0.011	0.011
<i>B. niluferensis</i>	0.032	0.030		0.010	0.010	0.010	0.010	0.011
<i>B. cyclolepis</i>	0.048	0.050	0.045		0.012	0.012	0.012	0.012
<i>B. anatolicus</i>	0.060	0.058	0.056	0.064		0.004	0.004	0.004
<i>B. escherichii</i>	0.062	0.060	0.058	0.069	0.009		0.000	0.002
<i>B. tauricus</i>	0.062	0.060	0.058	0.070	0.009	0.000		0.002
<i>B. oligolepis</i>	0.064	0.061	0.060	0.071	0.010	0.002	0.002	

TABLE 2 The variable nucleotide substitutions in the 652 base pairs long mtDNA COI barcode region

Species	Variable nucleotide positions							
	111	11111111122	2222222333	3333333333	3344444444	4444455555	5555666666	
	124578011	4666789902	2468889001	1123345556	7800112345	5669911235	5779112244	
	4683868325	5069816920	9750132470	3954705687	809237051	4037947900	3185362809	
<i>B. xanthos</i>	CTCCCCACCT	GATTAGGAAG	ACAACGCTAT	AGAGTGTTAA	GAAGAGAATA	TCACGATATA	TACACAAATT	
	T	A						
<i>B. pergamonensis</i>G...A...ACA....	A.G..A.G..	.T.....	
<i>B. niluferensis</i>	TCT..T....	.G.CGAT.GA	..G..A....	...A.A....	A.G....G..	..G.A..G..	..T.....C	
<i>B. cyclolepis</i>	TC.....C	...C.ATG..	G..G.A.C.C	GA.A.A...G	AG....GG.G	C.GTA...C	CG....GGC.	
<i>B. anatolicus</i>	TC.TT...T.	..CC.AT.GA	.T...AT...	GAG..CCC.G	A.CA...GC.	.TGTAGC..G	...CTG....	
<i>B. tauricus</i>	TC.TT...T.	A.CC.AT.GA	.T..TAT.G.	.A...CCC.G	A.CA...GC.	.TGTAGC..G	...CTG....	
<i>B. escherichii</i>	TC.TT...T.	A.CC.AT.GA	.T..TAT.G.	.A...CCC.G	A.CAG..GC.	.TGTAGC..G	...CTG....	
<i>B. oligolepis</i>	TC.TT...T.	A.CC.AT.GA	.T..TAT.G.	.A...CCCG	A.CA...GC.	.TGTAGC..G	...CTG....	

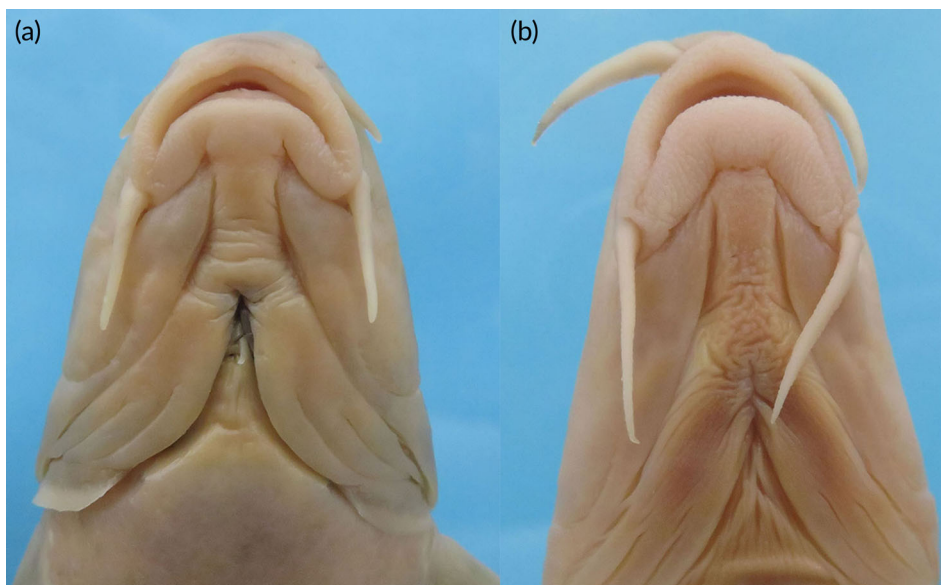
**FIGURE 4** Ventral view of head: (a) *B. xanthos*, IFC-ESUF 03-0470, paratype, 177 mm SL and (b) *B. pergamonensis*, IFC-ESUF 03-0451, 152 mm SL



FIGURE 5 Last unbranched dorsal-fin ray. Upper row, from left: *Barbus xanthos*, IFC-ESUF 03-0470 paratype, 181 mm SL, 132 mm SL, 128 mm SL, 125 mm SL; lower row, from left: *B. pergamonensis*, IFC-ESUF 03-0451, 112 mm SL, 137 mm SL, 135 mm SL, 128 mm SL

3.10 | Key to species of *Barbus* in western Anatolia and Black Sea basin

- 1a - 39–44 total vertebrae; small-sized fish reaching about 200 mm SL.
2.
 1b - 45–49 total vertebrae; large-sized fish reaching usually 300–500 mm SL.
5.
 2a - 43–44 total vertebrae; 62–72 total lateral line scales.
 *B. niluferensis*.
 2b - 39–42 total vertebrae; 50–61 total lateral line scales.
3.

- 3a - Interorbital distance 24%–29% SL.
 *B. cyclolepis*.
 3b - Interorbital distance 30%–34% SL.
4.
 4a - Last unbranched dorsal-fin ray 52–72% ossified; posterior margin of dorsal-fin slightly concave.
 *B. pergamonensis*.
 4b - Last unbranched dorsal-fin ray 33–50% ossified; posterior margin of dorsal-fin straight or slightly convex.
 *B. xanthos*.
 5a - Length of the anal fin equal in both sexes; outer margin of the dorsal fin markedly concave.
 *B. anatolicus*.
 5b - Length of the anal fin in female longer than that of the male; outer margin of the dorsal fin straight or slightly concave.
6.
 6a - 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*.
 6b - 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.
7.
 7a - Head length 1.2–1.5 times in body depth; snout length 1.5–1.7 times interorbital distance.
 *B. oligolepis*.
 7b - Head length 1.0–1.2 times body depth; snout length 1.4–1.5 times interorbital distance.
 *B. tauricus*.

3.11 | Phylogenetic placement of *B. xanthos*

COI barcode region sequences were analysed in eight *Barbus* species in south-western Anatolia and the Thrace region. Fifty individuals were sequenced by this study and seven sequences obtained from Genbank. *Barbus* species were divided into two main clades in the phylogenetic analysis supported by high bootstrap values. The first clade consisted of *B. xanthos*, *B. pergamonensis*, *B. niluferensis* and *B. cyclolepis* while the other clade contained *B. anatolicus*, *B. oligolepis*, *B. escherichii* and *B. tauricus*. *B. xanthos* constituted a highly supported clade sister to *B. pergamonensis* (Figure 7). Intrageneric K2P distances between species ranged from 0.0% (*B. escherichii* and *B. tauricus*) to 7.1% (*B. cyclolepis* and *B. oligolepis*). K2P distance is 1.6% between *B. xanthos* and closest relative *B. pergamonensis* and 3.2% between *B. xanthos* and *B. niluferensis* (Table 3). *B. xanthos* differs from its most closely related congener, *B. pergamonensis*, by nine nucleotide substitution sites and 1.6% K2P distance in the mitochondrial DNA (mtDNA) COI barcoding region. Seventy variable nucleotide positions in the COI barcoding region were determined between *Barbus* species. *B. xanthos* was differentiated from all other *Barbus* species in south-western Anatolia by three diagnostic and unique nucleotide

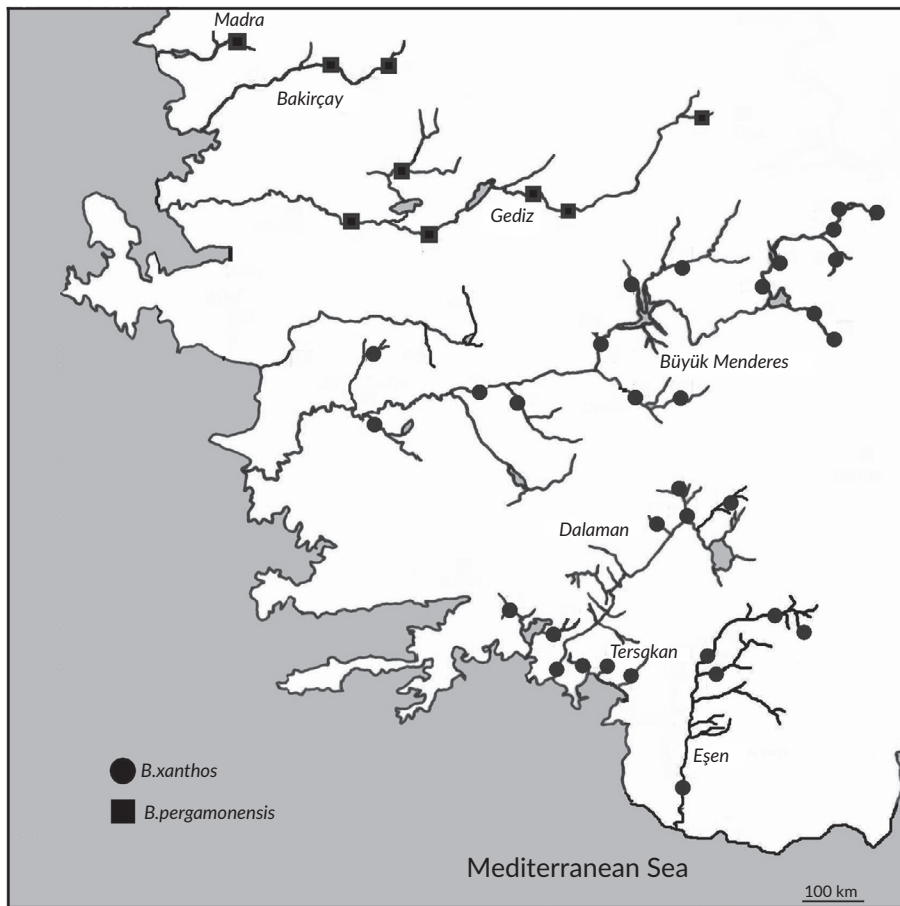


FIGURE 6 Distribution of *Barbus* species in the south-eastern Aegean

substitution sites in the COI barcoding region (Table 4). While *B. escherichii* and *B. tauricus* share the same haplotype, the other studied barbels have unique haplotypes as distinctly illustrated in the haplotype network (Figure 8).

4 | DISCUSSION

There are eight species of *Barbus* (*B. xanthos*, *B. pergamonensis*, *B. niluferensis*, *B. cyclolepis*, *B. tauricus*, *B. escherichii*, *B. anatolicus*, *B. oligolepis*) in the western Anatolia and Thrace region. The differences between *B. xanthos* and *B. pergamonensis*, *B. niluferensis* and *B. cyclolepis* are given in detail in the 3.4. diagnosis section. *B. xanthos* is also distinguished from *B. tauricus*, *B. escherichii*, *B. anatolicus* and *B. oligolepis* by smaller size (maximum size about 200 mm SL vs. about 500 mm SL), fewer total vertebrae (41–43 vs. 45–49) and maximum anal-fin length equal to or slightly greater than caudal-fin length (vs. maximum anal-fin length usually less than caudal-fin length). *B. xanthos* is further distinguished from *B. oligolepis* by having fewer gill rakers on the first gill arch (7–10 vs. 11–14), a less ossified last simple dorsal-fin ray (33–50% vs. 71–73%) and a smaller head (22–27% SL vs. 27–30%). *B. xanthos* is further distinguished from *B. escherichii* by having a less ossified last simple dorsal-fin ray (33–50% vs. 73–77%), fewer black or dark-brown spots on the back and flank (a few vs. numerous), lower lip with median pad (vs. lower lip with

median lobe) and dorsal-fin origin slightly in front of the vertical of the pelvic-fin origin (vs. dorsal-fin origin above the pelvic-fin origin). *B. xanthos* is further distinguished from *B. anatolicus* by having fewer lateral line scales (53–60 vs. 58–71), a less ossified last simple dorsal-fin ray (33–50% vs. 70–80%), fewer black or dark-brown spots on the back and flank (vs. numerous) and lower lip with median pad (vs. lower lip with median lobe). *B. xanthos* is further distinguished from *B. tauricus* by having more gill rakers on the first gill arch (7–10 vs. 11–13) and less developed lips (slightly developed vs. well developed, especially in specimens larger than about 200 mm SL).

5 | COMPARATIVE MATERIAL

5.1 | Material used for morphometric and meristic comparison

Morphometric and meristic data for *B. escherichii*, *B. niluferensis*, *B. oligolepis* and *B. tauricus* are from Turan *et al.* (2009, 2018a).

5.1.1 | *Barbus cyclolepis*

FFR 768, 15, 82–156 mm SL; Turkey: Kırklareli Prov.: Ergene Stream at Saray, Meriç River drainage 41°26'20"N, 27°55'39"E.

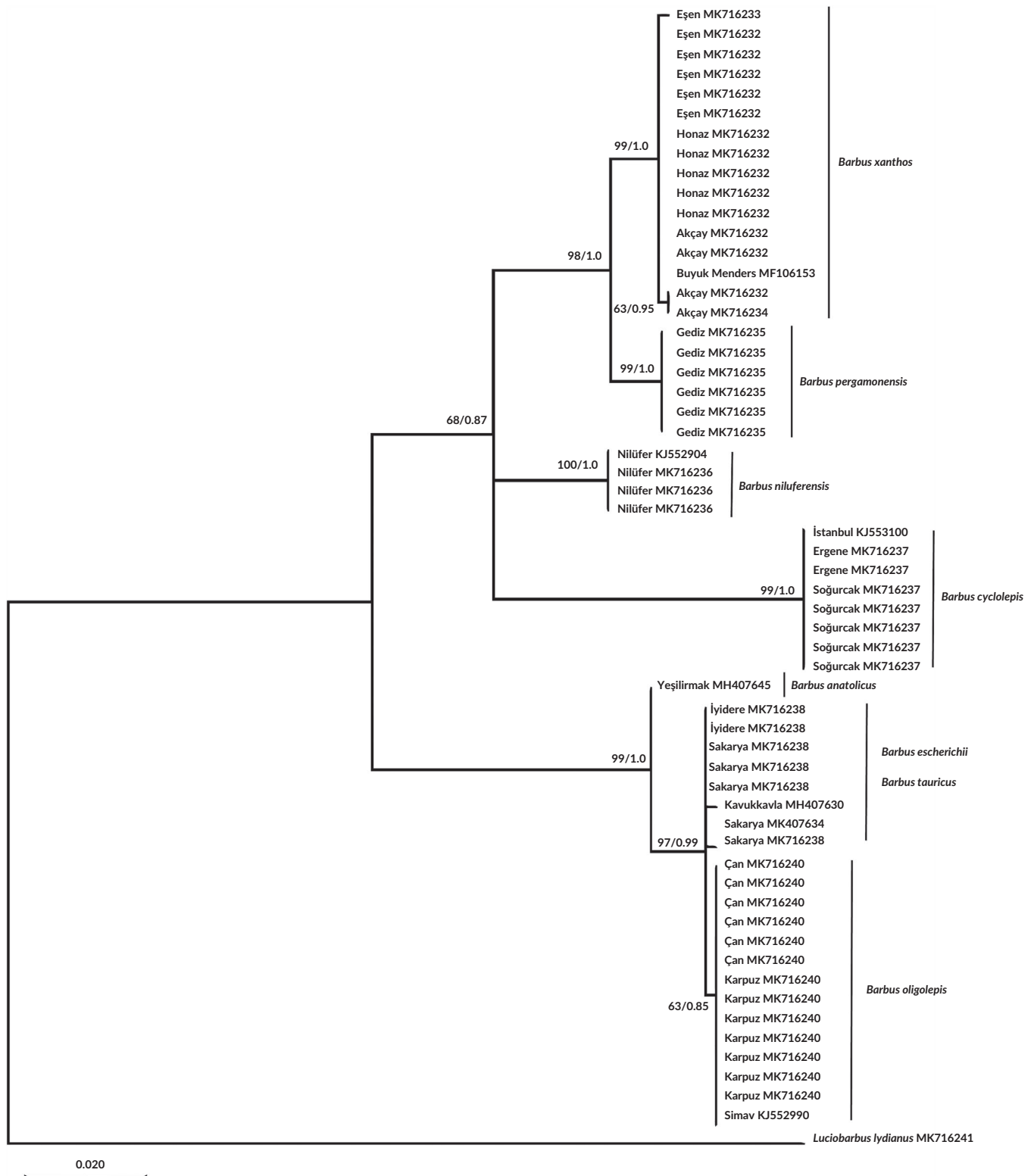


FIGURE 7 Maximum likelihood tree based on mitochondrial COI gene sequences of *Barbus* species in the Büyük Menderes, Black Sea basin and adjacent waters. Maximum likelihood and Bayesian inference analyses resulted in congruent trees. Bootstrap and posterior probability values are shown above nodes on tree if 50% or higher

5.1.2 | *Barbus pergamonensis*

FFR 00527, 10, 93–124 mm SL; Turkey: Manisa prov.: Stream Kelebek at Ahmetli, 38°30'11"N, 27°56'31"E. — FFR 00260

(Figures 9 and 10, and Table 5) 26, 68–140 mm SL; Turkey: Uşak prov.: Gediz River at Derbent, 38°46'36"N, 29°12'43"E. — FFR 8816, 3, 89–118 mm SL; Turkey: İzmir prov.: Stream Karadere at Kınık, Bakır River drainage, 39°07'47.7"N, 27°22'20.2"E. — IFC-ESUF 03-0451,

	Holotype and paratype			
	Holotype	Range	Mean	S.D.
Percentage of standard length				
Head length	23.1	21.9–26.9	24.6	2.5
Body depth at dorsal-fin origin	24.0	19.0–27.9	22.4	1.9
Predorsal length	51.2	50.5–56.3	52.6	1.4
Prepelvic length	50.8	49.5–54.1	51.2	1.0
Preanal length	75.2	72.0–80.2	76.3	1.6
Distance between pectoral and anal-fin origins	54.9	51.5–57.5	54.6	1.4
Distance between pectoral and pelvic-fin origins	28.8	24.5–32.3	29.3	1.4
Distance between pelvic and anal-fin origins	25.1	21.7–28.9	25.4	1.4
Length of caudal peduncle	20.0	15.1–20.3	17.9	1.3
Depth of caudal peduncle	10.3	10.0–12.7	10.9	0.7
Dorsal-fin depth	15.6	14.5–20.5	18.4	1.2
Pectoral-fin length	16.3	15.8–21.9	18.6	1.4
Pelvic-fin length	13.2	13.2–18.5	15.9	1.0
Anal-fin length	19.1	17.2–24.5	21.1	1.9
Upper caudal fin length	15.6	15.7–23.8	20.2	1.9
Percentage of head length				
Head width at anterior margin of eye	45	40–50	44.4	1.8
Head width at posterior margin of eye	56	52–63	56.7	2.6
Head width at occiput	70	55–71	61.7	3.7
Head depth at eye	59	43–59	49.2	3.6
Head depth at nape	74	56–74	64.4	4.0
Eye diameter	14	12–19	15.2	1.7
Snout length	41	37–45	41.1	1.9
Interorbital distance	40	30–42	34.9	2.5
Width of snout at nostrils	45	35–45	39.1	2.5
Depth of snout at nostrils	36	29–38	33.4	2.5
Distance between rostral barbels	23	17–28	22.1	2.1
Distance between maxillary barbels	30	23–26	28.2	2.3
Rostral barbel length	19	16–26	21.0	2.4
Maxillary barbel length	24	17–39	30.7	4.6

Note. The holotype and paratypes are included in the calculation of ranges, means and S.D.

TABLE 4 Frequency of meristic characters of two *Barbus* species in Aegean Sea drainages

	n	Lateral line scales												
		50	51	52	53	54	55	56	57	58	59	60	61	62
<i>B. xanthos</i>	25				2	4	5	3	7	3		1		
<i>B. pergamonensis</i>	25	2	3	2	3	3	2	4	4		1			1
		Scale rows between lateral line and												
		dorsal-fin origin						anal-fin origin						
	n	11	12	13		7	8							
<i>B. xanthos</i>	25	7	17	1		15	10							
<i>B. pergamonensis</i>	25	6	17	2		7	18							

11, 106–172 mm SL; Turkey: Kütahya prov.: Gediz River at Derbent-Gediz, 38°46'37"N, 29°12'41"E. — IFC-ESUF 03-0457, 13, 57–133 mm SL; Turkey: Manisa prov.: Gediz River at Yurtbaşı-

Kula, 38°36'09"N, 28°48'54"E. — IFC-ESUF 03-0473, 4, 61–83 mm SL; Turkey: Manisa prov.: Gediz River at Hamidiye-Kula, 38°40'08.70"N, 28°36'13.48"E.

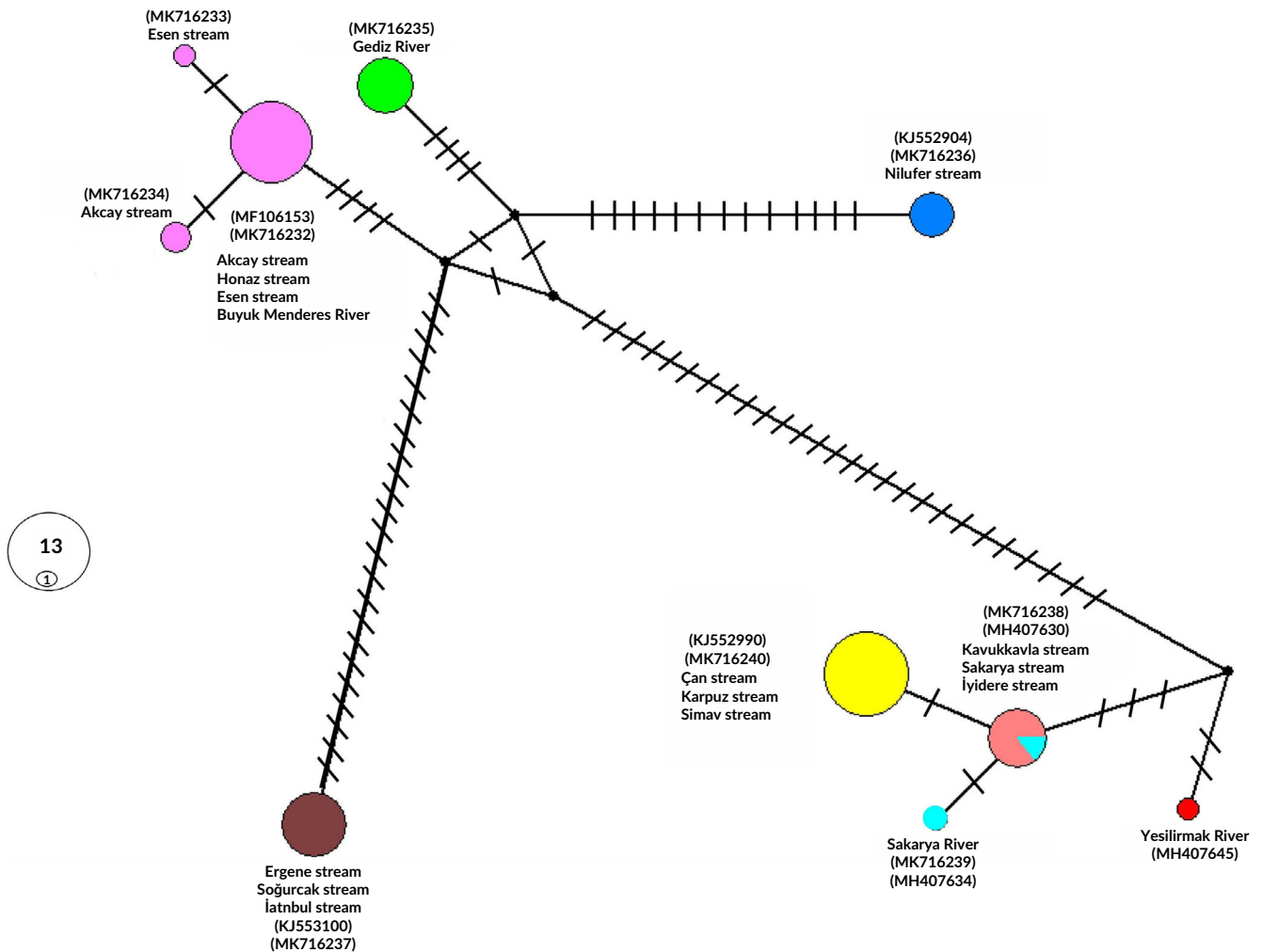


FIGURE 8 Median-joining network of the COI haplotypes. Circle size corresponds to sample size; one bar indicates an additional mutational step. Small black circles represent median vectors. Each small line represents one nucleotide difference (● *B. xanthos*, ● *B. pergamonensis*; ● *B. niluferensis*, ● *B. cyclolepis*; ● *B. oligolepis*, ● *B. tauricus*; ● *B. escherichii*, ● *B. anatolicus*)



FIGURE 9 *B. pergamonensis* (in live), FFR08816, 112 mm SL, Bakır Stream [Correction added on 19 March 2020, after first online publication: The captions of Figures 9 and 10 were inadvertently swapped and have been amended in this current version.]

5.2 | Material used in the molecular genetic analysis

5.2.1 | *Barbus xanthos*

FFR DNA 266, 6, Turkey: Muğla prov.: Eşen Stream at Seydikemer, 36°44'51"N, 29°23'15"E (GenBank accession number: MK716232, MK716233). –FFR DNA 268, 4, Turkey: Aydın prov.: Akçay Stream at



FIGURE 10 *B. pergamonensis*, FFR 00260, from top: 126 mm SL, 114 mm SL, 113 mm SL, Gediz River drainage

TABLE 5 Morphometric data of *B. pergamonensis* from the Gediz River [IFC-ESUF 03–0451 ($n = 10$); FFR 260 ($n = 25$)]

Drainage	Gediz River		
	Range	Mean	S.D.
Percentage of standard length			
Head length	24.3–31.3	26.2	1.9
Body depth at dorsal-fin origin	19.1–23.8	21.4	1.5
Predorsal length	50.4–56.7	53.5	1.6
Prepelvic length	48.4–54.3	51.6	1.6
Preal length	74.9–81.4	77.4	1.4
Distance between pectoral and anal-fin origins	59.6–57.4	54.2	2.0
Distance between pectoral and pelvic-fin origins	26–31	28.3	1.2
Distance between pelvic and anal-fin origins	22.2–28.3	26.0	1.4
Length of caudal peduncle	14.9–20.2	17.4	1.2
Depth of caudal peduncle	8.9–10.9	9.8	0.5
Dorsal-fin depth	17.2–23.4	20.0	1.6
Pectoral-fin length	18.2–21.5	19.8	0.1
Pelvic-fin length	14.9–18.7	16.9	1.0
Anal-fin length	20.1–25.9	23.4	1.4
Upper caudal fin length	18.8–23.6	21.2	1.3
Percentage of head length			
Head width at anterior margin of eye	39.6–50.1	43.4	3.1
Head width at posterior margin of eye	48.9–64.0	55.4	3.2
Head width at occiput	54.8–67.9	60.3	3.1
Head depth at eye	42.7–56.6	49.2	3.2
Head depth at nape	55.5–71.6	62.5	4.0
Eye diameter	12.4–19.6	16.9	1.6
Snout length	39.0–47.6	42.3	2.2
Interorbital distance	26.8–37.3	32.6	2.5
Width of snout at nostrils	33.2–42.0	37.8	2.1
Depth of snout at nostrils	29.6–37.9	33.9	1.9
Distance between rostral barbels	12.7–26.3	20.4	3.4
Distance between maxillary barbels	24.7–35.9	27.8	2.4
Rostral barbel length	14.8–23.7	19.2	1.8
Maxillary barbel length	23–29	26.2	1.7

Beğlerli, Büyük Menderes River, 37°45'34"N, 28°20'07"E (GenBank accession no: MK716232, MK716234). —FFR DNA 00274, 5, Turkey: Denizli prov.: Honaz Stream at Honaz, Büyük Menderes River, 37°47'21"N, 29°15'40"E (GenBank accession no: MK716232). Khaefi *et al.*, 2017 Turkey: Büyük Menderes (GenBank accession no: MF106153).

5.2.2 | *Barbus escherichii*

FFR DNA 00272, 3, Turkey, Bilecik prov.: Göynük Stream at Göynük, Sakarya River 40°23'07"N, 30°44'43"E (GenBank accession no:

MK716238). —FFR DNA 00275, 1, Turkey Kütahya prov.: Porsuk Stream, Sakarya River, 39°20'59"N, 30°02'17"E (GenBank accession no: MK716239). Turan *et al.*, 2018 Turkey: Sakarya River (GenBank accession no: MH407634).

5.2.3 | *Barbus niluferensis*

FFR DNA 00280, 3, Turkey, Bursa prov.: Hasanağa Stream, Nilüfer Stream, 40°10'07"N, 28°47'40"E (GenBank accession no: MK716236). Geiger *et al.*, 2014 Turkey: Simav drainage (GenBank accession no: KJ552904).

5.2.4 | *Barbus cyclolepis*

FFR DNA 768, 2, Turkey: Kırklareli prov.: Ergene Stream at Saray, Meriç River drainage 41°26'20"N, 27°55'39"E (GenBank accession no: MK716237). —FFR DNA 00285, 5, Turkey, Kırklareli prov.: Soğurcak Stream at Vize, 41°37'37"N, 27°35'37"E (GenBank accession no: MK716237). Geiger *et al.*, 2014 Turkey: İstanbul drainage (GenBank accession no: KJ553100).

5.2.5 | *Barbus oligolepis*

FFR DNA 00282, 7, Turkey, Yalova prov.: Karpuz Stream, Teşvikiye Stream at Armutlu, 40°36'04.4"N, 29°03'45.5"E (GenBank accession no: MK716240). —FFR DNA 00258, 6, Turkey, Çanakkale prov.: Çan Stream at Biga, 40°12'46"N, 27°14'37"E (GenBank accession no: MK716240). Geiger *et al.*, 2014 Turkey: Simav drainage (GenBank accession no: KJ552990).

5.2.6 | *Barbus pergamonensis*

FFR DNA 260, 6, Turkey: Uşak prov.: Gediz River at Derbent, 38°46'36"N, 29°12'43"E (GenBank accession no: MK716235).

5.2.7 | *Barbus tauricus*

FFR DNA 00131, 2, Turkey: Rize prov.: İyidere Stream at southwestern İyidere, 40°57'26"N, 40°24'44"E (GenBank accession number: MK716238). Turan *et al.*, 2018a Turkey: Kavukavla (GenBank accession no: MH407630).

5.2.8 | *Barbus anatolicus*

Turan *et al.*, 2018a Turkey: Yesilirmak River (GenBank accession no: MH407645).

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