

A new species of *Petroleuciscus* (Teleostei: Cyprinidae) from the Büyük Menderes River, southwestern Anatolia, Turkey

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Petroleuciscus ninae sp. nov. is described from the Büyük Menderes River drainage. The new species is distinguished by having a black lateral stripe from head to base of caudal fin, stripe distinct anteriorly and posteriorly, wider than eye diameter; numerous black pigments on anal-fin rays; body depth at dorsal-fin origin 27–30% standard length (L_S); head width at posterior margin of eye 16–19% L_S ; and eye diameter smaller than snout length. *Petroleuciscus ninae* is also distinguished from other species in adjacent waters by having six fixed diagnostic nucleotide substitutions in the mitochondrial DNA *col* barcode region.

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Key words: barcode region; *col*; new chub; taxonomy; Turkey.

INTRODUCTION

The genus *Petroleuciscus* Bogutskaya 2002 contains several species previously assigned to genus *Leuciscus* Cuvier 1816 (Bogutskaya, 2002). *Petroleuciscus* now comprises five valid species: *Petroleuciscus borysthenticus* (Kessler 1859) from the streams and rivers of the Black Sea, Marmara and northern Aegean Sea basins (Bogutskaya, 1996), *Petroleuciscus kurui* (Bogutskaya 1995) from the upper Tigris River drainage in Turkey (Bogutskaya, 1995), *Petroleuciscus smyrnaeus* (Boulenger 1896) from western Anatolia (Kottelat & Freyhof, 2007), *Petroleuciscus squaliusculus* (Kessler 1872) occurs in the Syr Darya drainage and *Petroleuciscus ulanus* (Günther 1899) from Orumeyeh Lake in Iran. Until recently, *Petroleuciscus* also contained *Petroleuciscus esfahani* Coad & Bogutskaya 2010 from the Zayandeh River basin in Iran (Bogutskaya, 1996, 2002; Coad & Bogutskaya, 2010; Perea *et al.*, 2010), but this sixth species has been found to be a synonym of *Alburnus doriae* De Filippi 1865 (Mohammadian-Kalat *et al.*, 2017).

In this study, specimens collected from the Madara, Bahçeköy and Karamandere streams (Black Sea Basin), İnce stream (a tributary of the Ergene River), Çamaşır stream (Lake Büyükçekmece Basin) and the Susurluk River basin have been examined

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and identified as *P. borysthenicus* and specimens collected from the Gediz River, Bakir stream, Lake Gölçük, the Küçük Menderes River and Lesbos (Aegean Sea basin) have been examined and identified as *Petroleuciscus smyrnaeus*. This study's comparison of specimens from the Büyük Menderes River (Aegean Sea basin) with *P. smyrnaeus* and *P. borysthenicus*, however, shows that the Büyük Menderes River specimens represent an unnamed species described here as *P. ninae*.

MATERIALS AND METHODS

Fish specimens and genetic tissues were catalogued at the Faculty of Fisheries (FFR) of the Recep Tayyip Erdogan University Zoology Museum. Measurements were made with dial callipers and recorded to 0.1 mm. All measurements are made point to point, never by projections. Counts and measurements follow Turan *et al.* (2016). Lateral line scale counts include scales on the base of the caudal fin. The last two branched dorsal and anal-fin rays articulating on a single pterygiophore were counted as 1½. Vertebral counts were obtained from radiographs and counted as total, predorsal, abdominal and caudal vertebrae following Bogutskaya & Coad (2009). Predorsal vertebrae included the Weberian vertebrae and abdominal vertebrae anterior to the first dorsal-fin pterygiophore. Abdominal vertebrae were counted from the first Weberian vertebra to the vertebra just anterior to the first caudal vertebra. The first caudal vertebra is the first vertebra with its haemal spine fully developed. Counts of total and caudal vertebrae include the last complex vertebra bearing hypurals. The meristic data for *P. ulanus* and *P. squaliusculus* are from Coad & Bogutskaya (2010).

DNA EXTRACTION, PCR AND SEQUENCING

Total DNA was extracted from fin tissue using the Wizard Genomic DNA Purification Kit (Promega; www.promega.com). Cytochrome c oxidase subunit 1 (*col*) barcode region (652 bp) was amplified using the FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') (Ward *et al.*, 2005) primer pairs. PCR products were purified using QIAquick PCR Purification Kit (Qiagen; www.qiagen.com) according to manufacturer's instructions and bidirectional sequencing of PCR products was performed with an ABI PRISM 3730x1 Genetic Analyser (Applied Biosystem; appliedbiosystems.com) using a BigDye Terminator 3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystem) at Macrogen Inc., Amsterdam (www.dna.macrogen.com).

MOLECULAR ANALYSES

COI barcode sequences were aligned using the Clustal W algorithm (Thompson *et al.*, 1994) implemented in Bioedit 7.2.5 (Hall, 1999). Haplotypes were identified using DnaSP 5.1 (Librado & Rozas, 2009) software and submitted to GenBank with accession numbers MF362183–MF362214. According to Akaike information criterion (AIC) and Bayesian information criterion (BIC) implemented in jModeltest 0.0.1 (Posada, 2008), the Hasegawa–Kishino–Yano (HKY) + G model (Hasegawa *et al.*, 1985) was the best-fit model for nucleotide substitution in the *col* barcode region. Phylogenetic relationships among haplotypes and species were estimated using maximum likelihood (ML) and maximum parsimony (MP) algorithms using PhyML (Guindon *et al.*, 2010) and PAUP* 4.0b10 software (Swofford, 2003). In addition, the Bayesian inference (BI) analysis was run using a Metropolis-coupled Markov chain Monte-Carlo (MCMC) algorithm from randomly generated starting trees for six million generations in the MrBayes 3.1.2 software (Ronquist & Huelsenbeck, 2003) with sampling every 1000 generations and the initial 25% of saved trees sampled in each MCMC run discarded as burn-in. *Squalius fellowesii* (Günther 1868) (MF362214), *Ladigesocypris irideus* (Ladiges 1960) (MF362212) and *Pseudophoxinus meandri* (Ladiges 1960) (MF362213) were selected as outgroup taxa for all phylogenetic analyses. Also *Acanthobrama persidis* (Coad 1981) (formerly known as *Petroleuciscus persidis* Coad 1981) sequences from GenBank were

included in the phylogenetic analysis. Pairwise genetic distances among species were estimated using the Kimura 2-parameter (K2P) distance model (Kimura, 1980) as implemented in MEGA 6.0 (Kumar *et al.*, 2016).

RESULTS

Nucleotide sequences of the *col* barcode region were examined in 29 individuals sequenced by this study and nine individuals from GenBank belonging to three species of *Petroleuciscus* in western Anatolia and the Thrace region (Table I). The phylogenetic analysis showed that these *Petroleuciscus* species are divided into two clades. The first clade consisted of *P. borysthenicus* while the other clade contains *P. smyrnaeus* and *P. ninae*. *Petroleuciscus ninae* constituted a highly supported clade sister to *P. smyrnaeus*, both taxa being clustered in a monophyletic group distinguished from *P. borysthenicus* (Fig. 1). ML, MP and BI analyses of mitochondrial *col* resulted in trees congruent with each other, supported by high bootstrap values. Intrageneric K2P distances between species were 1.6% (*P. ninae* and *P. smyrnaeus*), 3.3% (*P. borysthenicus* and *P. ninae*) and 3.5% (*P. borysthenicus* and *P. smyrnaeus*). In total, 28 variable nucleotide positions in the *col* barcode region were identified between *Petroleuciscus* species studied here. *Petroleuciscus ninae* was distinguished from all other *Petroleuciscus* species in western Anatolia by six unique and diagnostic nucleotide substitutions sites in the mtDNA *col* barcode region (Table II).

PETROLEUCISCUS NINAE NEW SPECIES

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Holotype (Fig. 2)

FFR 03856, 61 mm standard length (L_S); Turkey: Aydın Province: Akçay stream, 3 km west of Beğlerli, 37° 45' 34'' N, 28° 20' 07'' E.

Paratypes

FFR 03837, 54, 35–65 mm L_S ; same data as holotype.

Diagnosis

Petroleuciscus ninae is closely related to *P. smyrnaeus*. In preserved individuals it is distinguished from *P. smyrnaeus* by having a black stripe on flank extending from head to base of caudal fin, stripe wider than eye diameter (v. equal to or smaller than eye diameter) (stripe absent in life), numerous black pigments on anal-fin rays in life (v. pigments absent or very few orange pigments in life); a deeper body (body depth at dorsal-fin origin 27–30% L_S v. 24–27) and a wider head (head width at posterior eye margin 16–19% L_S v. 14–16). Also, *P. ninae* has an eye diameter that is smaller than snout length (v. eye diameter approximately equal to snout length).

Petroleuciscus ninae is distinguished from *P. borysthenicus* by having fewer lateral line scales (31–36 v. 36–41), fewer predorsal vertebrae (12–13, mode 13, v. 13–14, mode 14), a deeper body (body depth at dorsal-fin origin 27–30% L_S , v. 21–26), deeper caudal peduncle (12–14% L_S , v. 9–11), wider head (at posterior of eye 16–19% L_S ,

TABLE I. List of species used for molecular analysis of mitochondrial DNA *col*

Species	Accession number	Country	Drainage	Reference
<i>Petroleuciscus borysthenicus</i>	MF362192	Turkey	Karamandere	This study
<i>P. borysthenicus</i>	MF362193	Turkey	Bahçeköy	This study
<i>P. borysthenicus</i>	MF362194	Turkey	Bahçeköy	This study
<i>P. borysthenicus</i>	MF362195	Turkey	Bahçeköy	This study
<i>P. borysthenicus</i>	MF362196	Turkey	Bahçeköy	This study
<i>P. borysthenicus</i>	MF362197	Turkey	Çamaşır	This study
<i>P. borysthenicus</i>	MF362198	Turkey	Çamaşır	This study
<i>P. borysthenicus</i>	MF362187	Turkey	Çapraz	This study
<i>P. borysthenicus</i>	MF362183	Turkey	Sakar	This study
<i>P. borysthenicus</i>	MF362184	Turkey	Sakar	This study
<i>P. borysthenicus</i>	MF362185	Turkey	Sakar	This study
<i>P. borysthenicus</i>	MF362186	Turkey	Sakar	This study
<i>P. borysthenicus</i>	MF362188	Turkey	Çınarcık	This study
<i>P. borysthenicus</i>	MF362189	Turkey	Çınarcık	This study
<i>P. borysthenicus</i>	MF362190	Turkey	Ergene	This study
<i>P. borysthenicus</i>	MF362191	Turkey	Ergene	This study
<i>P. borysthenicus</i>	KJ554175	Bulgaria	Orljaska	Geiger <i>et al.</i> (2014)
<i>P. borysthenicus</i>	KJ554232	Bulgaria	Orljaska	Geiger <i>et al.</i> (2014)
<i>P. borysthenicus</i>	KX516732	Georgia	Rioni	Khafei <i>et al.</i> (2016)
<i>P. borysthenicus</i>	KX516733	Georgia	Rioni	Khafei <i>et al.</i> (2016)
<i>P. borysthenicus</i>	HM560280	Turkey	Bakakak	Perea <i>et al.</i> (2010)
<i>P. borysthenicus</i>	HM560281	Greece	Fotolivos	Perea <i>et al.</i> (2010)
<i>P. symrnaeus</i>	MF362199	Turkey	Gediz	This study
<i>P. symrnaeus</i>	MF362200	Turkey	Gediz	This study
<i>P. symrnaeus</i>	MF362201	Turkey	Gediz	This study
<i>P. symrnaeus</i>	MF362202	Turkey	Gediz	This study
<i>P. symrnaeus</i>	KJ554189	Turkey	Gediz	Geiger <i>et al.</i> (2014)
<i>P. symrnaeus</i>	KJ554102	Turkey	Gediz	Geiger <i>et al.</i> (2014)
<i>P. symrnaeus</i>	HM560284	Turkey	Gölcük	Perea <i>et al.</i> (2010)
<i>P. ninae</i>	MF362203	Turkey	Akçay	This study
<i>P. ninae</i>	MF362204	Turkey	Akçay	This study
<i>P. ninae</i>	MF362205	Turkey	Akçay	This study
<i>P. ninae</i>	MF362206	Turkey	Akçay	This study
<i>P. ninae</i>	MF362207	Turkey	Akçay	This study
<i>P. ninae</i>	MF362208	Turkey	Akçay	This study
<i>P. ninae</i>	MF362209	Turkey	Akçay	This study
<i>P. ninae</i>	MF362210	Turkey	Akçay	This study
<i>P. ninae</i>	MF362211	Turkey	Akçay	This study

v. 13–16) and longer pectoral-fin (20–24% L_S , v. 16–20). In *Petroleuciscus ninae*, the black stripe is markedly distinct in both the anterior and posterior part of the flank whereas in *P. borysthenicus*, the black stripe is indistinct at the anterior part of the body, faintly distinct in the posterior part and anal-fin rays have numerous black pigments (v. numerous orange pigments).

Petroleuciscus ninae is distinguished from *P. kurui* by having fewer lateral line scales (31–36, v. 51–55), fewer scale rows between the lateral line and dorsal-fin origin (6, v.



FIG. 1. Maximum likelihood tree based on mitochondrial *col* gene sequences of *Petroleuciscus* species. The maximum parsimony, 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.

8–11) and fewer scale rows between the lateral line and anal-fin origin (2–3, v. 4–6). Also, *Petroleuciscus ninae* is distinguished from *P. ulanus* and *P. squaliusculus* by having fewer lateral line scales (31–36, v. 36–45 in *P. ulanus*, 40–47 in *P. squaliusculus*) and fewer total vertebrae (35–37, v. 37–38 in *P. ulanus*, 38–40 in *P. squaliusculus*) and fewer gill rakers on the first gill arch (7–8, v. 12–16 in *P. ulanus*, 9–11 in *P. squaliusculus*).

Description

For general appearance see Fig. 2; morphometric and meristic data are provided in Tables III and IV.

TABLE II. List of the 28 diagnostic nucleotide substitutions found in the 652 bp long mitochondrial DNA *coI* barcode region

Species (n)	Variable nucleotide positions																												
	31	82	85	103	118	124	175	187	202	217	244	250	256	263	281	325	340	352	367	406	427	460	496	517	541	553	610	640	
<i>P. borys-thenticus</i> (16)	A	C	A	C	T/C	C	T/C	A	A	A	T	A	C	C	T	G	A	T	G	A/G	A	C	T	A	A/G	T/G	C	G/A	
<i>P. snyderi</i> (4)	C	T	G	T	C	C	T	A	C	G	T	G	T	C	C	A	T	C	C	A	G	T	C	G	G	G	T	T	G
<i>P. nitae</i> (9)	C	G	G	C	C	T	T	G	A	G	C	G	T	T	C	A	G	C	C	A	A	T	T	G	G	G	T	T	G

n, number of specimens.



FIG. 2. *Petroleuciscus ninae* (a) holotype, FFR 03856, 61 mm standard length (L_S) and paratypes FFR 03837 (b) 56 mm L_S and (c) 65 mm L_S .

Largest known individual 77 mm L_S . Body deep, slightly compressed laterally. Upper profile of body convex, lower profile slightly convex. Head short, its length 0.9–1.1 times in body depth at dorsal-fin origin. Dorsal head profile convex, interorbital space straight or slightly convex. Caudal peduncle length 1.1–1.5 times its depth. Snout slightly rounded, snout length greater than eye diameter but smaller than interorbital width. Mouth terminal and lips coequal.

Lateral-line complete, with 31–36 scales, six scale rows between lateral-line and dorsal-fin origin and two or three scale rows between lateral-line and anal-fin origin. Gill rakers 2–3 + 4–6 = 7–8 on outer side of first gill arch. Dorsal fin with three simple and $7\frac{1}{2}$ – $8\frac{1}{2}$ branched rays, outer margin slightly convex. Pectoral fin with one simple and 13–15 branched rays, outer margin slightly convex. Pelvic fin with one simple and (7) 8 (9) branched rays, outer margin convex. Anal fin with three simple and $8\frac{1}{2}$ – $9\frac{1}{2}$ branched rays, outer margin slightly convex. Caudal fin forked, lobes slightly

TABLE III. Origin, sample size (n) and range in morphometry, including holotype (H) and mean (in parentheses) of four *Petroleuciscus* species in western Anatolia and Thrace region

	<i>P. ninae</i>		<i>P. smyrnaeus</i>	<i>P. borysthenticus</i>
Basin	Aegean Sea		Aegean Sea	Blacksea, Marmara Sea
Drainage	Büyük Menderes		Gediz	Susurluk
Stream				Rezve, Istranca, Kocaçay, Sakar
Number of specimens	30	H	21	52
Standard length (L_S , mm)	45.3–77.3	77.1	47.0–84.7	51.8–117.5
Morphometry as % L_S				
Head length	26.4–30.7 (27.9)	26.8	26.9–28.9 (28.1)	23.9–28.5 (26.1)
Body depth at dorsal-fin origin	26.7–30.0 (28.2)	29.1	24.4–27.3 (26.1)	21.0–27.4 (25.3)
Depth of caudal peduncle	11.5–13.6 (12.6)	13.6	11.5–13.7 (12.4)	9.0–12.0 (10.5)
Head width 1	11.5–13.6 (12.6)	13.6	8.6–11.9 (10.2)	9.4–12.9 (10.6)
Head width 2	15.6–18.7 (16.2)	16.1	13.7–16.1 (14.8)	12.5–16.2 (14.3)
Head width 3 at middle of opercle	15.3–18.2 (16.7)	17.0	14.2–17.0 (15.6)	13.7–16.5 (15.2)
Head depth 1 at through eye	13.3–17.9 (15.4)	16.1	13.7–16.6 (15.1)	12.8–17.0 (14.5)
Head depth 2 at nape	19.023.2 (21.0)	21.2	18.6–21.5 (20.2)	16.7–21.4 (19.0)
Eye diameter	6.0–9.3 (7.5)	6.1	6.4–8.8 (7.7)	5.7–8.2 (7.0)
Snout length	6.8–11.0 (8.5)	8.8	6.3–9.1 (7.9)	7.0–9.5 (8.0)
Interorbital width	9.4–13.2 (10.9)	10.7	8.9–11.6 (9.8)	8.4–10.8 (9.8)
Snout width at nostrils	8.9–12.3 (10.7)	11.4	7.3–10.4 (8.8)	8.5–11.2 (9.6)
Snout depth at nostrils	8.8–14.5 (10.5)	10.1	7.9–11.5 (9.2)	7.9–10.6 (9.2)
Width of mouth gape	6.5–11.3 (8.3)	8.3	5.5–8.3 (7.1)	5.8–8.8 (7.4)
Length of mouth gape	6.2–11.1 (8.6)	8.2	6.9–10.3 (8.3)	7.2–10.2 (8.1)
Predorsal length	56.3–60.8 (58.9)	60.8	55.5–61.1 (58.5)	55.1–60.4 (57.0)
Prepelvic length	49.4–55.6 (52.1)	52.5	49.7–56.1 (52.4)	40.2–62.1 (50.4)
Pre-anal length	70.9–77.7 (73.9)	74.6	60.9–74.8 (72.7)	67.9–74.0 (70.6)
Pectoral-fin origin to anal fin	47.0–52.6 (49.3)	51.0	43.8–50.3 (47.6)	44.5–51.3 (47.1)
Pectoral-fin origin to pelvic fin	24.1–31.0 (26.6)	27.5	21.3–28.4 (25.7)	19.6–30.2 (25.5)
Pelvic-fin origin to anal fin	20.8–25.4 (22.8)	22.0	19.0–23.8 (21.6)	16.3–29.3 (21.3)
Length of caudal peduncle	14.3–18.5 (16.6)	16.3	16.0–19.5 (17.8)	16.7–22.1 (19.2)
Dorsal-fin height	18.3–24.0 (22.1)	19.6	18.5–23.6 (21.2)	16.1–24.5 (20.2)
Pectoral-fin length	19.6–24.0 (22.2)	19.6	17.2–21.1 (18.7)	15.8–20.4 (15.6)
Pelvic-fin length	14.9–19.3 (17.5)	16.0	14.1–18.3 (15.8)	12.9–20.8 (15.6)
Anal-fin height	13.9–21.7 (16.5)	15.7	13.9–18.3 (16.0)	13.5–18.2 (15.7)
Length of upper caudal-fin lobe	21.1–22.8 (24.9)	21.8	21.0–28.2 (24.2)	19.5–25.2 (22.3)
Length of middle caudal-fin ray	13.8–17.5 (15.8)	14.0	12.6–20.3 (15.7)	13.2–18.0 (15.1)

TABLE IV. Frequencydistribution of meristic features of four *Petroleuciscus* species in western Anatolia and Thrace region

	Lateral-line scales											Scale rows between lateral-line and:							
												Dorsal-fin origin				Anal-fin origin			
	<i>n</i>	31	32	33	34	35	36	37	38	39	40	41	Mean	6	7	Mean	2	3	4
<i>P. ninae</i>	30	1	2	3	12	11	1					34.1	30	6.0	22	8		2.3	
<i>P. borysthenicus</i>	60						7	10	18	11	12	2	38.3	2	5.8	7.0		60	3.0
<i>P. smyrnaeus</i>	30			4	7	17	1	1				34.6	26	4	6.1	15	15		2.5

	Predorsal vertebrae				Abdominal vertebrae				Caudal vertebrae				Total vertebrae						
	12	13	14	Mean	18	19	20	21	Mean	15	16	17	18	Mean	34	35	36	37	38
<i>P. ninae</i>	10	2	8	12.8	3	7			18.7	2	8			16.8	6	2	2		35.6
<i>P. borysthenicus</i>	30	22	8	13.3	7	21	2		19.8	2	17	11		17.3		5	15	10	37.2
<i>P. smyrnaeus</i>	15	10	5	12.3		3	12		20.8	3	10	2		15.9	3	8	4		35.1

n, Sample size.

rounded. Pharyngeal teeth 2.5–5.2 or 2.5–5.1, hooked at tip, not serrated. Total vertebrae 35–37, predorsal vertebrae 12–13, abdominal vertebrae 18–19 and and caudal vertebrae 16–17. Abdominal region longer than caudal region and difference between abdominal and caudal vertebral numbers varies from one to three. Vertebral formulae 18 + 17 (2), 19 + 16 (4), 19 + 17 (2) and 19 + 18 (2).

Coloration

Formalin-preserved individuals brownish on back and upper part of flank, yellowish on lower part of flank and belly. A black stripe present on upper part of flank from posterior margin of operculum to hypural complex, stripe width greater than eye diameter. Stripe absent in life. Numerous dark-brown pigment cells on flank scales in most individuals. Caudal and dorsal fins grey; pectoral, pelvic and anal fins light grey or yellowish. Numerous black pigments on anal-fin rays.

Distribution and notes on biology

Petroleuciscus ninae is only known from tributaries of the Büyük Menderes River drainage (Fig. 3). It inhabits somewhat deep and slowly flowing waters with cobble and pebble substrata.

Etymology

The species is named for Nina Bogutskaya, who has contributed to the knowledge of the fish fauna of Europe and Asia.

COMPARATIVE MATERIALS

Petroleuciscus smyrnaeus

FFR 03835 (Fig. 4), 18, 41–86 mm L_S ; Turkey: Uşak province: Gediz River at Derbent, 38° 46' 36'' N, 29° 12' 41'' E. FFR 03842, 13, 45–75 mm L_S ; Turkey: Uşak province: Gediz River south of Yenikent, 38° 50' 14'' N, 29° 16' 10'' E.



FIG. 3. Map of western Anatolia and the Thrace region showing the distribution of *Petroleuciscus* specimens examined for this study: ●, *P. ninae*; ■, *P. smyrnaeus*; ▲, *P. borysthenticus*.

Petroleuciscus borysthenticus

FFR 03847, 9, 33–80 mm L_S ; Turkey: Kırklareli province: stream Madara at Demirköy, $41^{\circ} 52' 33''$ N, $27^{\circ} 54' 37''$ E. FFR 03829, 10, 63–81 mm L_S ; Turkey: İstanbul province: stream Çamaşır at Çatalca, $41^{\circ} 09' 48''$ N, $28^{\circ} 27' 33''$ E. FFR 03840 (Fig. 5), 12, 51–94 mm L_S ; Turkey: Kırklareli province: stream Bahçeköy at Bahçeköy, $41^{\circ} 34' 09''$ N, $28^{\circ} 04' 46''$ E. FFR 03841, 1, 88 mm L_S ; Turkey: İstanbul province: stream Karamandere at Karamandere, $41^{\circ} 22' 48''$ N, $28^{\circ} 17' 45''$ E. FFR 03833, 25, 53–70 mm L_S ; Turkey: Balıkesir province: stream Sakar at Manyas, $40^{\circ} 03' 00''$ N, $27^{\circ} 57' 46''$ E. FFR 03834, 3, 79–91 mm L_S ; Turkey: Çanakkale province: stream Çınarcık northern of Çınarcık, $39^{\circ} 57' 59''$ N, $27^{\circ} 11' 43''$ E. FFR 03838, 5, 52–69 mm L_S ; Turkey: Çanakkale province: stream Kocaçay south of Dura, $40^{\circ} 00'$



FIG. 4. *Petroleuciscus smyrnaeus* FFR 03835, 79 mm L_S ; Uşak prov.: Gediz River at Derbent.



FIG. 5. *Petroleuciscus borysthenicus* FFR 03840, 81 mm L_S ; Kiklarelî province: Bahçeköy Stream at Bahçeköy.

52'' N, 27° 48' 45'' E. FFR 03839, 21, 51–91 mm L_S ; Turkey: Balıkesir province: stream Alburak at Ilıca, 39° 52' 31'' N, 27° 46' 16'' E. FFR 03839, 15, 44–59 mm L_S ; Turkey: Bursa province: Lake Gölbaşı at Gölbaşı, 40° 12' 42'' N, 29° 19' 07'' E. FFR 03836, 1, 63 mm L_S ; Turkey: Bursa province: stream Çapraz at Karacabey, 40° 11' 47'' N, 28° 21' 09'' E.

Petroleuciscus kurui

FFR 03857, 6, 45–74 mm L_S ; Turkey, Hakkari province: Tigris River basin at Yüksekova wetland, 37° 26' 07'' N, 44° 21' 58'' E.

MATERIAL USED IN THE MOLECULAR GENETIC ANALYSIS

Petroleuciscus ninae

FFR DNA-03856, 9, Turkey: Aydın Prov.: stream Akçay, 3 km west of Beğlerli, 37° 45' 34'' N, 28° 20' 07'' E (GenBank accession number: MF362203–MF362211).

Petroleuciscus borysthenicus

FFR DNA-03829, 2, Turkey: İstanbul province: stream Çamaşır at Çatalca, 41° 09' 48'' N, 28° 27' 33'' E (GenBank accession number: MF362197, MF362198). FFR DNA-03840, 4, Turkey: Kiklarelî province: stream Bahçeköy at Bahçeköy, 41° 34' 09'' N, 28° 04' 46'' E (GenBank accession number: MF362193–MF362196). FFR DNA-03841, 1, Turkey: İstanbul province: stream Karamandere at Karamandere, 41°

22° 48' N, 28° 17' 45'' E (GenBank accession number: MF362192). FFR 03833DNA, 4, Turkey: Balıkesir province: stream Sakar at Manyas, 40° 03' 00'' N, 27° 57' 46'' E (GenBank accession number: MF362183–MF362186). FFR DNA-03834, 2, Turkey: Çanakkale province: stream Çınarcık northern of Çınarcık, 39° 57' 59'' N, 27° 11' 43'' E (GenBank accession number: MF362188, MF362189). FFR DNA- 03836, 1, Turkey: Bursa province: stream Çapraz at Karacabey, 40° 11' 47'' N, 28° 21' 09'' E (GenBank accession number: MF362187). FFR DNA-03846, 2, Turkey: Kırklareli province: Ergene River at İnce, 41° 41' 39'' N, 27° 04' 47'' E (GenBank accession number: MF362190, MF362191).

Petroleuciscus smyrnaeus

FFR DNA-03835, 4, Turkey: Uşak province: Gediz River at Derbent, 38° 46' 36'' N, 29° 12' 41'' E (GenBank accession number: MF362199–MF362202).

DISCUSSION

Historically, it has been known that two species of *Petroleuciscus* occurred in Turkey. A third species of *Petroleuciscus*, however, has been identified in this systematic revision of the genus using genetic and morphological data. *Petroleuciscus ninae* sp. nov. has specific morphological characters and diagnostic nucleotide substitutions based on the mtDNA barcode region. As seen in the *col* gene-tree topology, *P. ninae* is sister to *P. smyrnaeus*. The fact that the genetic distance between *P. ninae* and *P. borysthenticus* is greater than between *P. ninae* and *P. smyrnaeus* is also consistent with geographical distributions of these species.

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References

- Bogutskaya, N. G. (1995). *Leuciscus kurui*, a new cyprinid fish from the upper Tigris (Dicle) system. *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut* **92**, 149–154.
- Bogutskaya, N. G. (1996). Contribution to the knowledge of leuciscine fishes of Asia Minor. Part 1. Morphology and taxonomic relationships of *Leuciscus borysthenticus* (Kessler, 1859), *L. smyrnaeus* Boulenger, 1896 and *Ladigesocypris ghigi* (Gianferrari, 1927). *Publicaciones Especiales – Instituto Español de Oceanografía* **2**, 25–44.
- Bogutskaya, N. G. (2002). *Petroleuciscus*, a new genus for the *Leuciscus borysthenticus* species group (Teleostei: Cyprinidae). *Zoosystematica Rossica* **11**, 235–237.
- Bogutskaya, N. G. & Coad, B. W. (2009). A review of vertebral and fin-ray counts in the genus *Alburnoides* (Teleostei: Cyprinidae) with a description of six new species. *Zoosystematica Rossica* **18**, 126–173.
- Coad, B. W. & Bogutskaya, N. G. (2010). *Petroleuciscus esfahani*, a new species of fish from central Iran (Actinopterygii: Cyprinidae). *Zootaxa* **2534**, 37–47.
- Geiger, M. F., Herder, F., Monaghan, M. T., Almada, V., Barbieri, R., Bariche, M., Berrebi, P., Bohlen, J., Casal-Lopez, M., Delmastro, G. B., Denys, G. P., Dettai, A., Doadrio, I., Kalogianni, E., Karst, H., Kottelat, M., Kovacic, M., Laporte, M., Lorenzoni, M., Marcic, Z., Ozulug, M., Perdices, A., Perea, S., Persat, H., Porcelotti, S., Puzzi, C., Robalo, J., Sanda, R., Schneider, M., Slechtova, V., Stoumboudi, M., Walter, S. & Freyhof, J. (2014). Spatial heterogeneity in the Mediterranean biodiversity hotspot affects barcoding accuracy of its freshwater fishes. *Molecular Ecology Resources* **14**, 1210–1221.

- Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**, 307–321.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95–98.
- Hasegawa, M., Kishino, H. & Yano, T. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22**, 160–174.
- Khaefi, R., Esmaeili, H. R., Sayadzadeh, G., Geiger, M. F. & Freyhof, J. (2016). *Squalius namak*, a new chub from Lake Namak basin in Iran (Teleostei: Cyprinidae). *Zootaxa* **4169**, 145–159.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120.
- Kottelat, M. & Freyhof, J. (2007). *Handbook of European Freshwater Fishes*. Cornol & Berlin: Kottelat & Freyhof.
- Kumar, S., Stecher, G. & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870–1874.
- Librado, P. & Rozas, J. (2009). DnaSPv5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452.
- Mohammadian-Kalat, T., Esmaeili, H. R., Aliabadian, M. & Jörg, F. (2017). Re-description of *Alburnus doriae*, with comments on the taxonomic status of *A. amirkabiri*, *A. mossulensis*, *A. sellal* and *Petroleuciscus esfahani* (Teleostei: Cyprinidae). *Zootaxa* **4323**, 487–502.
- Perea, S., Bohme, M., Zupancic, P., Freyhof, J., Sanda, R., Ozulug, M., Abdoli, A. & Doadrio, I. (2010). Phylogenetic relationships and biogeographical patterns in circum-Mediterranean subfamily Leuciscinae (Teleostei, Cyprinidae) inferred from both mitochondrial and nuclear data. *BMC Evolutionary Biology* **10**, 265.
- Posada, D. (2008). jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253–1256. <https://doi.org/10.1093/molbev/msn083>
- Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Swofford, D. L. (2003). *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4*. Sunderland, MA: Sinauer Associates.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680.
- Turan, D., Bektaş, Y., Kaya, C. & Bayçelebi, E. (2016). *Alburnoides diclensis* (Actinopterygii: Cyprinidae), a new species of cyprinid fish from the upper Tigris River, Turkey. *Zootaxa* **4067**, 79–87. <https://doi.org/10.11646/zootaxa.4067.1.6>
- Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R. & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B* **360**, 1847–1857.