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Oxynoemacheilus kottelati, a new species from the Havran and Karınca streams in Northern Aegean Basin, Türkiye (Teleostei, Nemacheilidae)

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Abstract

The taxonomic status of the *Oxynoemacheilus* from the Karınca and Havran streams in the north Aegean Basin was evaluated, and it was concluded that these populations contain a new species. The new species, *Oxynoemacheilus kottelati* **sp. nov.**, is distinguished by a body with a marbled pattern, a deeper caudal peduncle, a shorter caudal peduncle, a wider interorbital distance, and a shorter middle caudal-fin lope. *Oxynoemacheilus kottelati* **sp. nov.** is differentiated from the closest species, *O. marmaraensis*, in possessing 51 nucleotide substitution sites, a genetic distance of 8.40%, the presence of an axillary lobe at the base of the pelvic fin (vs. absent), and a narrower median incision in the upper lip (vs. absent). Three species delimitation tests (ASAP, ABGD, and PTP) and phylogenetic analyses reinforce the validity of *O. kottelati* **sp. nov.** as a distinct species.

Key Words

Anatolia, COI, freshwater fish, loach, taxonomy

Introduction

The species-rich genus *Oxynoemacheilus*, established by Banarescu and Nalbant in 1966 within the family Nemacheilidae, exhibits a wide distribution across the Eastern Mediterranean, the southern Caucasus, Anatolia, Mesopotamia, and Central Iran (Freyhof et al. 2011; Kottelat 2012). Despite its extensive range, our understanding of its diversity remains limited, particularly in the Asian portion of its distribution, where several new species have been described in recent years, notably from the Anatolian and Tigris River drainages. A total of 67 species within this genus have been described or reported in the literature (Erk'akan et al. 2007; Freyhof et al. 2011; Erk'akan 2012; Kamangar et al. 2014; Freyhof 2016; Sayyadzadeh et al. 2016; Freyhof and Abdullah 2017; Freyhof et al. 2017; Freyhof and Özuluğ 2017; Turan et al. 2019; Bektaş et al. 2022; Yoğurtçuoğlu et al. 2022; Çiçek et al. 2023; Turan et al. 2023a, b). Forty-eight of these species are distributed in inland waters throughout Türkiye, with 36 of them being endemic to the region. The Tigris and Euphrates basins harbor the highest number of *Oxynoemacheilus* species in Türkiye (n = 16), followed by the Mediterranean (n = 14), the Black Sea (n = 6), the Caspian (n = 4), the Aegean (n = 4), Konya (n = 2), Marmara (n = 2), and the Van basins (n = 1). Notably, the *Oxynoemacheilus* species distributed in the Aegean basin of Türkiye have not been adequately investigated.

In the Aegean region, Erk'akan et al. (2007) and Erk'akan (2012) described three species from the Büyük Menderes River: *Barbatula germencicus* from around Aydın-Germencik, *Barbatula cinica* from Cindere Stream between Kütahya and Denizli, and *Barbatula mesudae* from Büyük Menderes around Çivril. Yoğurtçuoğlu et al.

(2022) and Bektaş et al. (2022) later reported these species as synonyms of *O. germencicus*. Stoumboudi et al. (2006) described *O. theophilii* from Lesbos Island in Greece, based on three individuals. Subsequently, Yoğurtçuoğlu et al. (2022) recognized three species in the Aegean basin: *O. theophilii* (Bakır Stream), *O. germencicus* (Büyük Menderes and Gediz rivers), and *O. eliasi* (Tahtalı reservoir, Küçük Menderes, and Gediz rivers). Additionally, Turan et al. (2024) described *O. fatmae* from the Güzelhisar Stream. Considering the reported species, *Oxynoemacheilus* appears to be in a poor region in terms of species composition. However, in the detailed field studies we have carried out recently, it has been seen that there are still undescribed species in the Aegean region.

In this study, specimens from Havran and Karınca streams were examined and compared with other species from the Aegean and adjacent basins. The comparison results revealed that the materials from Havran and Karınca streams belong to a new species named *O. kottelati*.

Materials and methods

The fish samplings and experiments conducted in this study were approved by the Recep Tayyip Erdoğan University Local Ethics Committee for Animal Experiments in the Republic of Türkiye, under permit reference number 2020/4. Following anesthesia, sample fixation was initially carried out in 5% formaldehyde, with subsequent immersion in 70% ethanol whenever feasible. Alternatively, some samples were directly fixed in absolute ethanol for tissue collection for genetic analysis. Measurements were performed using a dial caliper set precisely to 0.1 mm, adhering to the stringent point-to-point measurement procedures outlined in the guidelines provided by Kottelat and Freyhof (2007). Morphometric data for *O. angorae*, *O. simavicus*, and *O. marmaraensis* were sourced from Turan et al. (2019) and Turan et al. (2023b), respectively.

DNA extraction, PCR, sequencing, and molecular analysis

DNA was isolated from fin clips via Hibrigen genomic DNA extraction kits. DNA quality was checked by agarose gel electrophoresis. The vertebrata *COI* barcode region (624 bp) was amplified with the FishF1 and FishR1 (Ward et al. 2005) primer pairs. The PCR reactions' conditions and components' volumes were detailed in Turan et al. (2023a). The PCR products were visualized with a gel documentation system, and eligible PCR products were sent to Macrogen Europa Inc. (Amsterdam, Netherlands) for purification and Sanger sequencing with the ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems; appliedbiosystems.com). We have used the six newly produced COI barcodes and 41 sequences from previously published studies (Geiger et al. 2014; Turan et al. 2019; Bektas et al. 2022; Yoğurtçuoğlu et al. 2022) for the molecular data analysis. *Oxynoemacheilus bureschi* (Genbank number: KJ553692) and *Seminemacheilus lendlii* (Genbank number: MT077008) were added to the analysis as outgroup taxa. Multiple sequence alignment was done with Clustal W options (Thompson et al. 1994) in Bioedit v7.2.5 software (Hall 1999). Sequences were submitted to NCBI GenBank with accession numbers PP085162–PP085167. Phylogenetic correlations were determined with maximum likelihood (ML) and Bayesian inference (BI) analysis using MEGA X (Kumar et al. 2018) and MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) software. The nucleotide substitution model was estimated as the TrN+G model (Tamura and Nei 1993) concerning the Bayesian information criterion (BIC) in jModeltest v. 0.0.1 (Posada 2008). To estimate pairwise genetic distances, a *p*-distance model was used in MEGA X software. In total, three single-locus species delimitation methods were used as follows: ASAP (Puillandre et al. 2021), ABGD (Puillandre et al. 2012), and GMYC (Fujisawa and Barraclough 2013). Analysis parameters and settings were given in the study by Turan et al. (2023a, b).

Collection codes

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

Results

Phylogenetic placement of *Oxynoemacheilus kottelati*

COI barcode region sequences were analyzed in eleven *Oxynoemacheilus* species distributed in the Aegean and Marmara basins. The species were divided into two main clades in all the phylogenetic analyses, supported by high bootstrap values. The first clade consists of *O. kottelati* sp. nov. and *O. marmaraensis*. The second clade consists of *O. anatolicus*, *O. angorae*, *O. eliasi*, *O. fatmae*, *O. germencicus*, *O. mediterraneus*, *O. nasreddini*, *O. simavicus*, and *O. theophilii*. *Oxynoemacheilus kottelati* sp. nov. constituted a highly supported clade sister to *O. marmaraensis* (Fig. 5). The uncorrected *p* distance between species ranged from 1.00% (*O. mediterraneus* and *O. nasreddini*) to 11.2% (*O. kottelati* sp. nov. and *O. simavicus*). The p-distance was determined to be 8.4% between *O. kottelati* sp. nov. and its closest relative, *O. marmaraensis* (Table 3). *Oxynoemacheilus kottelati* sp. nov. differed from its most closely related congener, *O. marmaraensis*, by 51 nucleotide substitution sites.

In the ASAP analysis, we found 10 OTUs. ASAP's best partition (score = 3.50) results from a *p*-distance threshold of 0.012428. However, the PTP determined 13 clusters, and ABGD resulted in 12 groups. Some barcoding analyses tended to over-split; however, *O. kottelati* sp. nov. was predicted as a candidate species in all three barcoding analyses.

Morphological differences and comparisons

The genetically closest species to *Oxynoemacheilus kottelati* sp. nov. is *O. marmaraensis*, distributed in the Susurluk River (Marmara basin). It is distinguished from *O. marmaraensis* by body color and pattern (marbled vs. vermiculated), presence of an axillary lobe at the base of the pelvic fin (vs. absent), a narrower median incision in the upper lip (vs. absent), and 4–6 small irregularly shaped narrow greyish or brownish saddles on the dorsal part of the caudal peduncle (vs. 3–4). *Oxynoemacheilus kottelati* sp. nov. differs from *O. theophilii* by having a greater interorbital distance (28–36% SL, vs. 20–28), the absence of the dorsal and ventral adipose crest on the caudal peduncle (vs. slightly developed), and no black bars or blocks on the flank (vs. 10–13 small irregularly shaped black bars or blocks on the flank in most individuals). *Oxynoemacheilus kottelati* sp. nov. differs from *O. eliasi* in having a shorter caudal peduncle (13–16, vs. 17–21), a greater interorbital distance (28–36% SL, vs. 20–27), the depth of the caudal peduncle 1.0–1.3, vs. 1.5–1.7 times in its length, and the body marbled pattern (vs. more or less black or brown blocks on the flank in most individuals). *Oxynoemacheilus kottelati* sp. nov. differs from *O. germencicus* by having a shorter caudal peduncle (13–16, vs. 16–22), the depth of the caudal peduncle 1.0–1.3, vs. 1.3–2.2 times in its length, and the body marbled pattern (vs. more or less black or brown blocks on the flank in most individuals). *Oxynoemacheilus kottelati* sp. nov. differs from *O. angorae* by having a shorter postdorsal distance (31–36% SL, vs. 38–42), a deeper caudal peduncle (12–14% SL, vs. 10–12), a shorter caudal peduncle (13–16, vs. 16–19), the depth of caudal peduncle 1.0–1.3, vs. 1.4–1.8 times in its length, and the body marbled pattern (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). *Oxynoemacheilus kottelati* sp. nov. differs from *O. simavicus* by having a longer head (24–27% SL, 19–22), a deeper caudal peduncle (12–14% SL, vs. 6–10), a deeper body (body depth at dorsal-fin origin 18–22% SL, vs. 12–17), the depth of the caudal peduncle 1.0–1.3, vs. 2.2–3.1 times in its length, and the body marbled pattern (vs. 2–8 dark brown blocks on flank). It differs from *O. fatmae* by the body color and pattern (marbled vs. having 4–8 irregularly shaped narrow black bars commonly on the posterior part of the flank and anterior part of the flank with a marbled pattern), having a deeper caudal peduncle (12–14% SL, vs. 10–12), a shorter caudal peduncle (14–16% SL, vs. 17–20), a longer middle caudal-fin lobe (21–24% SL, vs. 16–19), and a greater interorbital distance (28–36% HL, vs. 21–26).

Thus, we describe *Oxynoemacheilus* populations from Karınca and Havran streams as a new species, *Oxynoemacheilus kottelati* sp. nov.

***Oxynoemacheilus kottelati* sp. nov.**

https://zoobank.org/FDB3F47E-E4B4-411F-8D82-F4AB05BC2F3A
Figs 1, 2

Type material. ***Holotype***: FFR 15655, 47 mm SL, male; Türkiye, Balıkesir prov., Havran Stream, F. Aksu, S. Aksu, 26 October 2023, 39°30'33.3"N, 27°09'39.0"E.

Figure 1. *Oxynoemacheilus kottelati* sp. nov., FFR 15655, **a, b.** Holotype, male, 47 mm SL; FFR 15656; **c.** Paratype, female, 49 mm SL; Türkiye, Balıkesir prov., Havran Stream.

Figure 2. *Oxynoemacheilus kottelati* sp. nov., FFR 15657, paratypes: **a.** Male, 47 mm SL; **b.** Female, 46 mm SL; **c.** Male, 45 mm SL; Havran Stream; FFR 15656; **d.** Female, 48 mm SL; Karınca Stream; Türkiye, Balıkesir prov.

***Paratypes*:** FFR 15657, 21, 39–50 mm SL; same data as holotype. FFR 15656, 34, 35–54 mm SL; Türkiye, Balıkesir prov., Karınca Stream, F. Aksu, S. Aksu, 26 October 2023, 39°27'12.2"N, 27°00'30.9"E.

Material used in molecular genetic analysis. FFRDNA 15657, 6; Türkiye, Balıkesir prov., Karınca Stream, 39°27'12.2"N, 27°00'30.9"E. (GenBank accession numbers: PP085162–PP085167).

Diagnosis. *Oxynoemacheilus kottelati* sp. nov. is distinguished from other species in the Aegean and adjacent basins due to a distinctive combination of characteristics: a body with a marbled pattern (vs. more or less irregularly shaped dark brown or pale brown blocks or bars on the flank in *O. germencicus*, *O. theophilii*, *O. eliasi*, *O. simavicus*, *O. fatmae*, and *O. angorae*), a deeper caudal peduncle (caudal peduncle depth 1.0–1.3 in its length, vs. 1.3–2.2, except *O. theophilii*), and a wider interorbital distance (28–36% HL, vs. 20–28, except *O. germencicus* and *O. angorae*). It is distinguished from *O. marmaraensis* by the presence of an axillary lobe at the base of the pelvic fin (vs. absent) and a narrower median incision in the upper lip (Fig. 3a, b; vs. absent; Fig. 3c, d).

Description. The general appearance of the species is depicted in Figs 1, 2, with accompanying morphometric data provided in Table 1. The body is deep and compressed at the caudal peduncle, with the greatest depth occurring slightly in front of the dorsal-fin origin and gradually decreasing towards the base of the caudal fin. There is no noticeable hump at the nape, and the greatest body width is observed at the pectoral-fin base. The head is pointed, featuring a straight upper profile at the interorbital area and a convex profile on the snout. The snout is somewhat long and slightly pointed at the tip. Mouths are narrow and arched, with slightly developed lips, and there is a narrow median interruption in the lower lip and a narrow median incision in the upper lip. Males typically exhibit a suborbital groove. Barbels are somewhat long, with the inner rostral barbel typically reaching to the base of the maxillary barbel in most individuals and the outer rostral barbel reaching vertically through the anterior eye margin. The maxillary barbel usually extends to the posterior eye margin in most individuals. The caudal peduncle is deep and laterally compressed, with a length 1.0–1.3 times longer than its depth. An axillary lobe is present at the pelvic-fin base, albeit very slightly devel-

Table 1. Morphometric data of *Oxynoemacheilus kottelati* sp. nov. (holotype FFR 115655 and paratypes FFR 15657, n = 21).

	***O. kottelati* (n = 21)**		SD
	H	**Range (mean)**	
Standard length (mm)	47	39–50	
In percent of standard length			
Head length	25.5	23.6–27.1 (25.3)	0.8
Body depth at dorsal–fin origin	19.3	17.8–21.6 (19.2)	0.9
Body width at dorsal–fin origin	14.0	11.9–15.4 (13.5)	0.8
Predorsal length	53.0	49.5–54.1 (51.4)	1.3
Postdorsal length	34.9	31.3–35.9 (34.2)	1.3
Preanal length	74.7	72.1–80.0 (75.3)	1.6
Prepelvic length	50.9	48.7–54.2 (51.1)	1.3
Dist. betw. pectoral and pelvic-fin origins	28.4	26.0–31.2 (28.6)	1.2
Dist. between pelvic and anal-fin origins	23.2	21.2–26.2 (23.5)	1.2
Depth of caudal peduncle	13.1	11.6–14.2 (12.8)	0.6
Length of caudal peduncle	15.2	13.0–16.2 (15.0)	0.9
Dorsal-fin depth	23.3	20.1–26.2 (22.5)	1.2
Anal-fin depth	18.4	16.6–22.1 (18.4)	1.3
Pectoral-fin length	22.7	20.0–25.0 (22.2)	1.7
Pelvic-fin length	17.7	14.8–19.6 (17.2)	1.1
Caudal-fin length	27.1	24.2–30.2 (27.5)	1.4
Middle lop of caudal-fin length	24.3	20.7–24.3 (22.6)	1.1
In percent of head length			
Head depth at eye	43.5	37.8–47.1 (41.7)	2.5
Snout length	38.3	32.4–42.6 (37.5)	2.9
Eye diameter	16.5	16.5–25.6 (20.1)	2.3
Postorbital distance	48.5	45.2–59.8 (50.6)	3.7
Maximum head width	57.2	49.9–63.8 (57.3)	3.7
Interorbital width	30.8	27.7–35.6 (30.9)	2.4
Length of inner rostral barbel	30.9	24.8–40.7 (29.2)	3.4
Length of outer rostral barbel	37.7	32.0–42.8 (36.0)	2.3
Length of maxillary barbel	25.6	25.6–36.8 (32.1)	3.0
In percent of caudal peduncle length			
Depth of caudal peduncle	1.2	1.0–1.3 (1.2)	0.7
In percent of body depth at dorsal-fin origin			
Caudal peduncle depth	69.4	69–86 (79)	5.1
In percent of length of caudal-fin length			
Length of middle caudal-fin lope	89.6	76–90 (82)	4.1

oped and fully attached to the body. The pelvic-fin origin typically lies below the first or second branched dorsal-fin ray, and the anal-fin origin is positioned vertically behind the dorsal-fin tip. In males, the pectoral fin almost reaches vertically through the tip of the dorsal-fin origin, whereas in females, it falls short of this mark. There are no dorsal or ventral adipose crests present on the caudal peduncle. The lateral line is complete, extending to the base of the caudal fin. The body is covered by embedded scales on the flank, back, and belly. The dorsal fin typically possesses 7½–8½ branched rays, with its outer margin being straight. The anal fin typically has 5½ branched rays along with a straight outer margin. The pectoral fin usually comprises 10–12 rays, with its outer margin either straight or slightly convex. The pelvic fin typically consists of 7–8 rays, with its outer margin straight or slightly convex. The caudal fins are deeply emarginated, with lobes that are slightly rounded.

Coloration. The body has a marbled pattern; the general body color is brownish in live specimens and grayish in preserved individuals. In the population of the Karınca Stream, the head and cheeks are plain without any discernible color pattern ventrally, whereas in the Havran population, the head and cheeks display a modeled pattern. There is no pigmentation below a line extending from the pectoral-fin base to the anus. A small, irregularly shaped, dark-brown blotch is present at the origin of the dorsal fin. The flank appears plain grayish with a marbled pattern, while the back may exhibit zero to six small, slightly distinct brownish blotches anterior to the dorsal fin origin. The dorsal part of the caudal peduncle bears 4–6 small, irregularly shaped, small, and narrow greyish or brownish saddles. Additionally, one vertically elongated black spot is observed on the base of the caudal peduncle fin. The dorsal fin typically displays 2–3 fine, irregularly shaped black bands on its rays, while the caudal fin may feature 2–4 similar bands. The anal, pectoral, and pelvic fins present a yellowish hue, with the pectoral fins occasionally exhibiting a few small black spots on the rays.

Distribution. *Oxynoemacheilus kottelati* sp. nov. was found in the Havran and Karınca streams, which are drainages in the Northern Aegean Sea basin (Fig. 4).

Etymology. This species is named in honor of Maurice Kottelat, whose contributions significantly advanced the understanding of the world's fish fauna.

Discussion

The newly identified species, *Oxynoemacheilus kottelati* sp. nov., exhibits a close genetic relationship to *O. marmaraensis*, as indicated by the genetic dataset, showing a genetic distance of 8.4% between the two species. Both genetic divergences sufficiently support their distinctiveness, as the two species are clearly distinguished from each other morphologically (see comparison section above).

We obtained some morphometric data from the Şaşal Stream (a drainage of Tahtalı reservoir), Büyük Menderes, and Gediz rivers (Table 2). The morphometric data for *O. germencicus* (FFR 1523, n = 7, 52–58 mm SL; FFR 1528, n = 12, 39–56 mm SL; IFC-ESUF 19-0015, n = 11, 44–65 mm SL; IFC-ESUF 19-0016, n = 7, 47–59 mm SL) are as follows: body depth at dorsal-fin origin 15–23% SL; caudal peduncle depth 9–13% SL; caudal peduncle depth 1.3–2.2 times in length; interorbital distance 19–32% HL; and caudal-fin length 21–32% SL. The morphometric data for *O. eliasi* (FFR 1558, n = 7, 38–41 mm SL and IFC-ESUF 19-0015, n = 11, 44–65 mm SL; IFC-ESUF 19-0016, n = 7, 47–59 mm SL) are: body depth at dorsal-fin origin 19–22% SL in Şaşal and 17–22% SL in Gediz; caudal peduncle depth 12–13% SL in two populations; caudal peduncle length 1.5–1.7 times its depth in two populations; interorbital distance 21–26 in Şaşal and 20–27% SL in Gediz; and caudal-fin length 21–29% SL in two populations. The morphometric data obtained from *O. theophilii* from the Bakır Stream (FFR 15538, n = 14, 38–55 mm SL) are: body depth at dorsal-fin origin 17–19% SL; caudal peduncle depth 12–14% SL; caudal peduncle length 1.2–1.5 times its depth; interorbital dis-

Table 2. Morphometric data of *Oxynoemacheilus germencicus* (FFR 1523, n = 7, 52–58 mm SL; FFR 1528, n = 12, 39–56 mm SL; IFC-ESUF 19-0015, n = 11, 44–65 mm SL; IFC-ESUF 19-0016, n = 7, 47–59 mm SL), *O. theophilii* (FFR 15538, n = 14, 38–55 mm SL) and *O. eliasi* (FFR 1558, n = 7, 38–41 mm SL; IFC-ESUF 19-0015, n = 11, 44–65 mm SL; IFC-ESUF 19-0016, n = 7, 47–59 mm SL)).

	***O. germencicus* n = 37**		***O. theophilii* n = 14**		***O. eliasi* n = 25**	
	Range (mean)	**SD**	**Range (mean)**	**SD**	**Range (mean)**	**SD**
Standard length (mm)	39–65		38–55		38–65	
In percent of standard length						
Head length	22.7–27.8 (25.2)	1.2	23.6–25.7 (24.6)	0.7	23.0–27.6 (24.9)	1.1
Body depth at dorsal–fin origin	14.8–22.8 (18.8)	1.7	16.9–19.0 (18.1)	0.7	17.2–22.3 (19.2)	1.3
Body width at dorsal–fin origin	10.1–16.8 (13.5)	1.6	11.6–14.3 (12.7)	0.9	10.2–16.6 (13.3)	1.5
Predorsal length	48.3–55.3 (51.8)	1.5	46.7–53.8 (51.2)	1.8	39.0–54.5 (51.1)	3.0
Postdorsal length	32.5–39.5 (36.0)	1.7	32.6–37.1 (35.4)	1.5	33.2–41.1 (37.6)	2.1
Preanal length	70.9–77.3 (74.1)	2.9	74.6–79.5 (76.5)	1.4	70.1–78.6 (74.7)	2.2
Prepelvic length	48.1–56.3 (52.2)	1.7	51.6–54.6 (52.6)	1.0	48.6–59.9 (51.6)	2.5
Dist. betw. pectoral and pelvic-fin origins	24.1–32.2 (28.2)	2.8	26.7–32.2 (29.2)	1.7	25.6–33.7 (29.2)	2.2
Dist. between pelvic and anal-fin origins	20.8–27.4 (24.1)	1.4	20.6–27.3 (23.4)	1.7	18.4–25.0 (22.3)	1.8
Depth of caudal peduncle	9.4–13.4 (11.4)	0.1	11.9–14.4 (12.8)	1.2	11.6–13.1 (12.2)	0.4
Length of caudal peduncle	15.9–22.0 (19.0)	1.5	15.2–18.8 (17.0)	0.7	17.1–21.1 (18.9)	1.0
Dorsal-fin depth	19.4–25.4 (22.5)	1.0	17.6–24.5 (20.9)	1.6	16.0–23.6 (18.9)	1.8
Anal-fin depth	16.0–23.8 (19.9)	2.3	15.8–20.1 (18.0)	1.2	14.8–21.0 (17.6)	1.6
Pectoral-fin length	19.6–28.6 (24.1)	2.0	20.1–23.7 (21.9)	1.1	16.1–28.6 (23.9)	2.7
Pelvic-fin length	15.5–23.9 (9.7)	1.8	15.7–19.9 (17.7)	1.5	15.7–19.5 (17.9)	1.2
Caudal-fin length	20.8–31.8 (26.3)	2.6	22.8–29.2 (25.8)	2.0	20.8–29.0 (26.6)	1.9
Middle lop of caudal-fin length	16.4–23.6 (20.0)	1.8	18.7–22.8 (20.3)	1.2	18.3–24.4 (20.9)	1.7
In percent of head length						
Head depth at eye	34.6–55.8 (47.4)	3.5	33.6–48.6 (42.8)	3.9	34.0–49.3 (42.2)	4.2
Snout length	32.8–49.2 (41.0)	3.8	34.5–44.9 (39.3)	2.6	30.5–46.9 (39.4)	3.6
Eye diameter	12.2–27.9 (20.0)	3.8	16.2–25.5 (19.6)	2.6	14.9–29.9 (19.1)	5.8
Postorbital distance	42.6–54.6 (48.6)	2.6	47.5–63.0 (53.2)	5.2	41.0–58.9 (50.8)	3.7
Maximum head width	52.8–66.2 (59.5)	3.6	52.9–64.5 (58.4)	3.7	50.4–64.5 (57.1)	3.8
Interorbital width	19.2–31.5 (25.3)	2.9	19.7–28.2 (24.1)	2.6	19.7–27.2 (24.0)	3.8
Length of inner rostral barbel	19.4–41.8 (30.1)	5.2	16.3–32.3 (24.7)	4.9	17.8–35.6 (25.1)	4.6
Length of outer rostral barbel	25.7–48.1 (36.9)	5.7	26.1–40.2 (31.2)	4.8	23.0–47.2 (33.4)	5.8
Length of maxillary barbel	23.9–59.0 (41.5)	7.1	20.8–37.5 (29.6)	4.0	21.9–42.2 (30.9)	5.6
In percent of caudal peduncle length						
Depth of caudal peduncle	1.3–2.1 (1.7)	1.9	1.2–1.5	1.2	1.5–1.7 (1.6)	0.7
In percent of body depth at dorsal-fin origin						
Caudal peduncle depth	52.6–70.0 (61.2)	4.8	65.1–83.0 (70.2)	4.8	53.4–72.7 (63.5)	4.5
In percent of length of caudal-fin length						
Length of middle caudal-fin lope	63.4–67.9 (75.6)	6.8	73.3–83.1 (78.4)	3.4	69.8–94.4 (79.4)	6.6

Table 3. Pairwise distance values based on cytochrome oxidase sequences of *Oxynoemacheilus* species. (Intraspecific genetic diversity is shown in gray).

	O. kottelati	***O. marmaraensis***	***O. eliasi***	***O. theophilii***	***O. angorae***	***O. fatmae***	***O. mediterraneus***	***O. nasreddini***	***O. germencicus***	***O. anatolicus***	***O. simavicus***
O. kottelati	0.001										
O. marmaraensis	0.084	0.002									
O. eliasi	0.100	0.108	0.003								
O. theophilii	0.106	0.110	0.037	0.001							
O. angorae	0.106	0.104	0.040	0.042	0.001						
O. fatmae	0.107	0.109	0.035	0.022	0.033	0.000					
O. mediterraneus	0.100	0.106	0.034	0.036	0.033	0.034	0.000				
O. nasreddini	0.101	0.102	0.030	0.029	0.030	0.027	0.010	0.001			
O. germencicus	0.103	0.105	0.041	0.036	0.041	0.038	0.033	0.030	0.005		
O. anatolicus	0.112	0.109	0.038	0.040	0.043	0.041	0.033	0.029	0.019	0.001	
O. simavicus	0.112	0.111	0.073	0.084	0.082	0.080	0.082	0.081	0.081	0.078	0.005

Figure 3. Mouth shape and structure: *Oxynoemacheilus kottelati*, 15655, **a.** Holotype, 49 mm SL, male; **b.** FFR 15657, paratype, 45 mm SL, female; Türkiye, Balıkesir prov., Havran Stream. *Oxynoemacheilus marmaraensis*, FFR 1511; **c.** 52 mm SL, males; **d.** 46 mm SL, female; Türkiye, Balıkesir prov., Susurluk River.

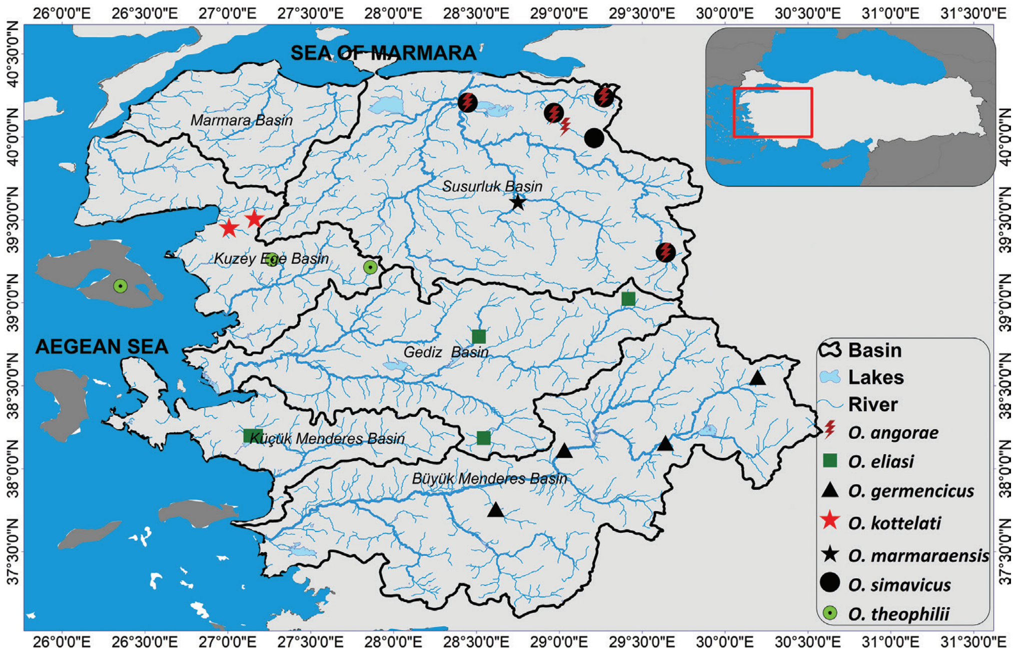

Figure 4. Distribution of *Oxynoemacheilus* species in Aegean and Marmara Sea basins of Anatolia.

tance 20–28% SL; and caudal-fin length 23–29% SL. Based on morphometric data, it is difficult to distinguish these three species (*O. germencicus*, *O. eliasi*, and *O. theophilii*) from each other. This might be due to the high morphological variation within *O. germencicus*, or it may include the possibility of one or more species in the Büyük Menderes River.

Comparative materials

Oxynoemacheilus marmaraensis, FFR 1511, 12, 46–59 mm SL; Türkiye, Balıkesir Province, stream Dursunbey 10 km east of Dursunbey, D. Turan, G. Kalaycı, and S. Aksu, 22.11.2022, 39°36'32.4"N, 28°45'01.9"E.

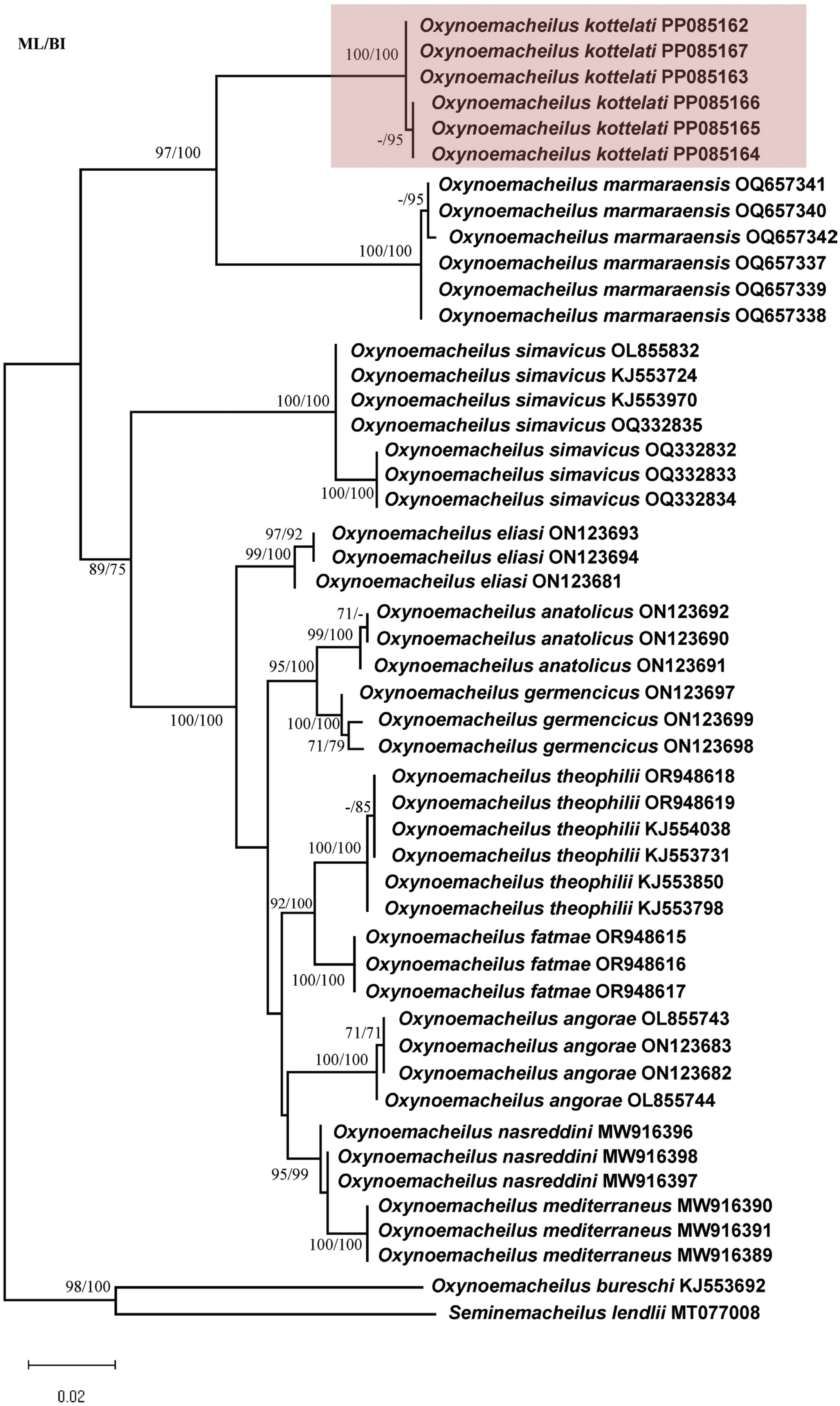

Figure 5. Maximum likelihood tree based on mitochondrial cytochrome oxidase subunit I (*COI*; 624 bp) gene sequences of *Oxynoemacheilus* spp. Bootstrap values (ML/BI) are shown above or below nodes on the tree if they are 70% or higher.

Oxynoemacheilus angorae, FFR01549, 50, 20–59 mm SL; Türkiye, Ankara prov., stream Peçenek, 7 km east of Şereflikoçhisar, D. Turan, C. Kaya, and E. Bayçelebi, 01.10.2015, 40°28'15.6"N, 32°39'18.0"E.

Oxynoemacheilus simavicus, FFR01505, 28, 32–56 mm SL; Türkiye, Balıkesir prov, stream Sakar at Manyas, 29.08.2014, D. Turan, C. Kaya, and E. Bayçelebi, 40°03'00.0"N, 27°57'43.2"E.

Oxynoemacheilus theophilii, FFR 15538, 5, 27–36 mm SL; Türkiye, İzmir prov., Çağlayan Stream, a tributary of Bakırçay River 15 km east of Bergama, D. Turan, C. Kaya, and E. Bayçelebi, 16.07.2018, 39°27'12.2"N, 27°00'30.9"E.

Oxynoemacheilus eliasi, FFR 15658, 7, 38–41 mm SL; Türkiye, İzmir prov., inlet of Tahtalı reservoir, under Şaşal bridge, D. Turan, C. Kaya, and E. Bayçelebi, 16.07.2018, 38°11'57.56"N, 27°08'09.79"E.—IFC-ESUF 19-0015, 14, 43-66 mm SL; Türkiye, Manisa prov., Derbent Stream, Gediz River, Uluderbent village bridge, Alaşehir, S.S. Güçlü, and G.K. Akyıldız, 28.04.2017, 38°11'3.43"N, 28°32'37.65"E.—IFC-ESUF 19-0016, 91, 38–62 mm SL; Türkiye, Manisa prov., Derbent Stream, Gediz River, Uluderbent village bridge, Alaşehir, S.S. Güçlü, and G.K. Akyıldız, 28.04.2017, 38°11'3.43"N, 28°32'37.65"E.—IFC-ESUF 19-0021, 7, 42–53 mm SL; Türkiye; Manisa prov., Demirci Stream, Gediz River, Saraycık village Demirci, F. Küçük, S.S. Güçlü, and G.K. Akyıldız, 01.11.2016, 38°47'48.36"N, 28°30'52.48"E.—IFC-ESUF 19-0022, 15, 37–74 mm SL; Türkiye, Manisa prov., Gediz River, Derbent bridge, Hacıbaba village, F. Küçük, and S.S. Güçlü, 22.06.2012, 39°01'23.50"N, 29°25'02.23"E.—IFC-ESUF 19-0011, 5, 41–58 mm SL; Türkiye, Kütahya prov., Gediz River, Bahçeler Creek, Dörtdeğirmen village bridge, Gediz, F. Küçük, S.S. Güçlü, and G.K. Akyıldız, 31.10.2016, 38°58'36.14"N, 29°23'43.66"E.—IFC-ESUF 19-0012, 3, 44–48 mm SL; Türkiye, Manisa prov., Gediz River, Hacıhalliler village, F. Küçük, S.S. Güçlü, and G.K. Akyıldız, 03.11.2016, 38°38'23.92"N, 27°32'37.74"E. —IFC-ESUF 19-0014, 5, 37–55 mm SL; Türkiye, Manisa prov., Gördük Stream, Gediz River, Zeytinbağı village, Akhisar, F. Küçük, S.S. Güçlü, and G.K. Akyıldız, 02.11.2016, 39°2'55.19"N, 27°55'39.27"E.—IFC-ESUF 19-0017, 2, 46–61 mm SL; Türkiye, Kütahya prov., Gediz River, Bahceler Creek, Dörtdeğirmen village bridge, Gediz, S.S. Güçlü, and G.K. Akyıldız, 27.04.2017, 38°58'36.14"N, 29°23'43.66"E.—IFC-ESUF 19-0018, 3, 35–37 mm SL; Türkiye, Manisa prov., Gördük Stream, Gediz River, Zeytinbağı village, Akhisar, S.S. Güçlü, and G.K. Akyıldız, 27.04.2017, 39°2'55.19"N, 27°55'39.27"E.—IFC-ESUF 19-0023, 1, 54 mm SL; Türkiye; Manisa prov., Akpınar Spring, Gölmarmara Lake, Gölmarmara, F. Küçük, and S.S. Güçlü, 21.06.2012, 38°42'04.40"N, 27°58'07.97"E.—IFC-ESUF 19-0024, 1, 43 mm SL; Türkiye, Kütahya prov., Gediz River, Gümüşlü DSI Regl., Gediz, F. Küçük, and S.S. Güçlü, 21.06.2012, 38°58'18.76"N, 29°28'01.56"E.—IFC-ESUF 19-0025, 9, 40–50 mm SL; Türkiye, Manisa prov., Gediz River, Yurtbaşı bridge, Kula, S.S. Güçlü, and H. Güçlü, 12.07.2010, 38°36'16.19"N, 28°48'54.68"E.—IFC-ESUF 19-0028, 1, 48 mm SL; Türkiye, Manisa prov., Demirci Stream, Gediz River, Saraycık village, Demirci, S.S. Güçlü, and G.K. Akyıldız, 25.04.2017, 38°47'48.36"N, 28°30'52.48"E.

Oxynoemacheilus germencicus, FFR 1523, 7, 52–58 mm SL; Türkiye, Denizli prov., Aksu Stream, Büyük Menderes River, 4 km north of Honaz, D. Turan, C. Kaya, and E. Bayçelebi, 19.08.2014, 37°47'21.55"N, 29°15'41.16"E.—FFR 1508, 22, 35–65 mm SL; Türkiye, Muğla prov., Çine Stream, Büyük Menderes River, a tributary of Adnan Menderes reservoir 8 km south of Çine, D. Turan, C. Kaya, and E. Bayçelebi, 25.08.2014, 37°32'34.15"N, 28°03'44.85"E.—FFR 1528, 12, 39–56 mm SL; Türkiye, Denizli prov., Suçıkan Stream, Büyük Menderes River, tributary of Lake Işıklı 1 km north of Çıtak, D. Turan, C. Kaya, and E. Bayçelebi, 18.08.2014, 38°09'20.15"N, 29°38'16.68"E.—FFR 1530, 61, 28–68 mm SL; Türkiye, Uşak prov., Banaz River, Büyük Menderes River, 8 km north of Sivaslı, D. Turan, C. Kaya, and E. Bayçelebi, 18.08.2014, 38°32'58.72"N, 29°37'12.98"E.—FFR 1597, 10, 47–63 mm SL; Türkiye, Aydın prov., Karacasu Stream, Büyük Menderes River, D. Turan, C. Kaya, and E. Bayçelebi, 18.08.2014, 37°48'22.96"N, 28°34'49.47"E—IFC-ESUF 19-0006, 10, 27–60 mm SL; Türkiye, Denizli prov., Cindere reservoir, Büyük Menderes River, Güney, F. Küçük, and S.S. Güçlü, 15.05.2017, 38°06'45.47"N, 29°01'47.65"E.—IFC-ESUF 19-0007, 14, 33–56 mm SL; Türkiye, Denizli prov., Büyük Menderes River, Çıtak Bridge, Çivril, O. Çetinkaya, 30.10.2017, 38°09'23.69"N, 29°38'24.29"E.—IFC-ESUF 19-0009, 9, 54–64 mm SL; Türkiye, Afyonkarahisar prov., Karadirek Stream, Büyük Menderes River, Karadirek, F. Küçük, and S.S.Güçlü, 29.10.2017, 38°33'08.29"N, 30°11'45.52"E.—IFC-ESUF 19-0026, 11, 52–61 mm SL; Türkiye, Aydın prov., Dandalas Stream, Büyük Menderes River, Karacasu, S.S. Güçlü, and H. Güçlü 14.07.2010, 37°45'26.00"N, 28°36'58.53"E.—IFC-ESUF 19-0010, 7, 36–67 mm SL; Türkiye, Denizli prov., Işıklı Lake canal, Büyük Menderes River, Çivril, O. Çetinkaya, 31.08.2017, 38°16'22.89"N, 29°54'23.64"E.—IFC-ESUF 19-0019, 6, 51–65 mm SL; Türkiye, Aydın prov., Şirindere Stream, Büyük Menderes River, İncirliova, F. Küçük, and S.S. Güçlü, 22.07.2019, 37°55'41.87"N, 27°46'39.37"E.—IFC-ESUF 19-0020, 7, 46–61 mm SL; Türkiye, Uşak prov., Banaz Stream, Büyük Menderes River, Ulubey, F. Küçük, and S.S. Güçlü, 27.07.2019, 38°31'48.46"N, 29°36'43.56"E.—IFC-ESUF 19-0027, 4, 38–40 mm SL; Türkiye, Denizli prov., Büyük Menderes River, Yenicekent DSI Regl., Sarayköy, F. Küçük, 04.06.1998, 38°02'15.45"N, 28°57'47.50"E.

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References

Bektaş Y, Aksu I, Kaya C, Bayçelebi E, Turan D (2022) DNA barcoding and species delimitation of the genus *Oxynoemacheilus* (Teleostei, Nemacheilidae) in Anatolia. Journal of Fish Biology 101(3): 505–514. https://doi.org/10.1111/jfb.15114

Çiçek E, Sungur S, Fricke R, Seçer B (2023) Freshwater lampreys and fishes of Türkiye; an annotated checklist. Turkish Journal of Zoology 47(6): 324–468. https://doi.org/10.55730/1300-0179.3147

Erk'akan F (2012) Two new *Oxynoemacheilus* (Teleostei, Nemacheilidae) species from western Turkey. Research Journal of Biological Sciences 7(2): 97–101. https://doi.org/10.3923/rjbsci.2012.97.101

Erk'akan F, Nalbant TT, Özeren SC (2007) Seven new species of *Barbatula*, three new species of *Schistura* and a new species of *Seminemacheilus* (Ostariophysi, Balitoridae, Nemacheilinae) from Turkey. Journal of Fisheries International 2: 69–85.

Freyhof J (2016) *Oxynoemacheilus karunensis*, a new species from the Persian Gulf basin Teleostei. Nemacheilidae. Zootaxa 4175(1): 94–100. https://doi.org/10.11646/zootaxa.4175.1.9

Freyhof J, Abdullah YS (2017) Two new species of *Oxynoemacheilus* from the Tigris drainage in Iraqi Kurdistan (Teleostei, Nemacheilidae). Zootaxa 4238(1): 73–87. https://doi.org/10.11646/zootaxa.4238.1.5

Freyhof J, Özuluğ M (2017) *Oxynoemacheilus hazarensis*, a new species of from Lake Hazar in Turkey with remarks on *O. euphraticus* (Teleostei, Nemacheilidae). Zootaxa 4247(4): 378–390. https://doi.org/10.11646/zootaxa.4247.4.2

Freyhof J, Erk'akan F, Özeren C, Perdices AJ (2011) An overview of the western Palaearctic loach genus *Oxynoemacheilus* (Teleostei, Nemacheilidae). Ichthyological Exploration of Freshwaters 22: 301–312.

Freyhof J, Kaya C, Turan D (2017) *Oxynoemacheilus kentritesensis*, a new species from the upper Tigris drainage in Turkey with remarks on the distribution of *O. frenatus* (Teleostei, Nemacheilidae). Zootaxa 4258(6): 551–560. https://doi.org/10.11646/zootaxa.4258.6.4

Fujisawa T, Barraclough TG (2013) Delimiting species using single-locus data and the generalized mixed yule coalescent approach: A revised method and evaluation on simulated data sets. Systematic Biology 62: 707–724. https://doi.org/10.1093/sysbio/syt033

Geiger MF, Herder F, Monaghan MT, Almada V, Barbieri R, Bariche M, Berrebi P, Bohlen J, Casal-Lopez M, Delmastro GB, Denys GPJJ, Dettai A, Doadrio I, Kalogianni E, Kärst H, Kottelat M, Kovačić M, Laporte M, Lorenzoni M, Marčić Z, Özuluğ M, Perdices A, Perea S, Persat H, Porcelotti S, Puzzi C, Robalo J, Šanda R, Schneider M, Šlechtová 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. https://doi.org/10.1111/1755-0998.12257

Hall TA (1999) BioEdit a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Kamangar BB, Prokofiev AM, Ghaderi E, Nalbant TT (2014) Stone loaches of Choman River system, Kurdistan, Iran (Teleostei, Cypriniformes, Nemacheilidae). Zootaxa 3755(1): 33–61. https://doi.org/10.11646/4364

Kottelat M (2012) Conspectus cobitidum: An inventory of the loaches of the world (Teleostei, Cypriniformes, Cobitoidei). The Raffles Bulletin of Zoology 26(Supplement): 1–199.

Kottelat M, Freyhof J (2007) Handbook of European Freshwater Fishes: Kottelat, Cornol and Freyhof, Berlin, [xiv +] 646 pp.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Posada D (2008) jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution 25(7): 1253–1256. https://doi.org/10.1093/molbev/msn083

Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology 21(8): 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

Puillandre N, Brouillet S, Achaz G (2021) ASAP: Assemble species by automatic partitioning. Molecular Ecology Resources 21(2): 609–620. https://doi.org/10.1111/1755-0998.13281

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Sayyadzadeh G, Eagderi S, Esmaeili HR (2016) A new loach of the genus *Oxynoemacheilus* from the Tigris River drainage and its phylogenetic relationships among the nemacheilid fishes (Teleostei, Nemacheilidae) in the Middle East based on mtDNA COI sequences. Iranian Journal of Ichthyology 3: 236–250.

Stoumboudi MT, Kottelat M, Barbieri R (2006) The fishes of the inland waters of Lesbos Island, Greece. Ichthyological Exploration of Freshwaters 17(2): 129–146.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10(3): 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Thompson JD, Higgins DG, Gibson TJ (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(22): 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Turan D, Kaya C, Kalaycı G, Baycelebi E, Aksu İ (2019) *Oxynoemacheilus cemali*, a new species of stone loach (Teleostei, Nemacheilidae) from the Çoruh River drainage. Journal of Fish Biology 94(3): 458–468. https://doi.org/10.1111/jfb.13909

Turan D, Aksu S, Kalaycı G (2023a) Two new *Oxynoemacheilus* species in western Anatolia (Teleostei, Nemacheilidae). Zoosystematics and Evolution 99(2): 439–455. https://doi.org/10.3897/zse.99.102575

Turan D, Bayçelebi E, Kalaycı G (2023b) *Oxynoemacheilus marmaraensis*, a new species from the Susurluk River, Türkiye (Teleostei, Nemacheilidae). Journal of Fish Biology 103(5): 1106–1112. https://doi.org/10.1111/jfb.15506

Turan D, Aksu S, Güçlü SS, Kalaycı G (2024) [in press] *Oxynoemacheilus fatmae*, a new species from the Güzelhisar Stream in Aegean Sea basin, Türkiye (Telostei: Nemacheilidae). Journal of Fish Biology. https://doi.org/10.1111/jfb.15779

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences 360(1462): 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Yoğurtçuoğlu B, Kaya C, Freyhof J (2022) Revision of the *Oxynoemacheilus angorae* group with the description of two new species (Teleostei, Nemacheilidae). Zootaxa 5133(4): 451–485. https://doi.org/10.11646/zootaxa.5133.4.1