



# Species of *Oxynoemacheilus* Bănărescu & Nalbant, 1966 (Actinopterygii: Nemacheilidae) in the Turkish Part of the Kura-Aras River System, with the First Detailed Evidence for the Occurrence of *O. bergianus* (Derjavin, 1934) and *O. cf. elsae* Eagderi et al., 2018

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**Abstract:** The study provides the first detailed evidence for the occurrence of *Oxynoemacheilus cf. elsae* Eagderi, Jalili & Cicek, 2018 and *O. bergianus* (Derjavin, 1934) in the Turkish parts of the Kura-Aras River system by reporting molecular (COI barcoding) and morphometric data. The taxonomic status of species of the genus *Oxynoemacheilus* Bănărescu & Nalbant, 1966 in the Turkish part of the Kura-Aras River is discussed. *Oxynoemacheilus cyri* (L. S. Berg, 1910) and *O. brandtii* (Kessler, 1877) are considered as restricted to the upper part of the Kura River drainages and endemic to Turkey. In contrast, *O. bergianus* and *O. cf. elsae* are reported for the first time from Turkish part of the Aras River, co-inhabiting with the widespread in the Aras River loach *O. veyselorum* Cicek, Eagderi & Sungur, 2018.

**Key words:** loach, taxonomy, phylogeography, freshwater

## Introduction

*Oxynoemacheilus* Bănărescu & Nalbant, 1966 is a genus of the family Nemacheilidae occurring in freshwaters from Albania to Cis-Caucasus, via Trans-Caucasus and Turkey to southern Iran and Mesopotamia (PROKOFIEV 2009). This genus consists of 59 nominal species (VAN DER LAAN 2020), 43 of which are found in Turkish freshwaters, with 26 being endemic (CICEK et al. 2015, 2016, 2018a). The taxonomic status of the members of this genus, similarly to other nemacheilids, needs further studies (PROKOFIEV 2009, 2010, POTTTELAT 2012, SAYY-

ADZADEH et al. 2016, 2017), particularly in the Middle East, mostly due to the high number of poorly described species from this area (FREYHOF et al. 2011, SAYYADZADEH et al. 2016). This is also valid for the species occurring in the Turkish part of the Kura-Aras River drainage. The Kura-Aras River system is mostly inhabited by species characteristic for the Caspian Sea Basin; it is inhabited by a number of endemic species, including several species of loaches of *Oxynoemacheilus*.

The taxonomic status of the members of this genus in the Kura-Aras River system was recently studied and five species, i.e. *O. bergi* (Gratzianov,

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1907), *O. bergianus* (Derjavin, 1934), *O. brandtii* (Kessler, 1877), *O. cyri* (Berg, 1910) and *O. veyselorum* Cicek, Eagderi & Sungur, 2018, were confirmed (CICEK et al. 2018b). However, the occurrence of *O. bergianus* in the Turkish part of the Kura-Aras River drainage has not been documented and needs to be confirmed. Hence, the present study aimed to document the occurrence of *O. cf. elsae* (described from Urmia lake basin, Iran) and *O. bergianus* by providing molecular (COI barcodes) and morphometric data of them. We review the taxonomic status of the species of the genus *Oxynoemacheilus* in the Turkish part of the Kura-Aras River based on our recent field studies.

## Materials and Methods

Sampling was carried out during 2014 and 2019 in the Turkish part of the Kura-Aras River system using a backpack electrofishing device (SAMUS 1000). Specimens were fixed in 5 % formaldehyde and stored in 70 % or directly fixed in 96 % ethanol. All measurements were made point-to-point with digital callipers to the nearest of 0.1 mm. Methods for counts and measurements followed KOTTELAT & FREYHOF (2007). The percentage ratios of morphometric characters in relation to their standard length (SL) and head length (HL) were calculated.

The following specimens were examined:

*Oxynoemacheilus araxensis*: NHVUIC 17006, 4, 52.9-61.4 mm SL, Turkey: Erzurum Prov.: Karasu River at Kandili, Euphrates-Tigris River system, 39°55'01"N 40°40'21"E. 1 August 2017, E. Cicek.

*Oxynoemacheilus cf. elsae*: NUIC 1507, 9, 25.2-58.2 mm SL; Turkey: Erzurum Prov.: Karasu Stream, Euphrates Basin, 39°42'20.5"N 41°51'40.0"E; E. Cicek and S. Sungur, 08 May 2015; NUIC 1711, 17, 39.6-71.9 mm SL, Turkey: Erzurum Prov.: Övenler Stream at Pasinler, Aras Basin, 39°58'38.5"N 41°38'28.8"E; E. Cicek, S. Sungur and B. Seçer, 27 August 2017; NUIC 1508, 42, 41.6-64.9 mm SL, Turkey: Kars Prov.: Derinöz Stream, Aras Basin, 40°22'37.2"N 43°24'44.2"E E. Cicek and S. Sungur, 01 May 2015; NUIC 1509, 2, 62.6-73.2 mm SL, Turkey: Kars prov.: Aras River, Aras Basin, 39°58'38.5"N 41°38'28.8"E; E. Cicek and S. Sungur, 08 May 2015.

*Oxynoemacheilus bergianus*: NUIC 1510, 8, 22.2-61.8 mm SL; Turkey: Ağrı Prov.: Sarısu Stream, Aras Basin, 39°26'58.5"N 44°23'16.0"E; E. Cicek and S. Sungur.

*Oxynoemacheilus brandtii*: NHVUIC 17004, 5, 46.2-66.4 mm SL, Turkey: Ardahan Prov.: Kura River, 40°50'32"N 42°48'57.2"E & 41°06'06.1"N

042°39'38.0"E, 2 August 2017.

*Oxynoemacheilus cyri*: NHVUIC 17005, 15, 40.5-73.7 mm SL, Turkey: Ardahan Prov.: Kura River, 40°50'32"N 42°48'57.2"E. 1 August 2017.

*Oxynoemacheilus veyselorum*: NUIC 1712, 28, 42.7-72.2 mm SL; Turkey: Erzurum Prov.: Övenler Stream at Pasinler, Aras Basin, 39°58'38.5"N 41°38'28.8"E; E. Cicek, S. Sungur and B. Seçer, 27 August 2017; NUIC 1511, 36, 33.5-67.9 mm SL, Turkey: Kars Prov.: Derinöz Stream, Aras Basin, 40°22'37.2"N 43°24'44.2"E E. Cicek and S. Sungur, 01 May 2015.

DNA was extracted from the collected pectoral-fin tissues using a modified phenol-chloroform method (SAMBROOK et al. 1989). The COI gene was amplified using primers FishF1-(5'-TCAACCAAC-CACAAAGACATTGGCAC-3') and FishR1-(5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') (WARD et al. 2005). Polymerase chain reaction (PCR) conditions were as follows: a 50 µl final reaction volume containing 25 µl of Taq 2X Mastermix red, 1 µl (10 µM) of each primer, 5 µl of total DNA and 18 µl of H<sub>2</sub>O. Amplification cycles were as follows: denaturation for 10 min at 94°C; 30 cycles at 94°C for 1 min, 58.5°C for 1 min, 72°C for 1 min and a final extension for 5 min at 72°C. PCR products were purified using a purification kit (Bioneer Inc, Daejeon, Korea). The PCR products were sequenced using the Sanger method by a robotic ABI-3130xl sequencer (Macrogen Inc, Daejeon, Korea). The forward and reverse primers were used to single strand sequencing.

The sequences were compared to published *Oxynoemacheilus* sequences using BLASTn basic local alignment search tool (SALTSCHUL et al. 1990). The retrieved sequences of the other members of the genus *Oxynoemacheilus* from GenBank database (NCBI) following blast search are shown in Table 1. For phylogenetic reconstructions, the datasets were analysed with the Bayesian Inference (BI) using MrBayes 3.1.2 (RONQUIST et al. 2011) and maximum likelihood (ML) method in IQTREE 1.6.0 (HOANG 2017). We determined the best-fit model of molecular evolution for the given data and reconstructed the mitochondrial relationships between the studied taxa using the Bayesian information criterion scores (BIC) in IQTREE 1.6.0 (KALYAANAMOORTHY et al. 2017). MrBayes was run with six substitution types (nst=6) and considered gamma-distributed rate variation across sites and a proportion of invariable sites (GTR) for the COI datasets. For BI, we ran four simultaneous Monte Carlo Markov Chains for 10,000,000 generations, sample frequency every 1000 generations, chain temperature 0.2. Log-likeli-

**Table 1.** List of species of the genera *Oxy-noemacheilus* and *Paracobitis* used for molecular analysis and their GenBank accession number.

Species	Locality	Accession number	Reference
<i>O. bergianus</i>	Sefid River, Caspian Sea, Iran	MK546451	GEIGER (2019)
	Murat River, Euphrates Basin, Turkey	MK546450	GEIGER (2019)
	Şeryan River, Turkey	MK546449	GEIGER (2019)
	Adiyaman: Euphrates Basin, Turkey	MK546448	GEIGER (2019)
	Adiyaman: Euphrates Basin, Turkey	MK546447	GEIGER (2019)
	Qare Sou, Caspian Sea, Iran	MK546446	GEIGER (2019)
	Iran	MK546445	GEIGER (2019)
	Kordan River, Namak Lake Basin, Iran	KX833723	SAYYADZADEH et al. (2016)
	Taleghan River, Caspian Sea, Iran	KX833722	SAYYADZADEH et al. (2016)
	Qare Sou River: Erdebil, Iran	KX833721	SAYYADZADEH et al. (2016)
	Talkhehrud River, Iran	KX833720	SAYYADZADEH et al. (2016)
	Talkhehrud River, Iran	KX833719	SAYYADZADEH et al. (2016)
	Sefid River, Caspian Sea, Iran	KX833718	SAYYADZADEH et al. (2016)
	Sefid River, Caspian Sea, Iran	KX833717	SAYYADZADEH et al. (2016)
	Qare Sou River, Caspian Sea, Iran	KX833716	SAYYADZADEH et al. (2016)
	Qare Sou River, Caspian Sea, Iran	KX833715	SAYYADZADEH et al. (2016)
	Sefid River, Caspian Sea, Iran	KX946211	JOULADEH-ROUDBAR et al. (2016)
	Sefid River, Caspian Sea, Iran	KX946210	JOULADEH-ROUDBAR et al. (2016)
Sefid River, Caspian Sea, Iran	KX946209	JOULADEH-ROUDBAR et al. (2016)	
<i>O. brandtii</i>	Kura River, Turkey	KU928281	FREYHOF et al. (2016)
<i>O. cemali</i>	Coruh River, Turkey	MH018880	TURAN et al. (2018)
	Coruh River, Turkey	MH018879	TURAN et al. (2018)
<i>O. cyri</i>	Kura River, Turkey	KJ723513	FREYHOF et al. (2014)
	Kura River, Turkey	KJ723498	FREYHOF et al. (2014)
<i>O. araxensis</i>	Euphrates Basin, Turkey	MH469258	TURAN et al. (2018)
	Euphrates Basin, Turkey	MH469257	TURAN et al. (2018)
<i>O. angorae</i>	Ilgın River, Turkey	KJ553824	GEIGER et al. (2014)
<i>P. malapterura</i>	Kordan River, Namak Lake Basin, Iran	KJ723500	FREYHOF et al. (2014)

hood stability was attained after 10,000 generations and we excluded the first 1000 trees as burn-in. The remaining trees were used to compute a 50% majority-rule consensus tree. For ML analyses, we conducted heuristic searches (1000 runs) according to TN+F+G4 model. The genetic distances were investigated based on the Kimura two-parameter (K2P) distances (KUMAR et al. 2008). As outgroups, *Cobitis saniae* and *Misgurnus fossilis* (accession numbers: KP050509 and KM286765, respectively) were retrieved from GenBank.

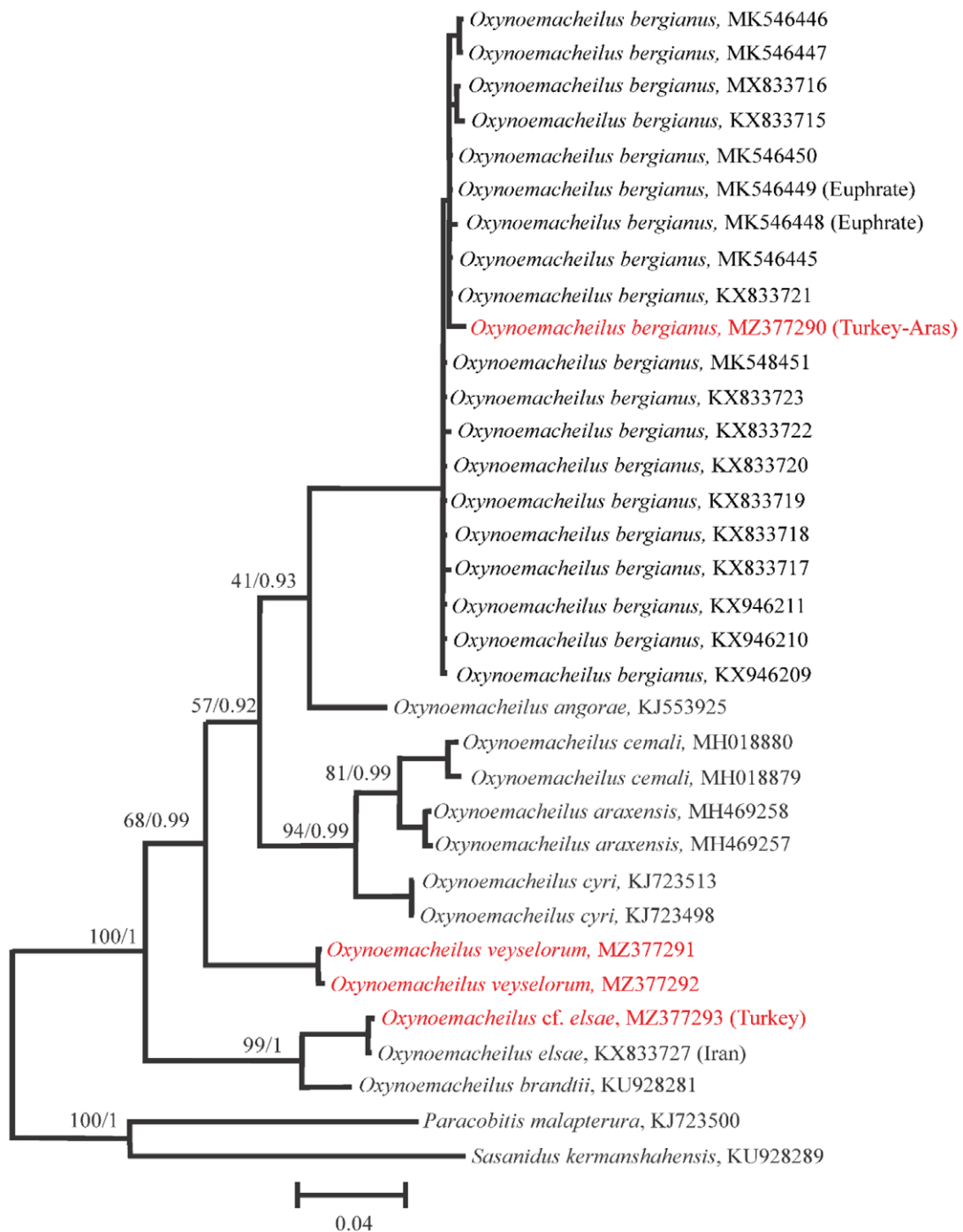
## Results

During the field studies at the Turkish part of the Kura-Aras River system, five species of the genus *Oxy-noemacheilus* were collected. These were *O. brandtii*

and *O. cyri* from the Kura River and *O. bergianus*, *O. cf. elsae* and *O. veyselorum* from the Aras River (Fig. 1). The specimens of *O. bergianus* were collected from Sarısu Stream, Ağrı Province (39°26'52.85"N 44°23'25.97"E) (Fig. 1) and Karasu Stream, Erzurum Province (39°38'03.28"N 41°56'11.45"E). Individuals of *O. cf. elsae* were also sampled. They originated from Karasu Stream, Erzurum Province (39°42'20.5"N 41°51'40.0"E); Övenler Stream at Pasinler, Erzurum Province (39°58'38.5"N 41°38'28.8"E); Derinöz Stream, Kars Province (40°22'37.2"N 43°24'44.2"E); Aras River, Erzurum Province (39°50'16.0"N 41°50'18.2"E) and Aras River, Kars Province (40°10'03.3"N 43°11'37.8"E). For the morphological characteristics of *O. bergianus* from Sarısu Stream and *O. cf. elsae* from the Karasu Stream, see Table 1.



Fig. 1. Lateral view of (from top to down) *Oxynoemacheilus bergianus* (Sarısu Stream, Aras River), *Oxynoemacheilus cyri* (Balcesmeh Stream, Kura River), *O. brandtii* (Derinöz Stream, Kura River), *O. bergi* (Balcesmeh Stream, Aras River) and *O. veyselorum* (Bozkuş River, Aras River).



**Fig. 2.** Bayesian estimation of the phylogenetic relationships based on the mitochondrial COI barcode region. Values at nodes correspond to ML bootstrap / BI posterior probability.

We generated four COI barcodes, including one for *O. cf. elsae*, one for *O. bergianus* and two for *O. veyselorum*. Additional 30 sequences of nine species were downloaded from Genbank (Table 1). Two phylogenetic approaches, Bayesian Inference (BI) and Maximum Likelihood (ML), gave the same tree topologies and thus one is presented (Fig. 2). Table 3 lists estimates of the average evolutionary divergence found in the mtDNA COI

barcode region of the analysed species. According to the results, *O. bergianus* from the Sarisu Stream (Aras River basin) was nested within species of *O. bergianus* and *O. cf. elsae* from Digor River (Aras River drainage) placed in the same clade with that of *O. elsae* from Iran. *Oxynoemacheilus elsae* was sister species of *O. brandtii*, with a genetic distance 4.6 %. *Oxynoemacheilus angorae* is the closest species to *O. veyselorum*, with 9.2% genetic distance.

**Table 2.** Morphometric data of *Oxynoemacheilus bergianus* and *O. cf. elsae* from the Aras and Sefid Rivers, Caspian Sea Basins.

	<i>O. bergianus</i>		<i>O. cf. elsae</i>	
	Min-Max	mean±SD	Min-Max	mean±SD
<b>TL(mm)</b>	<b>47.7-76.0</b>	<b>58.6±8.7</b>	<b>45.40-64.67</b>	<b>54.18±5.48</b>
<b>In percent of Standard length</b>				
Body Depth Maximal	17.4-23.1	19.3±1.7	12.76-16.62	15.18±1.16
Caudal Peduncle Depth	7.1-8.5	7.7±0.5	6.45-7.97	7.21±0.41
Predorsal Length	48.9-52.5	50.4±1.8	47.66-52.97	51.15±1.35
Postdorsal Length	31.2-38.0	34.8±1.8	32.57-35.61	34.19±0.87
Prepelvic Length	48.5-54.3	52.5±1.8	51.62-55.56	53.42±1.21
Preanal Length	72.0-76.1	73.9±1.5	71.46-75.89	73.49±1.36
Caudal Peduncle Length	15.2-21.4	18.9±2.0	17.47-20.53	19.25±0.76
Dorsal-Fin Base Length	12.1-17.4	14.3±1.7	13.48-16.28	15.24±0.74
Dorsal-Fin Depth	15.1-22.2	20.5±2.1	20.10-24.35	21.69±1.10
Anal-Fin Base Length	6.4-10.1	8.2±1.4	6.36-9.17	7.96±0.97
Anal-Fin Depth	12.9-18.4	16.6±1.9	16.93-20.29	18.41±1.02
Pectoral Fin Length	20.6-25.5	23.4±1.7	21.76-24.98	22.82±0.84
Pelvic Fin Length	17-24.5	18.9±2.4	16.57-20.34	18.54±0.88
Pectoral-Pelvic-Fin Origin Distance	23.7-31.7	29.2±2.6	28.39-32.90	30.26±1.25
Pelvic-Anal-Fin Origin Distance	19.0-28.0	21.6±2.6	18.09-21.21	19.79±0.88
Caudal-Fin Length	22-25.6	23.0±1.1	19.85-25.11	22.71±1.63
Body Width	12.1-16.6	14.3±1.7	12.79-15.57	13.99±0.85
Caudal Peduncle Width	3.2-4.6	4.0±0.4	5.90-7.74	6.55±0.47
Head Length	22.0-24.0	23.1±0.8	21.66-24.60	23.26±0.84
<b>In percent of Head length</b>				
Snout Length	34.8-39.1	37.6±1.4	38.97-45.66	42.22±1.90
Eye Horizontal Diameter	15.9-20.7	18.5±1.8	14.30-18.33	16.20±0.98
Postorbital Distance	41.8-60.9	45.9±5.8	34.20-43.69	39.54±2.71
Head Depth at Nape	59.1-66.7	61.8±2.8	50.81-60.99	54.92±2.93
Head Width	53.3-70.2	60.4±5.6	53.41-68.67	58.82±3.87
Inter Orbital	26.5-31.9	28.7±2.0	24.06-28.73	26.80±1.27
Inter Nasal	20.4-25.8	22.9±1.9	19.44-25.17	21.80±1.72
Mouth Width	18.0-25.3	21.2±2.5	18.25-26.83	22.30±2.23
Inner Rostral Barbel	24.8-38.5	31.6±4.3	16.06-27.67	21.01±3.21
Outer Rostral Barbel	17.3-24.2	20.3±2.5	27.00-39.16	33.24±3.18
Maxillary Barbel	25.5-35.1	29.8±3.6	25.83-36.32	32.05±2.62

**Table 3.** K2P mean distances of some species genus *Oxy-noemacheilus*.

Species		1	2	3	4	5	6	7
<i>O. bergianus</i>	1							
<i>O. brandtii</i>	2	13.2						
<i>O. cemali</i>	3	13.3	13.9					
<i>O. cyri</i>	4	10.6	13.3	5.4				
<i>O. araxensis</i>	5	12.5	14.3	2.7	4.6			
<i>O. cf. elsae</i>	6	12.1	4.6	15.6	15.7	14.9		
<i>O. veyselorum</i>	7	12.6	13.6	12.0	10.7	11.3	14.4	
<i>O. angorae</i>	8	8.1	13.2	10.6	9.7	9.6	13.9	9.2

## Discussion

Several species and subspecies of *Oxy-noemacheilus* have been reported from the Kura-Aras River system. These are *O. angorae* (Steindachner, 1897), *O. araxensis* Banarescu & Nalbant, 1978, *N. brandti gibbusnazus* Elanidze, 1983, *N. angorae alasanicus* Elanidze, 1983 and *O. lenkoranensis* (Abdurakhamanov, 1962). However, the former two are restricted to the Western and Central Anatolian Black Sea Basin, Turkey, and the upper parts of the Karasu River, Euphrates-Tigris River system, while the later three are synonyms of *O. bergi* and *O. bergianus* (see

CICEK et al. 2018b). Therefore, only *O. bergi*, *O. bergianus*, *O. brandtii*, *O. cyri* and *O. veyselorum* occur in this river system (CICEK et al. 2018b). In addition, our results revealed the presence of *O. elsae* in the Kura-Aras River system that already described from Urmia lake basin of Iran (EAGDERI et al., 2018).

CICEK & SUNGUR-Birecikligil (2016) have reported *O. araxensis* from the Aras River Basin. However, its type locality is Karasu (Kandilli, Erzurum), Euphrates River Basin; therefore, it is listed by mistake for the fish fauna of the Kura-Aras River system. In addition, the type locality of *O. angorae* is in the Ankara Region and this species is distributed in the Kızılırmak and Sakarya Basins of Turkey. Recently, *O. phoxinoides* from the Iznik Lake (Susurluk Basin) has been assigned as junior synonym of *O. angorae* (see CICEK et al. 2019). In previous studies, *O. angorae* was reported incorrectly from the Kura-Aras River Basin (BERG 1949, KURU 1975, CICEK et al. 2015).

*Oxynoemacheilus bergianus* (Derjavin, 1934) has been described from the Sefid River (southern Caspian Sea Basin) and is found in the Namak Lake, Urmia Lake and probably the Dasht-e Kavir Basins of Iran (BANARESCU & NALBANT 1966, SAADATI 1977, HOLCIK & OLAH 1992, ABBASI et al. 1999, ESMAEILI et al. 2018). In addition, KAYA et al. (2016) have reported it from the Turkish part of the Tigris and Euphrates River drainages, respectively, but there are no reports of *O. bergianus* from the Kura-Aras River system in Turkey. Hence, the present study is the first report of *Oxynoemacheilus* from the Turkish part of the Kura-Aras River system providing evidence for its occurrence.

The presence of the genus *Oxynoemacheilus* from Albania to the central region of Iran may indicate its origin in the Persian Gulf Basin and subsequent expansion from southern Asia toward west (SAWADA 1982). Along with the distribution of this genus, the ancestor of *O. bergianus* probably moved toward the Aras-Kura River Drainage via the Taurus Mountains in the east of Turkey, where the drainages of the Tigris and Aras-Kura Rivers originate from its southern and northern slopes, respectively. The presence of *O. bergianus* in the Caspian Sea Basin (the Aras River) and the Urmia Lake Basins may show their connection in the past due to geological events as further migration path of this species toward the Sefid River in the Caspian Sea Basin.

*Oxynoemacheilus cyri* (Berg, 1910) has been described as a subspecies of *O. tigris* and, later on, has been elevated to the species level (FRICKE et al. 2007). This species is endemic to Turkey and restricted to some springs and tributaries of the upper part of the Kura River Drainage. Furthermore, *O. brandtii*

(Kessler, 1877) is also endemic for Turkey and only found in the upper parts of the Kura River Drainage but not sympatric with *O. cyri*. *Oxynoemacheilus brandtii* has been previously reported from the drainages of the Aras (BERG 1949, KURU 1975) and Kızılırmak rivers (FREYHOF et al. 2011). This species has not been found in the Kızılırmak River or other basins of Turkey. We have recorded *O. elsae* gene from the Aras River; it has been found with *O. veyselorum* in the Kars River. This *Oxynoemacheilus* species from the Aras drainage was clustered as sister to *O. elsae* was identified as *O. brandtii* by FREYHOF et al (2021) based on its morphological characters. But, since it was clustered with *O. elsae* based on molecular data, we keep it as *O. cf. elsae* until further studies. *Oxynoemacheilus veyselorum* has recently been described from the Baykuş River, a tributary of the Aras River; during the period of the present study, it has been registered as widespread in all parts of the Aras River in Turkey. We expect this species to be found in other countries overlapping the drainage system of the Aras River.

*Oxynoemacheilus araxensis* and *O. cemali* are sister species and form a monophyletic group with *O. cyri*, with k2p genetic distance of 4.6 and 5.4%, respectively. Previous studies (KRUPP 1983, HRBEK et al. 2004, VASILYAN et al. 2014, BEKTAS et al. 2017) reported that the palaeo-Coruh, Aras-Kura and Upper Euphrates Basins have been isolated from each other as a result of the Pliocene (5.3–2.6 Ma) uplift of the eastern Anatolia region, including their intersection points. Therefore, the separation times among present-day Coruh, Kura-Aras and Euphrates populations coincide with the Pliocene uplift that has isolated these basins in the region.

*Oxynoemacheilus bergi* (Gratzianov, 1907) has been described from the Akstafa River, a tributary of the Kura River, Azerbaijan. Later, this species was considered as a junior synonym of *O. angorae* (see COAD 1995, PROKOFIEV 2009, FREYHOF et al. 2012) until CICEK et al. (2018b) revalidated it on the basis of the absence of *O. angorae* in the Kura-Aras River and unpublished molecular data from the type locality of the latter species. However in the present study, this species did not find in the Turkish part of the Kura-Aras River System.

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