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Two new *Oxynoemacheilus* species in western Anatolia (Teleostei, Nemacheilidae)

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Abstract

Oxynoemacheilus sakaryaensis **sp. nov.**, is restricted to the Sakarya River basin, and *O. melenicus* **sp. nov.**, is distributed in both the Sakarya River and Büyükmelen Stream. *Oxynoemacheilus sakaryaensis* is distinguished by having a flank plain or with numerous irregularly shaped pale brownish bars and a caudal-peduncle depth 2.8–3.2 times in its length. *Oxynoemacheilus melenicus* is distinguished by having a flank with 10–13 irregular shaped brownish bars or blotches and the caudal peduncle depth 1.9–2.8 times in its length. *Oxynoemacheilus banarescui*, *O. samanticus*, *O. simavicus*, *O. fatsaensis*, *O. sakaryaensis*, and *O. melenicus* are valid, which belong to the *O. bergianus* species group. *O. melenicus* and *O. sakaryaensis* were differentiated from all other *Oxynoemacheilus* species in western Anatolia by two diagnostic and unique nucleotide substitution sites in the COI barcoding region. Also, species delineation tests (ABGD, GMYC, ASAP) and phylogenetic analyses support the validity of *O. melenicus* and *O. sakaryaensis* as distinct species.

Key Words

Cytochrome oxidase I, freshwater fish, molecular identification, Northwestern Anatolia, species delineation, taxonomy

Introduction

Nemacheilid loaches of the genus *Oxynoemacheilus* are widespread fishes all over the Eastern Mediterranean, the southern Caucasus, Anatolia, Mesopotamia, and Central Iran (Freyhof et al. 2011, 2022; Kottelat 2012). Freyhof et al. (2011) list 41 species as valid in that genus, and Kottelat (2012) also included *O. oxianus* from the Central Asian Amu-Darya drainage. Since, Çiçek et al. (2018); Erk`akan (2012); Freyhof (2016); Freyhof et al. (2017, 2019, 2021a, 2021b, 2022); Freyhof and Abdullah (2017); Freyhof and Geiger (2021); Freyhof and Özuluğ (2017); Kamangar et al. (2014); Kaya et al. (2020, 2021); Saygun et al. (2021); Sayyadzadeh et al. (2016); Sungur et al. (2017); Turan et al. (2019); Yoğurtçuoğlu et al., (2021a, 2021b); Yoğurtçuoğlu et al. (2022) described and re-validated additional species and we recognize 62 species of *Oxynoemacheilus* as valid. *Oxynoemacheilus* is one of the largest genera of freshwater fishes in the Western Palearctic. It has been reviewed comprehensively in the global distribution zone due to the many species. Indeed, most *Oxynoemacheilus* species are distributed in small ranges except *O. bergianus*, which lives in both the Caspian Sea basin and the Persian Gulf basin (Freyhof 2016; Freyhof and Abdullah 2017; Freyhof and Özuluğ 2017; Freyhof et al. 2017; Freyhof et al. 2022). In recent years, new species (*O. veyseli*, *O. elsae*, *O. ciceki*) have been discovered from stretching Anatolia and nearby basins (Çiçek et al. 2018; Eagderi et al. 2018; Sungur et al. 2017; Freyhof et al. 2022).

Freyhof et al. (2022) treated four species within *O. bergianus* species group as valid, corresponding to *O. banarescui*, *O. bergianus*, *O. fatsaensis*, and *O. simavicus*. Bektaş et. al (2022) reported *O. bergianus*, *O. samanticus*, *O. fatsaensis*, *O. banarescui* and O. *simavicus* as valid species. Freyhof et al. (2022) reported O. *simavicus* from drainages in the southern shores of the Marmara Sea, as well as the Sakarya and Büyükmelen Stream. In this study, these populations of the species in Sakarya and Büyükmelen rivers are described as new species as *O. sakaryaensis* and *O. melenicus*. These two species are included in the *O. bergianus* species group, which has a slender caudal peduncle and lack the two distinct black or dark-brown spots on the caudal fin base and the presence of suborbital flap or groove.

Material and methods

The care of experimental animals was consistent with the Republic of Turkey's animal welfare laws, guidelines, and policies. After anesthesia, fishes were fixed in 5% formaldehyde stored in 70% ethanol or directly fixed in 99% ethanol. Measurements were made with a dial caliper and recorded to 0.1 mm. All measurements were made point-to-point, never by projections. Methods for counts and measurements followed Kottelat and Freyhof (2007) and the terminology of head canals followed Kottelat (1990). Standard length (SL) was measured from the tip of the snout to the end of the hypural complex. The length of the caudal peduncle was measured from behind the base of the last anal-fin ray to the end of the hypural complex, at mid-height of the caudal-fin base. The last two branched rays articulating on a single pterygiophore in the dorsal and anal fins are counted as "1½". Simple rays of dorsal and anal fins were not counted as they were deeply embedded. The holotype was included in the calculation of means and SD. Males of several *Oxynoemacheilus* show an exposed lachrymal bone, which is often called a suborbital flap or groove. We distinguish between a suborbital flap and a suborbital groove. In the present study, all *Oxynoemacheilus* having an exposed lachrymal bone possess a suborbital groove.

We see no application in developing identification keys for several species in large geographic areas. Therefore, we provide keys for western Anatolia only. Differential species diagnoses are given against related species as derived by the COI molecular analysis presented here and against geographically adjacent species. No differential diagnosis is provided against largely unrelated and/or geographically distant species.

Abbreviations used: **SL**, standard length; **K2P**, Kimura 2-parameter. Collection codes: **FFR**, Recep Tayyip Erdogan University Zoology Museum of the Faculty of Fisheries, Rize. Materials examined are listed at the end of the study.

DNA extraction, PCR and sequencing

Total DNA was extracted from fin clips via Qiacube automated DNA/RNA purification system using Qiagen DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany). DNA quality and quantity were checked on a NanoDrop 2000/c spectrophotometer (Thermo Scientific, Rockford, IL, USA) and 0.8% agarose gel electrophoresis. The standard vertebrate DNA barcode region of *COI* gene (645 bp) was amplified using a universal *COI* barcoding primer pair, the FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') (Ward et al. 2005). PCR reactions were performed in a 50 µL reaction volume containing 5 µL 10× PCR buffer, 100 ng template DNA, 0.5 mM dNTPs mix, 3 mM $MgCl_2$, 0.5 mM of each primer, and 1 µL Taq DNA polymerase (New England Biolabs). The polymerization was carried out under the following conditions: initial denaturation at 95 °C for 30 s, denaturation at 95 °C for 30 s, annealing at 58 °C for 45 s, extension at 68 °C for 1 min through 35 cycles, and a final extension at 68 °C for 5 min using Biorad T100 (Bio-Rad, Hercules, CA, USA) thermal cycler. The PCR products were also run and visualized under UV Quantum–Capt ST4 system (Vilber Lourmat, France), purified, and sequenced at Macrogen Europa Inc. (Amsterdam, Netherlands).

Molecular data analysis

We have used the newly generated 30 DNA barcodes from the present study and included additional 42 specimens from earlier studies deposited to NCBI GenBank (Geiger et al. 2014, Geiger 2019, Turan et al. 2019, Bektas et al. 2022, Freyhof et al. 2022) (Table 1). The dataset also consisted of DNA barcodes from individuals *Seminemacheilus lendlii* and *Oxynoemacheilus cemali* as outgroup taxon. Clustal W algorithm (Thompson et al. 1994) in Bioedit v7.2.5 (Hall 1999) was used to align COI barcode sequences, and the sequences were submitted to NCBI GenBank with accession numbers OQ332806–OQ332835. Phylogenetic relationships among species were carried out using both maximum likelihood (ML) and Neighboor Joining (NJ) analysis using MEGA 11 (Tamura et al. 2021). TrN+G model (Kimura 1980) was chosen as the best nucleotide substitution model according to the Bayesian information criterion (BIC) in jModeltest v. 0.0.1 (Posada 2008). The K2P distance model (Kimura 1980) in MEGA 11 was used to estimate pairwise genetic distances among species. POPART (1.7) (Leigh and Bryant 2015) was used to generate and display the haplotype network. We used three single-locus species delimitation methods relying on different operational criteria for species delimitation being implemented: ABGD, Automatic Barcode Gap Discovery (Puillandre et al. 2012), Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al. 2021) and GMYC, the General Mixed Yule Coalescent method, single-threshold version (Fujisawa and Barraclough 2013). All species delimitation methods were performed using the COI sequences (not haplotypes). The ABGD species delimitation was performed via the ABGD web server with default settings (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html, accessed on 1 May 2023) and ASAP species delimitation was performed via the ASAP web server (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html, accessed on 1 May 2023) and the following parameters:

Table 1. List of COI sequences downloaded from NCBI GenBank with information on drainage and country of origin.

Species	Accession N.	Drainage	Reference
O. melenicus	OK316642	Asar stream, Büyükmelen River	Freyhof et. al. 2022
O. melenicus	MH018854	Büyükmelen drainage	Turan et. al. 2019
O. melenicus	MH018856	Büyükmelen drainage	Turan et. al. 2019
O. melenicus	OK316651	Asar stream, Büyükmelen	Freyhof et. al. 2022
O. melenicus	OK316688	Doğançay, Sakarya River	Freyhof et. al. 2022
O. melenicus	MH018855	Büyükmelen drainage	Turan et. al. 2019
O. melenicus	OK316739	Asar stream, Büyükmelen River	Freyhof et. al. 2022
O. melenicus	OK316620	Allıkova stream, Sakarya River	Freyhof et. al. 2022
O. melenicus	OK316798	Allıkova stream, Sakarya River	Freyhof et. al. 2022
O. sakaryaensis	OK316616	Bayındır stream, Sakarya River	Freyhof et. al. 2022
O. sakaryaensis	OK316622	Bayındır stream, Sakarya River	Freyhof et. al. 2022
O. sakaryaensis	OK316635	Bayındır stream, Sakarya River	Freyhof et. al. 2022
O. sakaryaensis	OK316735	Bayındır stream, Sakarya River	Freyhof et. al. 2022
O. sakaryaensis	OK316743	Bayındır stream, Sakarya River	Freyhof et. al. 2022
O. sakaryaensis	OK316796	Bayındır stream, Sakarya River	Freyhof et. al. 2022
O. simavicus	KJ553724	Simav stream	Geiger et. al. 2014
O. simavicus	KJ553970	Simav stream	Geiger et. al. 2014
O. fatsaensis	OL855789	Tersakan stream, Yesilirmak	Bektaş et. al. 2022
O. fatsaensis	OL855790	Tersakan stream, Yesilirmak	Bektaş et. al. 2022
O. fatsaensis	OL855791	Tersakan stream, Yesilirmak	Bektaş et. al. 2022
O. banarescui	OK316694	Yenice River	Freyhof et. al. 2022
O. banarescui	MH469261	Devrekani stream	Turan et. al. 2019
O. banarescui	MH469262	Devrekani stream	Turan et. al. 2019
O. samanticus	MH018861	Terme stream, Kızılırmak River	Turan et. al. 2019
O. samanticus	OK316652	Kızılırmak River	Freyhof et. al. 2022
O. samanticus	MH018863	Vezirkoprü stream, Kızılırmak River	Turan et. al. 2019
O. bergianus	MH469265	Murat River	Turan et. al. 2019
O. bergianus	MH469266	Murat River	Turan et. al. 2019
O. bergianus	OL855763	Murat River	Bektaş et. al. 2022
O. bergianus	OL855764	Merziman stream, Euphrates	Bektaş et. al. 2022
O. bergianus	OL855765	Goksu river, Euphrates	Bektaş et. al. 2022
O. bergianus	OL855766	Sogutlucay stream, Euphrates	Bektaş et. al. 2022
O. bergianus	OL855767	Sogutlucay stream, Euphrates	Bektaş et. al. 2022
O. bergianus	OK316672	Yalekhlou, Caspian sea basin	Freyhof et. al. 2022
O. bergianus	OK316691	Murat River	Freyhof et. al. 2022
O. bergianus	MK546446	Caspian sea basin	Geiger 2019
O. bergianus	MK546447	Euphrates	Geiger 2019
O. bergianus	MK546448	Euphrates	Geiger 2019
O. bergianus	MK546449	Şerhan stream, Euphrates	Geiger 2019
O. angorae	OL855744	Karasu stream, Iznik lake	Bektaş et. al. 2022
O. angorae	OL855745	Purtek stream, Sakarya	Bektaş et. al. 2022
O. angorae	OL855747	Zamanti stream, Seyhan	Bektaş et. al. 2022

Pmin = 0.001, Pmax = 0.1, 1000 replicates, and the Kimura evolutionary model, with TS/TV = 2.0. The same parameters were applied under ABGD analysis. GMYC analysis was applied by the single-threshold version of the method, which usually outperforms the multiple-threshold version (Fujisawa and Barraclough 2013). The input ultrametric phylogenetic tree was made in BEAST v.1.8.4 (Drummond et al. 2012) with the following parameters: strict clock, Speciation:Yule process as the tree prior with 5 million generations, and sampling frequency of 1000. The resulting ultrametric tree was imported into R 3.1.3 (R Core Team 2013), and the single threshold ST-GMYC analysis was carried out using the R packages (Splits; Ezard et al. (2009) and Ape libraries; Paradis et al. (2004). In the concordant outcome of these methods, the resulting delimitation appears more logical (Dellicour and Flot 2018).

Results

Key to *Oxynoemacheilus bergianus* species group in the Anatolia

1	Snout length equal or greater than postorbital length	*O. samanticus*
–	Snout length equal or smaller than postorbital length	2
2	Flank with marmalade pattern or with numerous irregularly shaped dark brown blotches as two or three horizontal rows	*O. fatsaensis*
–	The flank plain or with two to numerous irregularly shaped dark brown blotches	3
3	The flank with plain yellowish or with numerous irregularly shaped pale brown bars in most individuals	*O. sakaryaensis*
–	The flank dark brownish with 2–13 irregular shaped dark brownish bars or blotches	4
4	Maxillary barbells approximately equal or greater outer rostral barbells	*O. banarescui*
–	Maxillary barbells always shorter than outer rostral barbells	5
5	The flank dark brownish with 10–13 irregular shaped dark brownish bars or blotches	*O. melenicus*
–	The flank with 2–9 irregularly shaped brown bars or blotches	6
6	Interorbital width 15–24% HL	*O. simavicus*
–	Interorbital width 26–34% HL	*O. bergianus*

***Oxynoemacheilus sakaryaensis* sp. nov.**

https://zoobank.org/6006326D-484D-435C-BD03-AFAE9A84DEE3

Figs 1, 2

Type material. *Holotype.* FFR15629, 1, 58 mm SL; Turkey: Ankara prov.: stream Kirmir 3 km north of Güdül, a tributary of Sakarya River, 40.236°N, 32.606°E.

Examined materials. *Paratypes.* FFR15514, 20, 51–62 mm SL; same data holotype. – FFR01527, 17, 50–62 mm SL; Turkey: Ankara prov.: stream Kirmir about 3 km north of Güdül, 40.236°N, 32.261°E. – FFR01387, 9, 39–58 mm SL; Turkey: Ankara prov.: stream İlhan at İlhan Village, 40.093°N, 32.245°E. – FFR15621, 27, 37–65 mm SL; Turkey: Ankara prov.: stream İlhan at İlhan Village, 40.097°N, 32.250°E. – FFR15623, 21, 49–60 mm SL; Turkey: Ankara prov.: stream Kirmir about 6 km north of Güdül 40.259°N, 32.268°E.– FFR15624, 14, 47–60 mm SL; Turkey: Ankara prov.: stream Bayındır at Gümele Village, 40.314°N, 32.466°E. –FFR 1364, 16, 25–64 mm SL; Turkey: Ankara prov: stream Kirmir at Kızılcahamam, 40.483°N, 32.653°E.–FFR 1386, 13, 49–62 mm SL; FFR 15515, 1, 62 mm SL; Turkey: Ankara prov: stream Öz at Kızılcahamam, 40.463°N, 32.653°E.– FFR 1387, 9, 39–58 mm SL; FFR 1527, 17, 50–62 mm SL; Turkey: Ankara prov: stream İlhan 5 km west of Gökçebağ, 40.093°N, 32.245°E. – FFR 15514, 1, 59 mm SL; Turkey: Ankara prov: stream Kirmir 3 km north of Güdül, 40.236°N, 32.261°E.

Material used in molecular genetic analysis. FFR DNA 15623, 4, Turkey: Ankara prov.: stream Kirmir about 6 km north of Güdül, 40.259°N, 32.268°E. (GenBank accession numbers OQ332822–OQ332825) – FFR DNA 15629, 3, Turkey: Ankara prov: stream Kirmir about 3 km north of Güdül 40.236°N, 32.606°E. (GenBank accession numbers OQ332826–OQ332828) – FFR DNA 15621, 3, Turkey: Ankara prov: stream İlhan at İlhan Village, 40.097°N, 32.250°E. (GenBank accession numbers OQ332829–OQ332831) – FFR DNA 1527, 2, Turkey: Ankara prov: stream Kirmir about 3 km north of Güdül, 40.236°N, 32.261°E. (GenBank accession numbers OQ332820–OQ332821)

Diagnosis. *Oxynoemacheilus sakaryaensis* is distinguished from *O. melenicus* by having the flank plain yellowish or numerous irregularly shaped pale brownish bars (vs. the flank with 10–13 irregular shaped dark brownish bars or blotches, 0–2 irregularly shaped brownish saddle in front of dorsal-fin origin (vs. 3–4) and caudal peduncle depth 2.8–3.2 times in its length (vs. 1.9–2.8). *Oxynoemacheilus sakaryaensis* is distinguished from *O. angorae* by having a caudal-peduncle depth 2.8–3.2 times in its length (vs.1.4–1.8), the flank with plain yellowish or with numerous irregularly shaped brown bars in most individuals (vs. showing a dark-brown mid lateral stripe or a series of fused, dark-brown blotches interrupted by a whitish or pale-brown lateral line (Fig. 3), a groove in upper lip in males (vs. absent), an axillary lobe at the base of pelvic fin (vs. absent) and the caudal-fin moderately forked (vs. emarginated) *Oxynoemacheilus sakaryaensis* is distinguished from *O. banarescui* by the flank with plain yellowish or with numerous irregularly shaped brown bars in most individuals (vs. 7–9 brownish blotches on flank (Fig. 4), the caudal peduncle depth 2.8–3.2 times in its length (vs. 1.9–2.8) and maxillary barbells always shorter than outer rostral barbells (vs. equal or shorter) *Oxynoemacheilus sakaryaensis* is distinguished from *O. simavicus* by the flank with plain yellowish or with numerous irregularly shaped pale brown bars in most individuals (vs. flank with 2–8 dark brownish blotches (Fig. 5) and 7–9 small and very pale brownish saddles on back (vs. 4–5 large dark brownish saddles on back). *Oxynoemacheilus sakaryaensis* is distinguished from *O. samanticus* by having a snout length smaller than postorbital length (vs. the snout length longer than the postorbital length), more slender caudal peduncle (caudal peduncle depth 2.8–3.2 times in its length, vs. 2.2–2.7) and the flank with plain yellowish or with numerous irregularly shaped brown bars in most individuals (vs. 5–10 black or dark brown bars or blocks on flank (Fig. 6). *Oxynoemacheilus sakaryaensis* is distinguished from *O. fatsaensis* by the flank with plain yellowish or with numerous irregularly shaped brown bars in most individuals (vs. the flank with marmalade pattern or with numerous irregularly shaped dark brown blotches as two or three horizontal rows (Fig. 7) and more slender caudal peduncle (caudal peduncle length 2.8–3.2 times greater than its length (vs. 2.0–2.6). *Oxynoemacheilus sakaryaensis* is distinguished from *O. bergianus* by the flank with plain yellowish or with numerous irregularly shaped brown bars in most individuals (vs. 4–9 black or dark brown bars or blocks on flank (Fig. 8) and a greater distance between anus and anal-fin origin (3–5% SL, vs. 2–3).

Oxynoemacheilus sakaryaensis is distinguished from *O. seyhanensis* by having the caudal-peduncle length 2.8–3.2 times greater than its depth (vs.1.2–1.4), the flank with plain yellowish or with numerous irregularly shaped brown bars in most individuals (vs. the body with marmorate pattern or numerous small irregularly shaped and spaced dark-brown bars on flank (Fig. 9), a suborbital groove in males (vs. absent), an axillary lobe at base of pelvic fin (vs. absent), a forked caudal fin (vs. slightly emarginate), lacking dorsal and ventral adipose crest on caudal peduncle (vs. present) and a more slender caudal peduncle (depth 2.7–3.5 times in its length, vs. 2.0–2.6, vs. 1.2–1.4). *Oxynoemacheilus sakaryaensis* is distinguished from *O. cemali* by having the flank with plain yellowish or with numerous irregularly shaped brown bars in most individuals (vs. 9–15 irregularly shaped dark-gray bars on the flank), a forked caudal-fin (vs. slightly forked), and having a slender caudal peduncle (the caudal-peduncle length 2.8–3.2 times greater than its depth (vs.1.4–2.0).

Description. See Figs 1, 2 for general appearance and Table 2 for morphometric data. Adult size large (maximum 65 mm SL). Body slender, compressed at caudal peduncle, greatest depth about midline between nape and dorsal-fin origin, slightly decreasing towards caudal-fin base. No hump at nape. Greatest body width at pectoral-fin base. Head pointed, upper head profile slightly convex on snout,

Figure 1. *Oxynoemacheilus sakaryaensis*, FFR15629 holotype, 58 mm SL; Turkey: stream Kirmir.

Figure 2. *Oxynoemacheilus sakaryaensis*, FFR 15623 paratypes, top to bottom 56 mm SL, 61 mm SL; Turkey: stream Kirmir.

Figure 3. *Oxynoemacheilus angorae*, FFR01513, 55 mm SL, Turkey: stream Berçin, FFR01526, 64 mm SL; Turkey: stream Kirmir.

Table 2. Morphometric data of *Oxynoemacheilus sakaryaensis* (holotype, FFR15629, paratypes, FFR 15514; n = 20).

	Holotype	paratypes			
		mean	min	max	SD
Standard length (mm)	58		51	62	
In percent of standard length					
Head length	23.8	23.1	20.4	24.8	1.1
Body depth at dorsal–fin origin	14.2	16.5	14.2	17.7	0.8
Predorsal length	52.7	50.3	47.7	53.0	1.7
Postdorsal length	35.9	35.6	32.0	39.7	2.0
Preanal length	74.2	71.0	68.4	74.2	1.5
Prepelvic length	52.4	49.5	46.6	52.4	1.4
Distance between pectoral and pelvic-fin origins	32.0	29.6	27.0	32.0	1.1
Distance between pelvic and anal-fin origins	22.0	21.1	18.0	27.5	1.9
Distance between vent and anal-fin origin	3.8	4.0	3.2	5.1	0.5
Depth of caudal peduncle	6.3	7.2	6.4	7.9	0.4
Length of caudal peduncle	23.1	21.4	19.7	23.7	1.3
Dorsal-fin depth	17.6	18.8	16.6	20.7	1.2
Anal-fin depth	15.0	16.3	14.5	18.2	0.9
Pectoral-fin length	20.0	22.2	18.3	26.3	1.7
Pelvic-fin length	17.8	16.8	14.6	17.9	0.9
In percent of head length					
Head depth at eye	46.5	49.3	45.4	58.1	3.2
Snout length	33.6	42.2	35.6	37.9	3.1
Eye diameter	19.4	20.6	17.3	24.5	1.9
Postorbital distance	45.7	46.9	42.5	55.3	3.2
Maximum head width	61.6	62.0	54.6	69.5	3.3
Interorbital width	25.3	28.0	23.1	35.8	3.2
Length of inner rostral barbel	23.9	25.9	20.5	32.7	2.8
Length of outer rostral barbel	28.3	30.6	24.8	37.6	3.4
Length of maxillary barbel	26.7	26.6	22.4	34.1	2.7
Caudal peduncle length/depth	3.0	2.9	2.8	3.2	0.1

flattened on ventral surface. Snout slightly pointed at tip. Mouth narrow and arched, lips well developed. A narrower median interruption in lower lip. A narrower median incision in upper lip a very shallow groove. A suborbital groove in males. Barbels short, inner rostral barbel not reaching to base of maxillary barbel; outer almost not reaching to vertical through anterior eye margin. Maxillary barbell not reaching to posterior eye-margin in most individuals. Caudal peduncle slender, compressed laterally, length 2.8–3.2 times longer than deep. Axillary lobe presents at pelvic-fin base, fully attached to body. Pelvic-fin origin below first or second branched dorsal-fin ray. Anal-fin origin at vertically equal to dorsal-fin tip. The pectoral fin almost reaching vertical through tip of dorsal-fin origin in males. No dorsal or ventral adipose crest on caudal peduncle.

Lateral line complete, reaching caudal-fin base. Body covered by embedded scales on flank, back, and belly. Dorsal fin with 8½ branched rays, its outer margin straight or slightly concave. Anal fin with 5½ branched rays, its outer margin straight or slightly concave. Pectoral fin with 10–12 rays, outer margin straight. Pelvic fin with 7–8 rays, outer margin slightly convex. Caudal fin with 8+8, 8+9, and 9+9 branched rays, outer margin moderately forked and lopes slightly pointed.

Coloration. Body yellowish or brownish in life and preserved individuals. Head and cheek with small, plain brown mottling on top and cheeks, without color pattern ventrally. No pigmentation below a line from pectoral-fin base to anus. A large, irregularly shaped, dark-brown blotch at dorsal fin-origin. Flank plain yellowish with numerous irregularly shaped brown bars in most

Figure 4. *Oxynoemacheilus banarescui*, IUSHM 2018-1403, 62 mm SL, 46 mm SL; Turkey: stream Davulga.

Figure 5. *Oxynoemacheilus simavicus*, FFR 01544, mm SL, 52 mm SL; Turkey: stream Nilüfer.

Figure 6. *Oxynoemacheilus samanticus*, FFR015518, 64 mm SL, 62 mm SL; Turkey: stream Soruk.

Figure 7. *Oxynoemacheilus fatsaensis*, FFR 01566 paratypes, 74 mm SL, 67 mm SL; Turkey: stream Tersakan.

Figure 8. *Oxynoemacheilus bergianus*, FFR015506, 59 mm SL, 57 mm SL; Turkey, Murat River at Ballıbostan.

Figure 9. *Oxynoemacheilus seyhanensis*, FFR01577, 53 mm SL, 54 mm SL; Turkey: stream Soruk.

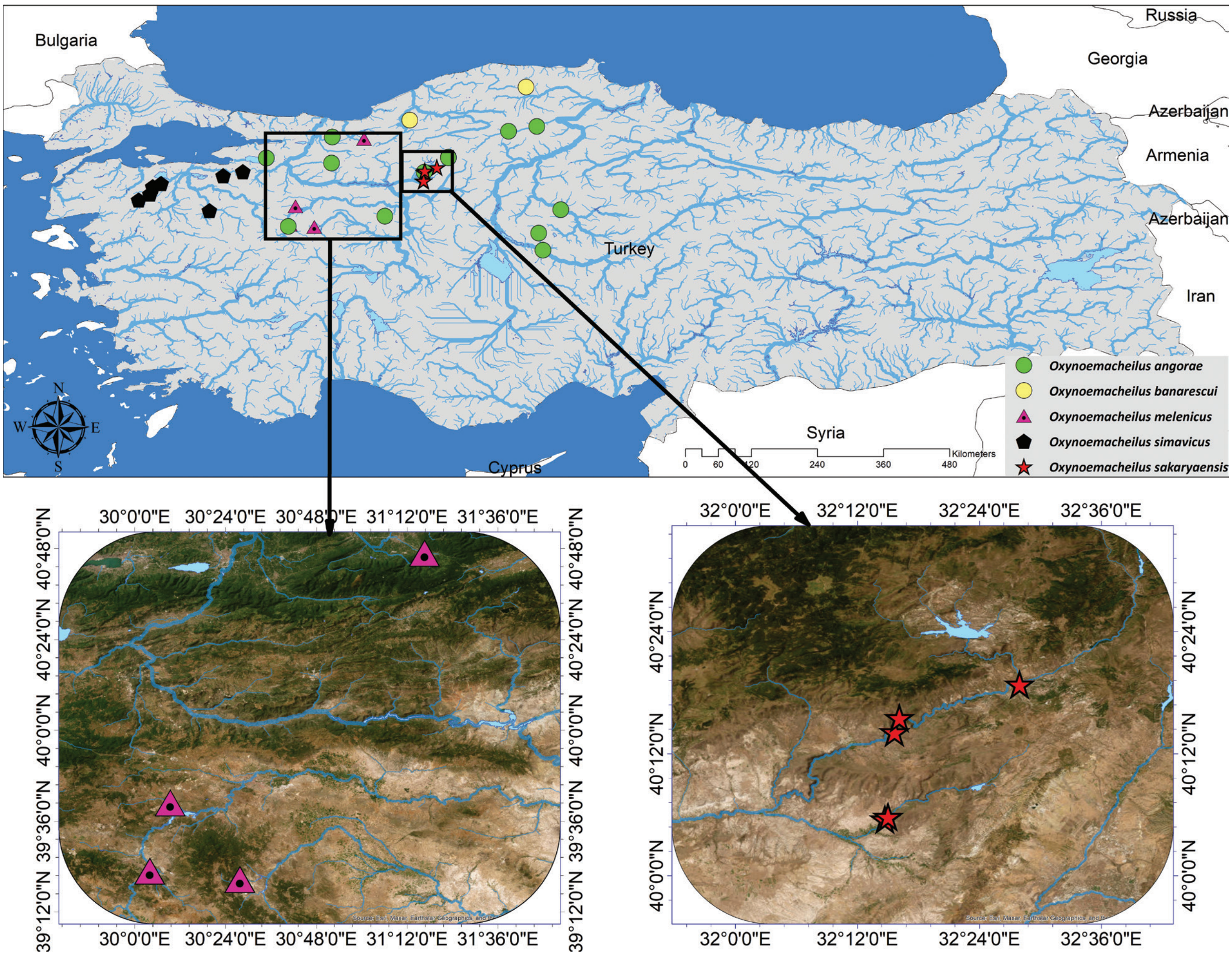

Figure 10. Distribution of *Oxynoemacheilus* species in the western Anatolia.

individuals. Back with zero to 2 pale blotches anterior to dorsal-fin origin. The dorsal part of caudal peduncle with 3–4 irregularly shaped pale saddle, not fused with mid-lateral blotches. One or two irregular shaped small black spots on caudal-fin base. Dorsal-fin with 1–2 and caudal fin with 2–3 fine, irregularly shapes black bands on rays. Anal, pectoral and pelvic fins greyish to yellowish, with numerous small black spots on rays.

Distribution. *Oxynoemacheilus sakaryaensis* was found in the Sakarya drainage in western Anatolia (Fig. 10).

Etymology. The name of the species is derived from the Sakarya River.

Oxynoemacheilus melenicus sp. nov.

https://zoobank.org/F530199C-A29C-4A1B-A3F1-493CDF0390CF

Figs 11, 12

Type material. ***Holotype***. FFR15627, 1, 65 mm SL; Turkey: Eskişehir prov.: stream Yarılgan at Gemiç Village, 39.343°N, 30.463°E.

Examined materials. ***Paratypes***. FFR15626, 39, 50–69 mm SL; same data holotype. FFR01378, 2, 45–57 mm SL; Turkey: Kütahya prov.: stream Porsuk at Kütahya 39.380°N, 30.067°E. – FFR01525, 1, 55 mm SL; Turkey: Kütahya prov.: stream Porsuk about 9 km south of Kütahya, 39.349°N, 30.038°E. – FFR15625, 4, 32–49 mm SL; Turkey: Kütahya prov: stream Porsuk at Porsuk Village, 39.348°N, 30.036°E. –FFR15630, 5, 61–63 mm SL; Turkey: Eskişehir prov.: stream Allıkova about 3km south of Kümbet, 39.681°N, 30.157°E. –FFR 01564, 10, 45–61 mm SL; Turkey: Düzce prov: stream Asar about 2 km west of Kaynaşlı a tributary of Büyükmelen drainage, 40.781°N, 31.277°E.

Material used in the molecular genetic analysis. FFR DNA 15630, 2, Turkey: Eskişehir prov.: stream Allıkova about 3km south of Kümbet, 39.681°N, 30.157°E (GenBank accession numbers OQ332806, OQ332808). – FFR DNA 15625, 3, Turkey: Kütahya prov.: stream Porsuk at Porsuk Village, 39.348°N, 30.036°E. (GenBank accession numbers OQ332809–OQ332811). – FFR DNA 15627, 4, Turkey: Eskişehir prov.: stream Yarılgan at Gemiç Village 39.343°N, 30.463°E. (GenBank accession numbers OQ332812–OQ332815). –FFR DNA 15631, 4, Turkey: Eskişehir prov.: a tributary of Sakarya River about 9 km northeast of Nasreddi Hoca Village, 39.553°N, 31.757°E. (GenBank accession numbers OQ332816–OQ332819). – FFR DNA 01524, 1, Turkey: Kütahya prov.: stream Porsuk at Porsuk Village 39.350°N, 30.038°E. (GenBank accession number OQ332807).

Figure 11. *Oxynoemacheilus melenicus*, FFR15627 holotype, 65 mm SL; Turkey: stream Yarılgan.

Diagnosis. *Oxynoemacheilus melenicus* is distinguished from *O. angorae* by having a more slender caudal pedincle (caudal pedinuncle depth 1.9–2.8 times in its length, vs.1.4–1.8), the flank with 6–13 irregular shaped brownish bars or blotches (a dark-brown midlateral stripe or a series of fused, dark-brown blotches interrupted by a whitish or pale-brown lateral line (Fig. 3), a suborbital groove in males (vs. absent), an axillary lobe at pelvic fin base (vs. absent), a forked caudal fin (vs. slightly emarginate), a more slender caudal peduncle (depth .5 times in its length, vs. 2.4–1.8).). *Oxynoemacheilus melenicus* is distinguished from *O. banarescui* by having the flank with 10–13 irregular shaped brownish bars or blotches (vs. the flank with 7–10 large, irregularly shaped dark-brown, vertically elongated blotches or bars along lateral midline behind dorsal-fin origin (Fig. 4) and maxillary barbells always shorter than outer rostral barbells (vs. equal or longer). *Oxynoemacheilus melenicus* is distinguished from *O. simavicus* by having the flank with 10–13 irregularly shaped brown bars or blotches (vs. 2–8 blotches (Fig. 5) and 6–8 irregularly shaped dark brown saddle on back (vs. 4–6) and a longer head (head length 22–26% SL, vs. 19–22). *Oxynoemacheilus melenicus* is distinguished from *O. samanticus* by having the with 10–13 irregular shaped brownish bars or blotches (vs. the flank with 5–10 irregular shaped brownish blotches along lateral line (Fig. 6) and the snout length smaller than postorbital length (vs. the snout length longer than postorbital length) and the pelvic-fin origin below first or second unbranched dorsal-fin ray (vs. equal with dorsal fin origin). *Oxynoemacheilus melenicus* is distinguished from *O. fatsaensis* by having the flank plain brownish or with 10–13 irregular shaped brownish bars or blotches (vs. flank marmalade pattern or with numerous irregularly shaped dark brown blotches as one or two horizontal rows (Fig. 7) and the pelvic-fin origin below the last unbranched dorsal-fin ray (vs. first or second branched dorsal-fin ray).

Oxynoemacheilus melenicus is distinguished from *O. bergianus* the flank with 10–13 dark brownish bars on flank (vs. 4–9 bars or blotches (Fig. 8) and the presence prominent bars or blotches in front of dorsal fin (vs. mostly absent or slightly prominent) and a greater distance between anus and anal-fin origin (4–6% SL, vs. 2–3). *Oxynoemacheilus melenicus* is distinguished from *O. seyhanensis* by having the with 10–13 irregular shaped brownish bars or blotches (vs. the body with marmorate pattern or numerous small irregularly shaped and spaced dark-brown bars on flank (Fig. 9), a suborbital groove in males (vs. absent), an axil-

Figure 12. *Oxynoemacheilus melenicus*, FFR15626 paratypes, top to bottom 60 mm SL, 63 mm SL; Turkey: stream Yarılgan.

lary lobe at pelvic fin base (vs. absent), a forked caudal fin (vs. slightly truncate) and a more slender caudal peduncle (caudal peduncle length 1.9–2.7 times its depth, vs. 1.1–1.4). *Oxynoemacheilus melenicus* is distinguished from *O. cemali* by having a forked caudal-fin (vs. slightly forked) and a more slender caudal peduncle (depth 1.9–2.6 times in its length, vs. 1.4–2.0).

Description. See Figs 11, 12 for general appearance and Table 3 for morphometric data. Adult size large (maximum 69 mm Ls). Body slender, compressed at caudal peduncle, greatest depth about midline between nape and dorsal-fin origin, slightly decreasing towards caudal-fin base. No hump at nape. Greatest body width at pectoral-fin base. Head pointed, upper head profile slightly convex on snout, flattened on ventral surface. Snout slightly pointed at tip. Mouth narrow and arched, lips well developed. A narrower median interruption in lower lip. A suborbital groove in males. No median incision in upper lip. Barbels short, inner rostral barbel not reaching to base of maxillary barbel; outer almost not reaching to vertical through anterior eye margin. Maxillary barbel, not reaching to posterior eye-margin in most individuals. Caudal peduncle slender, compressed laterally, length 1.9–2.7 times longer than deep. Axillary lobe present at pelvic-fin base, fully attached to body. Pelvic-fin origin below first or second branched dorsal-fin ray. Anal-fin origin at vertically equal to dorsal-fin tip. Pectoral fin not reaching vertical through tip of dorsal-fin origin in males. No dorsal or ventral adipose crest on caudal peduncle. Pelvic fin not reaching vertical of dorsal-fin tip, almost reaching to anus. Anal fin not reaching caudal-fin base. Caudal fin moderately forked.

Lateral line complete, reaching caudal-fin base. Body covered by embedded scales on flank, back, and belly.

Table 3. Morphometric data of *Oxynoemacheilus melenicus* (holotype, FFR15627; paratypes, FFR 15626; n = 20).

	Holotype	paratypes			
		mean	min	max	SD
Standard length (mm)	65		51	69	
In percent of standard length					
Head length	23.4	23.4	22.0	25.4	0.9
Body depth at dorsal–fin origin	15.5	15.7	14.3	17.0	0.8
Predorsal length	52.1	50.9	47.4	53.2	1.7
Postdorsal length	36.4	36.8	34.6	39.8	1.1
Preanal length	71.8	71.4	67.3	74.8	1.9
Prepelvic length	49.5	50.5	47.4	53.7	1.7
Distance between pectoral and pelvic-fin origins	27.5	29.4	27.2	31.9	1.4
Distance between pelvic and anal-fin origins	22.0	20.5	18.6	23.2	1.2
Distance between vent and anal-fin origin	4.4	4.6	3.7	4.6	0.7
Depth of caudal peduncle	7.8	7.3	6.2	8.3	0.4
Length of caudal peduncle	17.2	19.1	17.2	20.5	0.5
Dorsal-fin depth	18.1	18.3	16.8	20.3	0.9
Anal-fin depth	15.8	15.9	14.2	18.4	1.0
Pectoral-fin length	20.2	21.6	19.0	23.9	1.4
Pelvic-fin length	15.4	16.0	14.7	17.2	0.7
In percent of head length					
Head depth at eye	46.7	44.7	39.4	48.6	2.2
Snout length	44.7	43.2	36.0	37.5	2.8
Eye diameter	16.0	18.2	14.3	20.9	1.7
Postorbital distance	50.3	45.0	38.3	50.8	3.8
Maximum head width	67.3	59.2	53.3	67.3	2.6
Interorbital width	24.7	24.3	19.6	29.8	2.7
Length of inner rostral barbel	20.4	24.2	19.5	29.7	3.0
Length of outer rostral barbel	34.1	29.3	21.1	34.1	3.3
Length of maxillary barbel	26.5	27.2	21.3	35.4	3.6
Caudal peduncle length/depth	2.3	2.6	2.3	2.8	0.2

Table 4. Pairwise distance Kimura's two parameters (K2P) values based on cytochrome oxidase sequences of *Oxynoemacheilus* species.

	O. melenicus	***O. sakaryaensis***	***O. simavicus***	***O. fatsaensis***	***O. banarescui***	***O. samanticus***	***O. bergianus***
O. melenicus							
O. sakaryaensis	0,017						
O. simavicus	0,024	0,027					
O. fatsaensis	0,032	0,042	0,044				
O. banarescui	0,034	0,037	0,035	0,040			
O. samanticus	0,032	0,038	0,041	0,039	0,027		
O. bergianus	0,036	0,038	0,045	0,041	0,032	0,024	
O. angorae	0,076	0,079	0,092	0,076	0,075	0,076	0,080

Table 5. List of the variable nucleotide substitutions in the 645 base pairs long mt DNA COI barcode region.

Species	**Variable nucleotide positions**			
	111122	2222222233	3334444444	556
	2489078900	0133478915	6891267899	292
	1063670214	7314365451	0161387308	247
O. melenicus	CCTYATGGGY	RCAGGCGTGR	GTTRGYGAYC	RCC
O. sakaryaensis	T..T..WAR.	..R..T....	RC..R..GCG	..T
O. simavicus	TYW.WC.A..	.A.R.TACC.	..C...A.C.	SA.

(Y= C/T, R= A/G, W= A/T, S= C/G)

Dorsal fin with 7–8½ branched rays, its outer margin straight or slightly concave. Anal fin with 5½ branched rays, its outer margin straight. Pectoral fin with 10–11 rays, outer margin straight. Pelvic fin with 7–8 rays, outer margin slightly convex. Caudal fin with 8+8, 8+9, and 9+9 branched rays, outer margin forked, and lopes slightly pointed.

Coloration. Body yellowish in life and light brown in preserved individuals. Head and cheek plain or with small, plain brown mottling on top and cheeks, without color pattern ventrally. Numerous pigmentations below a line from pectoral-fin base to anus. A dark brown blotch at dorsal fin-origin. Flank plain brownish or with 10–13 irregular shaped brownish bars or blotches. Back in front of dorsal-fin with zero or four dark brownish blotch. Upper part of caudal peduncle with 3–4 irregularly shaped dark brownish blotches, not fused with midlateral bars or blotches in most individuals. One irregular shaped small black spots on caudal-fin base. Dorsal fin with 1–2 and caudal fin with 2–3 fine, irregularly shapes black bands on rays. Anal, pectoral and pelvic fins plain yellowish, and pectoral fin with few small black spots on rays.

Distribution. *Oxynoemacheilus melenicus* was found in the Büyükmelen Stream and Sakarya River drainage in western Anatolia (Fig. 10).

Etymology. The name of the species is derived from the Stream Büyükmelen.

Phylogenetic positions *of Oxynoemacheilus melenicus* and *Oxynoemacheilus sakaryaensis*

COI barcode region sequences were analyzed in seven *Oxynoemacheilus* species in western Anatolia. *Oxynoemcaheilus* species were divided into three main clades in the all phylogenetic analysis supported by high bootstrap values. The first clade consisted of *O. bergianus* group species which are *O. melenicus*, *O. sakaryaensis and O. simavicus O. banarescui*, *O. bergianus*, *O. samanticus*. The second and third clades included *O. fatsaensis* and *O. angorae*, respectively. *O. melenicus*, constituted a highly supported clade sister to *O. sakaryaensis* (Fig. 13). Intrageneric K2P distances between species ranged from 1.7% (*O. melenicus*, *O. sakaryaensis*) to 8.0% (*O. angorae and O. bergianus*). K2P distance is 1.7% between *O. melenicus* and its closest relative, *O. sakaryaensis* and, 2.4% between *O. melenicus and O. simavicus* (Table 4). Also, the K2P distance is 2.7% between *O. sakaryaensis* and *O. simavicus*. *O. melenicus* differs from its most closely related congeners, *O. sakaryaensis*, and *O. simavicus*, by 9 and 12 nucleotide substitution sites and *O. sakaryaensis* differs from *O. simavicus*, by 13 nucleotide substitution sites. *O. melenicus* and *O. sakaryaensis* were differentiated from all other *Oxynoemacheilus* species in western Anatolia by two diagnostic and unique nucleotide substitution sites in the COI barcoding region (Table 5). In the haplotype network analysis, the 32 distinct haplotypes were determined. The most common haplotype was H9, shared by many populations belonging to *O. sakaryaensis*. All species have unique haplotypes, as distinctly illustrated in the haplotype network (Fig. 14).

We found eight OTUs according to ABGD and GMYC analysis. The ASAP determined nine clusters for studied species of *Oxynoemacheilus*. The likelihoods of GMYC and null models were 600.9632 and 595.6004, respectively. The GMYC analysis was represented by eight ML entities (CI: 8–11). ASAP's best partition (score = 1.50) results from a p-distance threshold of 0.009 and both recursive and initial partition predicts ten subsets. ABGD analysis determined that the barcode gap is 0.012.

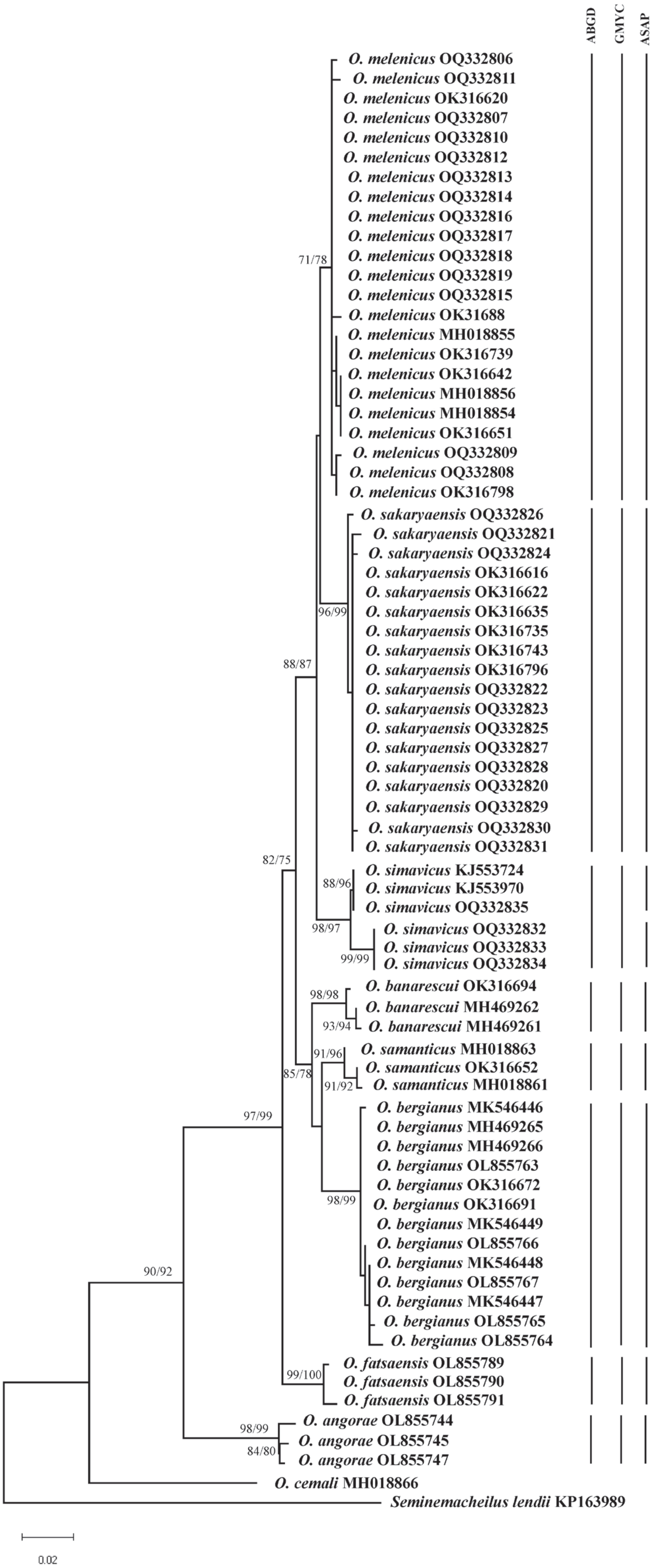

Figure 13. Maximum likelihood tree based on mitochondrial cytochrome oxidase subunit I (COI; 645 bp) gene sequences of *Oxynoemacheilus spp*. Maximum likelihood and Neighboor Joining analyses resulted in congruent trees. Bootstrap and posterior probability values are shown above nodes on tree if 70% or higher. ABGD, GMYC and ASAP clusters were indicated in vertical bars.

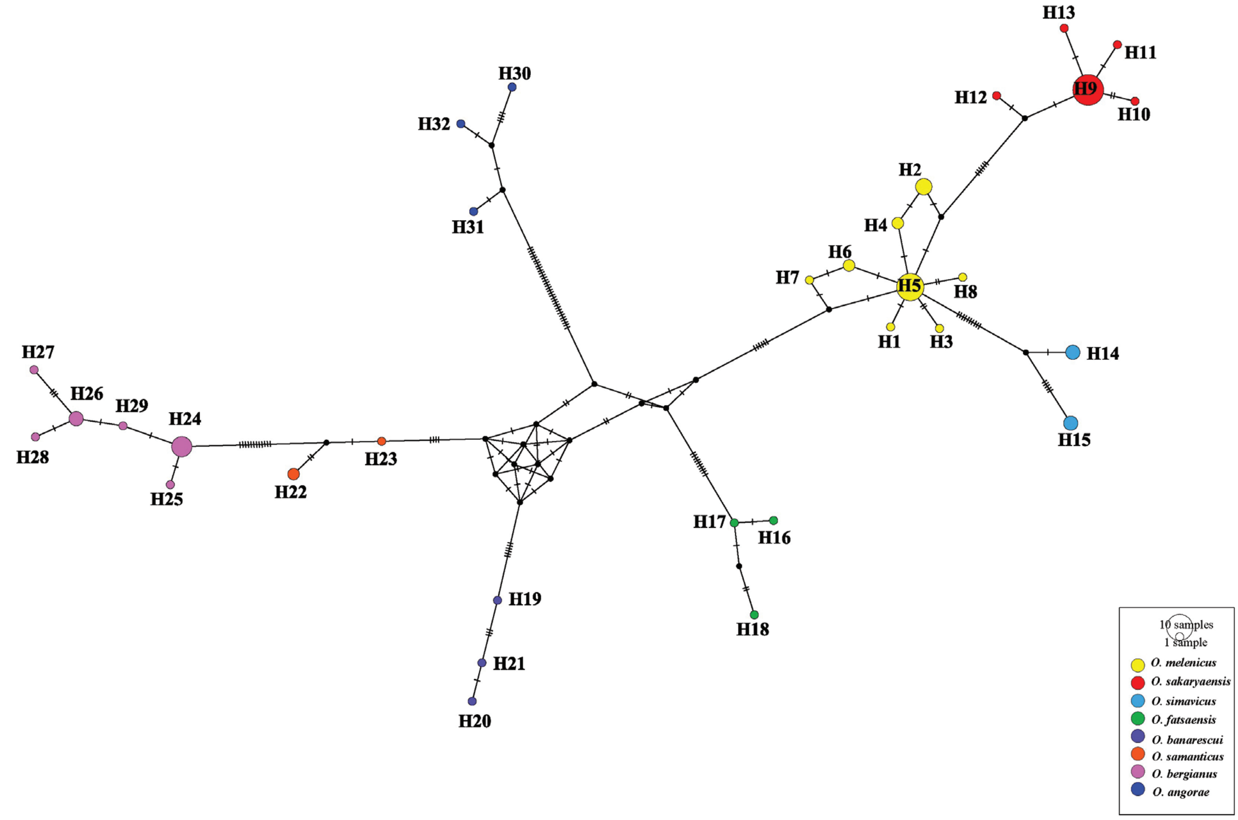

Figure 14. Median-joining network of the COI haplotypes. Circle size corresponds to sample size; one bar indicates an additional mutational step. Small black circles represent median vectors. Each small lines represent one nucleotide difference.

Discussion

Oxynoemacheilus melenicus and *O. sakaryaensis* were genetically identified in *O. bergianus* species group. Similar to this study Freyhof et al. 2022 revealed two unnamed molecular clades in the Western Black Sea (Büyükmelen and Sakarya River drainage), which could represent candidate species. However, they could not describe these species because morphological differences could not be found. In this study, besides morphological differences, the results from the species delineation tests (ABGD, GMYC, ASAP) and phylogenetic analyses support the validity of *O. melenicus* and *O. sakaryaensis* as distinct species.

Freyhof et al. 2022 detected 15 entities in the PTP analysis, while mPTP detected four entities representing putative species, and ASAP predicted 15 entities, which are mainly congruent to the PTP result. According to their results, *O. banarescui*, *O. fatsaensis*, *O. samanticus*, *O. simavicus*, and specimens from Sakarya and Büyük Melen were determined as different clusters, and *O. bergianus* was divided into two clusters. In our ABGD and GMYC analysis, eight entities were defined, which are *O. melenicus*, *O. sakaryaensis*, *O. simavicus*, *O. banarescui*, *O. bergianus*, *O. samanticus*. *O. fatsaensis* and *O. angorae*. Unlike ABGD and GMYC, *O. simavicus* was divided into two clusters in the ASAP analysis. In this regard, this study reflected that the species delineation tests agree with the previous research. Freyhof et.al. (2022) reported that *Oxynoemacheilus bergianus* group comprises ten molecular clades following congruently well-supported NJ, MP, and ML based entities. Species described as *O. bergianus*, *O. banarescui*, *O. fatsaensis*, and *O. simavicus* from Turkey, *O. lenkoranensis* from Azerbaijan, and *O. longipinnis* and *O. parvinae* from Iran belong to this species group. In addition to these, it also includes four unnamed molecular clades. We did not have the opportunity to examine *O. lenkoranensis*, *O. longipinnis*, *O. parvinae*, and four unnamed groups due to the lack of specimens from their localities. However, we examined many specimens from their distribution area from *Oxynoemacheilus bergianus*, *O. banarescui*, *O. fatsaensis*, *O. samanticus*. We concluded that *O. bergianus*, *O. banarescui*, *O. fatsaensis*, *O. samanticus*, and *O. simavicus*, together with *O. sakaryaensis* and *O. melenicus* are valid species.

Freyhof et al. (2022) did not make a detailed comparison, considering that the Sakarya and Büyükmelen populations are *O. simavicus*. They gave only brief information about the color and pattern characteristics of the *O. simavicus*. Freyhof et al. 2022 stated that *O. simavicus* with mottled and blotched individuals are common, including those from the Simav, Büyükmelen, and Sakarya River drainages. According to our results, *Oxynoemacheilus sakaryaensis* is characterized by having a flank with plain or with numerous irregularly shaped pale brownish bars and *O. melenicus* with a flank with 10–13 irregularly shaped brownish bars or blotches. However, *O. simavicus* is characterized by a flank with 2–8 dark brownish blotches.

Comparative material

***Oxynoemacheilus angorae*:** FFR001389, 2, 49–63 mm SL; Turkey: Ankara prov.: stream Berçin at Kızılcahamam, 40.471N 32.655E. – FFR01506, 5, 37–63 mm SL; Turkey: Bolu prov.: stream Uludere at Hendek, 40.809°N, 30.758°E. – FFR01513, 14, 41–60 mm SL; Turkey: Ankara prov.: stream Berçin at Kızılcahamam, 40.482°N, 32.653°E. – FFR01521, 6, 60–77 mm SL; Turkey: Bilecik prov.: stream Göynük 4 km west of Göynük, 40.386°N, 30.746°E. – FFR01524, 4, 51–63 mm SL; Turkey: Kütahya prov.: stream Porsuk on road from Kütahya to Uşak, 39.350°N, 30.038°E. – FFR01526, 6, 45–63 mm SL; Turkey: Ankara prov.: stream Kirmir 3 km north of Güdül, 40.236°N, 32.261°E. – FFR01547, 9, 46–54 mm SL; Turkey: İznik prov.: stream Karasu 6 km southeast of İznik, 40.468°N, 29.681°E. – FFR01549, 50, 20–59 mm SL; Turkey: Ankara prov.: stream Peçenek 7 km east of Şereflikoçhisar, 40.471°N, 32.655°E. – FFR01550, 2, 34–51 mm SL; Turkey: Yozgat prov.: stream Delice at 2 km southeast of Yerköy, 39.622°N, 34.490°E. – FFR01554,116, 32–62 mm SL; Turkey: Kastamonu prov.: stream Devrez 6 km southeast of Tosya, 40.984°N, 34.099°E. – FFR01557, 14, 29–43 mm SL; Turkey: Kırşehir prov.: Kızılırmak River at Kesikköprü, 38.961°N, 34.199°E. – FFR01558, 43, 20–67 mm SL; Turkey: Kırşehir prov.: stream Kılıçözü Kızılcahamam 2 km north of Özbağ, 39.241°N, 34.128°E. – FFR01559, 4, 51–61 mm SL; Turkey: Çankırı prov.: stream Devrez 2 km south of Ilgaz, 40.904°N, 33.638°E. – FFR01571, 5, 52–67 mm SL; Turkey: Eskişehir prov.: stream Pürtek 11 km southeast of Sivrihisar, 39.518°N, 31.618°E.

***Oxynoemacheilus banarescui*:** FFR15511, 4, 43–77 mm SL; Turkey: Kastamonu prov.: stream Devrekanı 8 km northeast of Devrekanı, 41.627°N, 33.922°E. – IUSHM 2018-1403, 10, 50 –62 mm SL; Turkey: Karabük prov.: stream Davulga at 15 km north of Mengen, 41.087°N, 32.023°E.

***Oxynoemacheilus cemali*:** FFR01359, 4, 53–73 mm SL; Turkey: Giresun prov: stream Dereli at Dereli, 40.740°N, 38.450°E. – FFR01440, 7, 31–64 mm SL; Turkey: Erzincan prov: stream Köroğlu 2 km south of Kürelik, 39.932°N, 38.749°E. – FFR01441, 12, 52–95 mm SL; Turkey: Gümüşhane prov: stream Değirmen at Yukarıözlüce village, 39.986°N, 39.540°E. – FFR01470, 16, 47–72 mm SL; Turkey: Erzincan prov: stream Köroğlu 2 km north of Refahiye, 39.920°N, 38.760°E. – FFR01515, 12, 30–83 mm SL; Turkey: Samsun prov: stream Tersakan 5 km northeast of Havza, 40.989°N, 35.717°E. – FFR01582, 26, 49–70 mm SL; Turkey: Erzurum prov: Çoruh River 500 m northwest of Adabaşı, 40.380°N, 40.320°E.– FFR 01583, 19, 68–88 mm SL; Turkey: Erzurum prov: stream Yağlı at 2 km southeast of İncesu, 40.308°N, 41.007°E. – FFR01584, 22, 36–88 mm SL; Turkey: Erzurum prov: stream Tortum 4 km west of Demirciler, 40.379°N, 40.320°E. – FFR01585, 3, 70–81 mm SL; Turkey: Erzurum prov: stream Tortum 600 m west of Engüzek Kapı Castle, 40.510°N, 41.523°E. – FFR01586, 16, 36–64 mm SL; Turkey: Erzurum prov: stream Tortum 2 km west of Altınçanak, 40.568°N, 41.596°E. – FFR01587, 27, 51–75 mm SL; Turkey: Erzurum prov: stream Oltu 2 km west of Ayvalı, 40.753°N, 41.854°E. – FFR01588, 8, 60–86 mm SL; Turkey: Artvin prov: stream Oltu 3 km southwestern of İşhanı, 40.774°N, 41.713°E. – FFR01589, 18, 48–72 mm SL; Turkey: Artvin prov: Çoruh River at 1 km north of Bademli, 40.445°N, 40.901°E. – FFR01590, 33, 67–78 mm SL; Turkey: Artvin prov: stream Ekşinar 1 km north of Ekşinar, 41.116°N, 42.056°E. – FFR01591, 4, 66–68 mm SL; Turkey: Artvin prov: stream Köprüler 3 km northwest of Ardanuç, 41.137°N, 42.038°E. – FFR01592, 14, 41–84 mm SL Turkey: Artvin prov: stream Okçular 2 km south of Eskikale, 41.263°N, 42.191°E. – FFR01593, 27, 49–83 mm SL; Turkey: Artvin prov: stream Göknar 1 km north of Çayağzı, 41.284°N, 42.232°E. – FFR01594, 54, 46–74 mm SL; Turkey: Gümüşhane prov: stream Şiran 15 km northwest of Şiran, 40.310°N, 39.031°E. – FFR01596, 17, 60–70 mm SL; Turkey: Giresun prov: stream Bağırsak Alucra, 40.310°N, 38.773°E.

Oxynoemacheilus fatsaensis: FFR15513, 1, 55 mm SL; Turkey: Samsun prov: stream Tersakan 5 km east of Havza 40.990°N, 35.717°E. – FFR01361, 5, 46–58 mm SL; Turkey: Samsun prov: stream İlhanlı at Ayvacık 40.990°N, 36.634°E. – FFR01362, 10, 37–64 mm SL; Turkey: Giresun prov: stream Aksu at Dereli 40.731°N, 38.460°E. – FFR01362, 10, 37–64 mm SL; Turkey: Tokat prov: stream Kelkit 9 km north of Erba 40.759°N, 36.515°E. – FFR01516, 31, 38–75 mm SL; same data as holotype.

Oxynoemacheilus seyhanensis: FFR01433, 9, 38–78 mm SL; Turkey: Sivas prov.: Kızılırmak River at Zara, 39.902°N, 37.763°E. – FFR01474, 32, 32 –79 mm SL; Turkey: Sivas prov.: Kızılırmak River 7 km north of Zara, 39.961°N, 37.739°E. – FFR01545, 20, 51–78 mm SL; Turkey: Çankırı prov.: stream Ulusu at Çerkeş, 40.820°N, 32.800°E. – FFR01551, 1, 69 mm SL; Turkey: Yozgat prov.: stream Delice 11 km northwest of Yerköy, 39.698°N, 34.362°E. – FFR01552, 7, 38–77 mm SL; Turkey: Yozgat prov.: stream Delice 2 km southeast of Yerköy, 39.622°N, 34.490°E. – FFR01578, 8, 50–67 mm SL; Turkey: Çankırı prov.: stream Ulusu at Çerkeş, 40.800°N, 32.883°E.

Oxynoemacheilus bergianus: FFR01457, 11, 64–72 mm SL; Turkey: Malatya prov.: stream Sultansuyu 8 km east of Akçadağ, 38.339°N, 38.064°E. – FFR01467, 28, 54–64 mm SL; Turkey: Erzurum prov.: stream Baş 10 km east of Aşkale, 39.948°N, 40.804°E.–FFR 1457, 11, 64 –72 mm SL; Turkey: Malatya prov.: stream Sultansuyu 8 km east of Akçadağ, 38.3388°N, 38.0620°E. –FFR 15506, 25, 33–59 mm SL; Turkey: Ağrı prov.: Murat River 17 km west of Taşlıçay, 39.6785°N, 43.1887°E.

Oxynoemacheilus samanticus: FFR01553, 2, 55–62 mm SL; Turkey: Yozgat prov.: stream Delice, 2 km southeast of Yerköy, 39.622°N, 34.490°E. – FFR01556, 3, 37–55 mm SL; Turkey: Sinop prov.: stream Gökırmak at Çarşak 11 km east of Boyabat, 41.453°N, 34.889°E. –FFR015518, 19, 54–62 mm SL; Turkey: Samsun prov.: stream Soruk 20 km east of Vezirköprü, 41.119°N, 35.227°E.

Oxynoemacheilus simavicus: FFR01380, 12, 38–50 mm SL; Turkey: Balıkesir prov: stream Koca at Manyas. –FFR01505, 28, 32–56 mm SL; Turkey: Balıkesir prov:

stream Sakar at Manyas 40.050°N, 27.962°E. – FFR01518, 8, 44–74 mm SL; Turkey: Balıkesir prov: stream Koca 4 km north of Balya 39.782°N, 27.596°E. – FFR01520, 8, 47–55 mm SL; Turkey: Balıkesir prov: stream Değirmenboğazı 13 km west of Manyas 40.000°N, 27.828°E. – FFR01522, 4, 47–51 mm SL; Turkey: Balıkesir prov: stream Koca at Ilıca 39.878°N, 27.780°E. – FFR01542, 4, 48–49 mm SL; Turkey: Bursa prov: stream Nilüfer at Narlıdere 40.244°N, 29.298°E. – FFR01544, 4, 48–49 mm SL; Turkey: Bursa prov: stream Nilüfer at Misi 40.180N 28.974E.

Material used in the molecular genetic analysis

Oxynoemacheilus simavicus

FFR DNA 01505, 1, Turkey: Balıkesir prov: stream Sakar at Manyas 40.050°N, 27.962°E. (GenBank accession number OQ332835) – FFR DNA 01512, 3, Turkey: Balıkesir prov: stream Dursunbey 4 km south of Adaören 39.609°N, 28.751°E. (GenBank accession numbers OQ332832–OQ332834)

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