

TAXONOMIC STATUS OF THE GENUS *SABANEJEWIA* (COBITIDAE) FROM KURA-ARAS RIVER SYSTEM (TURKEY)

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ABSTRACT

Sabanejewia is a member of the family Cobitidae found in Europe and Asia. In previous studies, *S. balcanica*, *S. caucasica*, *S. aurata* and *S. caspia* were reported from Turkey. Of these *S. aurata* and *S. caspia* were reported from the Kura-Aras Basin. We examined morphological characters and molecular data of the *Sabanejewia* specimens from Turkish parts of the Kura-Aras river system to determine their taxonomic status. We found no differences between the Kura and Aras rivers' populations in terms of morphology, but different populations showed a genetic distance of 0.1-1.9%. All the studied populations were identified as *S. aurata*. In addition, we could not find any evidence for the presence of *S. caucasica* and *S. caspia* in Turkey.

ZUSAMMENFASSUNG: Taxonomischer Status der Gattung *Sabanejewia* (Cobitidae) aus dem Einzugsgebiet des Kura-Aras Flusses (Türkei).

Sabanejewia ist ein Vertreter der in Europa und Asien vorkommenden Cobitidae. In vorangegangenen Studien wurden *S. balcanica*, *S. caucasica*, *S. aurata* und *S. caspia* aus der Türkei gemeldet. Von diesen wurden *S. aurata* und *S. caspia* aus dem Entwässerungsgebiet des Kura-Aras-Flusses angegeben. Um ihren taxonomischen Status zu bestimmen wurden morphologische charakteristische Merkmale und molekulare Daten der *Sabanejewia*-Exemplare aus den türkischen Bereichen des Kura-Aras-Flusssystems untersucht. Dabei wurden in Bezug auf die Morphologie keine Unterschiede zwischen den Populationen der Flüsse Kura und Aras festgestellt, aber verschiedene Populationen zeigten einen hohen genetischen Abstand von 0,1-1,9%. Alle untersuchten Populationen wurden als *S. aurata* identifiziert. Außerdem konnten wir in der Türkei keine Hinweise auf das Vorkommen von *S. caucasica* und *S. caspia* finden.

REZUMAT: Statutul taxonomic al genului *Sabanejewia* (Cobitidae) din sistemul fluvial Kura-Aras (Turcia).

Sabanejewia este un membru al familiei Cobitidae prezent în Europa și Asia. În studiile anterioare, *S. balcanica*, *S. caucasica*, *S. aurata* și *S. caspia* au fost raportate în Turcia. Dintre acestea, *S. aurata* și *S. caspia* s-au raportat în bazinul sistemului Kura-Aras. Am examinat caracterele morfologice și datele moleculare ale specimenelor de *Sabanejewia* din zonele turcești ale sistemului fluvial Kura-Aras pentru a determina statutul lor taxonomic. Nu am găsit diferențe între populațiile râurilor Kura și Aras în ceea ce privește morfologia, dar populații diferite au arătat o distanță genetică ridicată de 0,1-1,9%. Toate populațiile studiate au fost identificate ca *S. aurata*. În plus, nu am putut găsi nicio dovadă pentru prezența speciilor *S. caucasica* și *S. caspia* în Turcia.

INTRODUCTION

Sabanejewia Vladykov, 1929 is an Eurasian genus of the Cobitidae family consisting of ten nominal species (Sayyadzadeh et al., 2018; van der Laan, 2019) with four species, *S. aurata*, *S. balcanica*, *S. caucasica*, and *S. caspia* reported from inland waters of Turkey (Kuru et al., 2014; Çiçek et al., 2015; Çiçek and Sungur-Birecikligil, 2016). *Sabanejewia balcanica* (Karaman, 1922), described from the Vardar River of Greece, is found in the Thrace region of Turkey. De Filippi (1863) described *S. aurata* from the Sefid River basin at the Sartschem (Sarcham) of Iran, and it is widespread in the Kura-Aras river system. *Sabanejewia caspia* (Eichwald, 1838) was described from the Caspian Sea at Lenkoran, Azerbaijan, and *S. caucasica* (Berg, 1906) listed as a member of Turkish freshwater ichthyofauna by Kuru et al. (2014) without providing any evidence of its distribution area. In this study, we present our findings regarding the *Sabanejewia* populations in the Turkish part of the Kura-Aras river system, where some authors have reported the presence of three species, *S. aurata*, *S. caspia*, and *S. caucasica*, by providing their morphometric characters and phylogeny using COI barcoding to gain a much better understanding of *Sabanejewia* species in the Turkish part of this complex river system.

MATERIAL AND METHODS

Sampling and morphological study

Samplings were done using a backpack electrofishing device (SAMUS 1000), and the specimens were fixed into buffered 5% formaldehyde or directly fixed in 96% ethanol for molecular works. Then those formaldehyde-fixed specimens were stored in 70% ethanol. All measurements are made point-to-point with digital callipers to the nearest 0.1 mm. Methods for counts and measurements follow Kottelat and Freyhof (2007).

DNA extraction and PCR

DNA was extracted from fin-clips of the collected specimens using a modified Phenol-chloroform method (Sambrook et al., 1989). The COI gene was amplified using primers FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-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, one µl (10 µm) of each primer, five µl of total DNA and 18 µl of H₂O. Amplification cycles were as follows: denaturation for 10 minutes at 94°C; 30 cycles at 94°C for one min., 58.5°C for one min., 72°C for one min., and a final extension for five 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 using the manufacturer's protocol (Macrogen, Inc, Daejeon, Korea). The forward and reverse primers were used for single-strand sequencing.

Molecular data analysis

We newly generated four DNA barcodes, and the sequences were compared to the published *Sabanejewia* sequences using a basic local alignment search tool (BLASTn) (Altschul et al., 1990). The retrieved sequences of the other members of the genus *Sabanejewia* from GenBank database (NCBI) following blast search are shown in table 1. For phylogenetic reconstructions, the datasets were analysed by Bayesian Inference (BI) using MrBayes 3.1.2 (Ronquist et al., 2011) and the maximum likelihood (ML) method in IQTREE 1.6.0 (Hoang et al., 2018). We determined the best-fit model

of molecular evolution for the given data and to reconstruct 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 1,000 generations, chain temperature 0.2. Log-likelihood stability was attained after 10,000 generations, and we excluded the first 1,000 trees as burn-in. The remaining trees were used to compute a 50% majority-rule consensus tree. For ML analyses, we conducted heuristic searches (1,000 runs) according to TPM2+F+G4 model. The genetic distances were investigated based on 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.

Table 1: List of species used for molecular analysis and their GenBank accession number.

No.	Species	Accession no.
1.	<i>Sabanejewia aurata</i>	MK377023
2.	<i>Sabanejewia aurata</i>	MK377024
3.	<i>Sabanejewia balcanica</i>	KJ55470
4.	<i>Sabanejewia balcanica</i>	KJ554693
5.	<i>Sabanejewia baltica</i>	KR477107
6.	<i>Sabanejewia baltica</i>	KM287070
7.	<i>Sabanejewia caspia</i>	MK377022
8.	<i>Sabanejewia caspia</i>	MK377021
9.	<i>Sabanejewia caspia</i>	MK377020
10.	<i>Sabanejewia larvata</i>	KJ554832
11.	<i>Sabanejewia larvata</i>	KJ554664
12.	<i>Cobitis saniae</i>	KP050509
13.	<i>Misgurnus fossilis</i>	KM286765

The Mantel test was used to test for correlation between COI sequence data from each population and their geographical coordinates. The Mantel test was performed in PAST.

Materials used for morphological study

Sabanejewia aurata, IMNRF-UT-1085, 6, 47.8-61.6 mm SL; Iran: Ardabil prov.: Balikhlochay River at Baligulu, tributary of Aras River drainage, Caspian Sea basin, 38°09'11"N 48°11'07"E. – NHVUIC 1707, 3, 63.0-63.4 mm SL; Turkey: Erzurum prov.: Övenler Stream at Pasinler, Caspian Sea basin, 39°58'38.5"N 41°38'28.8"E. – NHVUIC 1807, 2, 76.1-81.0 mm SL; Turkey: Ardahan prov.: Kura River at Altaş Village, Caspian Sea basin, 41°09'33.9"N 42°52'06.5"E. – NHVUIC 1808, 4, 59.9-66.6 mm SL; Turkey: Ardahan prov.: Kura River at Sugöze Village, Caspian Sea basin, 41°06'06.1"N 42°39'38.0"E.

Sabanejewia caspia: IMNRF-UT-1084, 2, 45.5-50.1 mm SL; Iran: Gilan prov.: Rasht city, Anzali Wetland, Caspian Sea basin, 37°29'13"N 49°21'2"E.

Materials used for molecular analyses

All from Turkey: *Sabanejewia aurata*: – NHVUIC 1707-2 fin, Turkey: Erzurum prov.: Övenler Stream at Pasinler, 39°58'38.5"N 41°38'28.8"E. – NHVUIC 1807-2 fin, Turkey: Ardahan prov.: Kura River at Altaş Village, Caspian Sea basin, 41°09'33.9"N 42°52'06.5"E. GeneBank Accession number (ON025669, ON025670, ON025671, ON025672).

RESULTS

The body shape of the collected *Sabanejewia* specimens from the Aras and Kura rivers are displayed in figure 1, and their morphometric and meristic data are provided in tables 2 and 3. The morphometric characters of the *Sabanejewia* specimens from these two rivers largely overlap except prepelvic length (46.3-49.0 (in Aras) vs. 49.2-51.4 (in Kura) %SL) and postorbital distance (42.5-46.9 (in Aras) vs. 48.2-52.0 (in Kura) %HL). In addition, there was no difference between the studied populations in terms of the colour pattern.

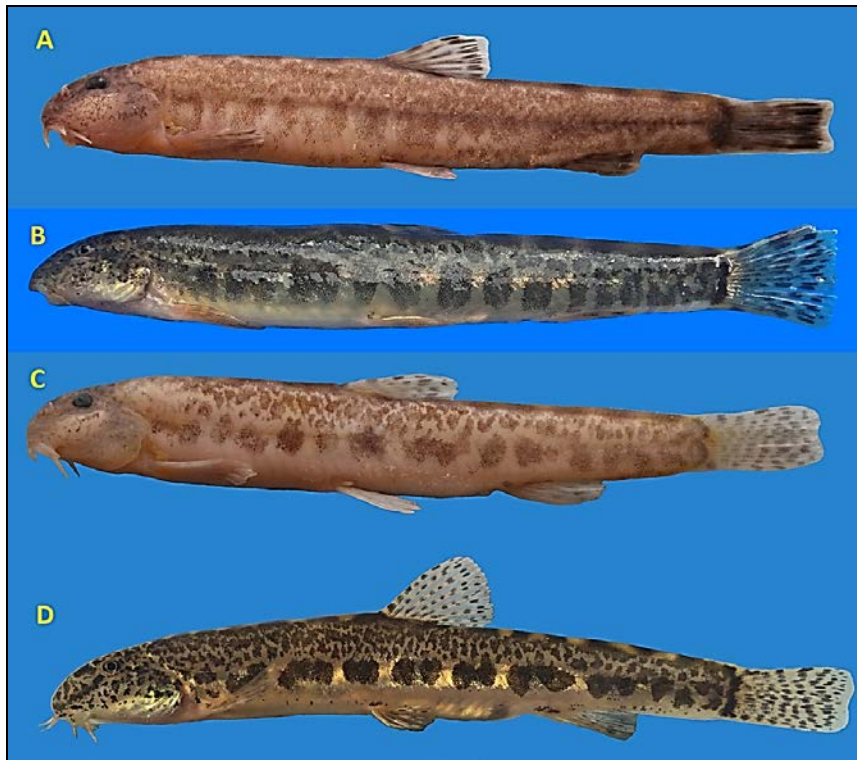


Figure 1: *Sabanejewia aurata* from the (A-B) Kura River, Turkey, (C) Aras River, Turkey, (D) Balikhlochay River, Iran.

Table 2: Morphometric data of the studied *Sabanejewia aurata* populations from the Kura and Aras rivers.

Morphometrics	Aras River (n = 3)		Kura River (n = 6)	
	Range	Mean±SD	Range	Mean±SD
Standard length (mm)	62.9-63.4	63.2±0.2	59.9-81.0	70.5±8.8
In percent of SL				
Head length	19.2-20.4	19.9 ± 0.6	19.1-21.1	20.1±0.7
Body depth at dorsal-fin origin	16.0-17.0	16.53 ±0.5	14.8-16.5	15.7±0.7
Body width at dorsal-fin origin	8.7-10.2	9.2±0.9	8.2-9.3	8.6±0.4
Predorsal length	47.2-48.9	47.95±0.8	48.5-50.7	49.6±0.8
Postdorsal length	40.3-43.4	42.2±1.7	39.1-42.8	41.5±1.3
Prepelvic length	46.3-49.0	46.8±1.0	49.2-51.4	50.1±0.8
Preanal length	71.3-73.6	71.5±0.1	72.3-76.4	74.9±1.3
Distance between pectoral and pelvic-fin origins	26.0-28.8	27.0±1.5	28.5-31.4	29.9±1.4
Distance between pelvic and anal-fin origins	22.6-24.3	23.7±0.9	23.7-26.7	25.2±1.0
Depth of caudal peduncle	10.4-10.7	10.59±0.1	9.1-10.8	9.5±0.5
Length of caudal peduncle	19.9-21.2	20.5±0.6	17.9-20.5	19.1±0.8
Dorsal-fin depth	16.7-16.9	16.8±0.1	16.2-17.3	16.7±0.5
Pectoral fin length	15.3-15.9	15.8±0.2	13.9-15.6	15.0±0.6
Pelvic fin length	12.6-13.7	13.1±0.6	11.6-13.9	12.9±0.8
In percent of HL				
Head depth at nape	65.9-67.9	66.9±1.0	62.1-73.7	69.0±4.6
Head depth at eye	47.1-49.3	48.4±1.1	48.6-58.8	52.5±3.9
Snout length	42.5-45.6	43.8±1.6	39.7-47.0	43.7±2.6
Eye diameter	16.9-17.8	17.4±0.4	14.4-18.9	15.8±1.6
Postorbital distance	42.5-46.9	45.0±2.3	48.2-52.0	50.0±1.6
Maximum head width	43.8-48.9	46.6±2.6	39.9-51.0	45.9±3.8
Interorbital width	19.1-21.1	20.0±1.0	14.5-19.5	17.3±1.3

Table 3: Meristic data of the studied *Sabanejewia aurata* populations from the Kura and Aras rivers.

	Branched dorsal-fin rays			
	N	6½	7½	mode
<i>Sabanejewia aurata</i> (Aras)	3	1	2	7
<i>Sabanejewia aurata</i> (Kura)	6	2	4	7
	Branched anal-fin rays			
	N	5½	6½	mode
<i>Sabanejewia aurata</i> (Aras)	3	3	–	5
<i>Sabanejewia aurata</i> (Kura)	6	6	–	5
	Pelvic-fin rays			
	N	6	7	mode
<i>Sabanejewia aurata</i> (Aras)	3	3	–	6
<i>Sabanejewia aurata</i> (Kura)	6	5	1	6
	Pectoral-fin rays			
	N	8	9	mode
<i>Sabanejewia aurata</i> (Aras)	3	1	2	9
<i>Sabanejewia aurata</i> (Kura)	6	2	4	9
	Caudal-fin rays			
	N	12	14	mode
<i>Sabanejewia aurata</i> (Aras)	3	3	–	12
<i>Sabanejewia aurata</i> (Kura)	6	6	–	12

According to the obtained results, *Sabanejewia* populations from the Aras and Kura rivers are nested in the same clade (Fig. 2).

The generated DNA barcode sequences of the *Sabanejewia* populations showed more than 98% similarity with the available sequences of *S. aurata*. In addition, a minimum K2P genetic distance between *S. aurata* from the Kura River and those from the Övenler Stream at Pasinler (a tributary of Aras River) and Balikhlochay River, entering to the Gharasu River, a tributary of the Aras River, were 1.3-1.5% and 1.6-1.9%, respectively. Furthermore, the distance between specimens from the Övenler stream and Balikhlochay River was 0.5-0.6%. The results revealed no correlation between the sequence data from each population and their geographical coordinates of the studied *S. aurata* ($P > 0.05$).

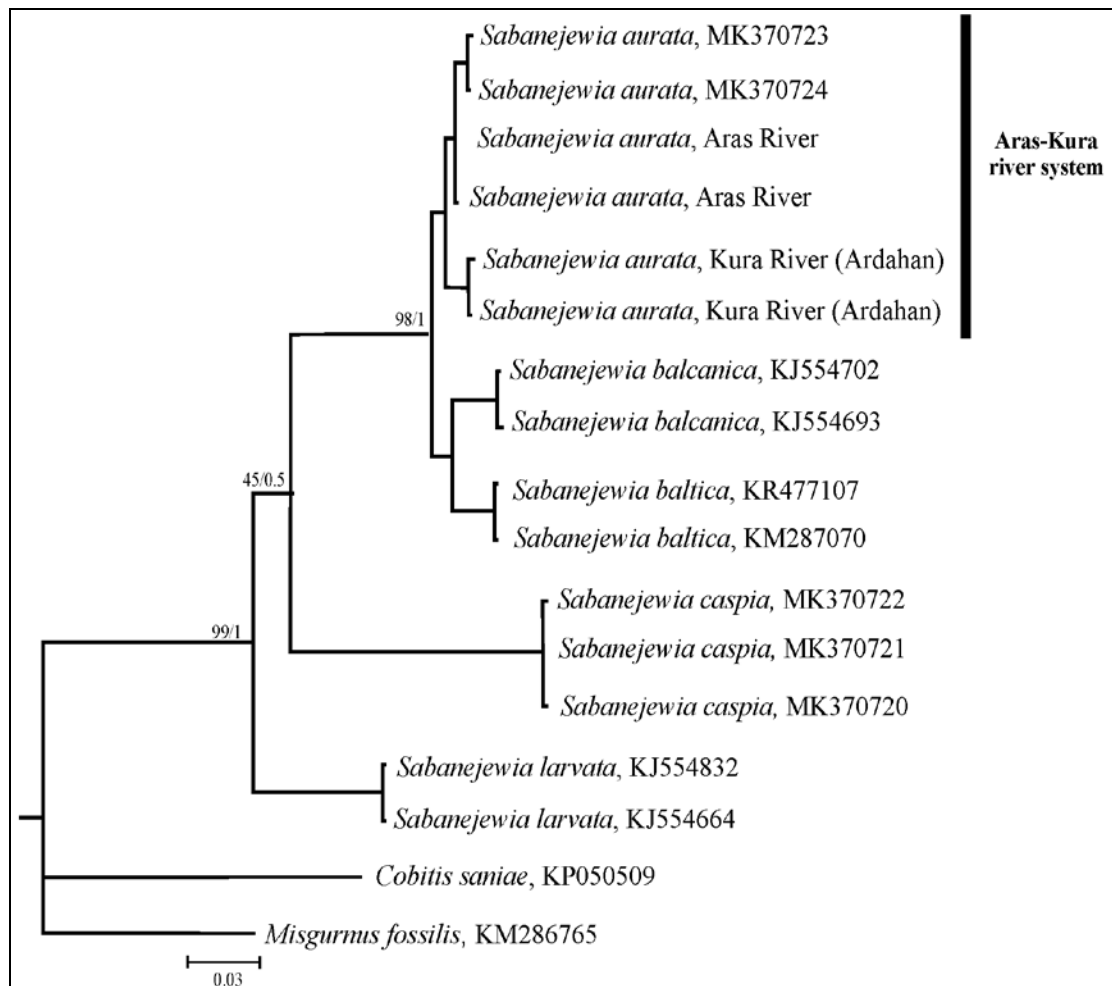


Figure 2: Maximum likelihood estimation of the phylogenetic relationships based on the mitochondrial COI barcode region.

Values at nodes correspond to ML bootstrap /BI posterior probability.

DISCUSSION

We found only *S. aurata* in the Aras and Kura rivers and their morphometric and meristic data were largely overlapping, except prepelvic length and postorbital distance. In a species with a widespread distribution area, we expect small morphological differences between populations found in different environmental conditions (Marcil et al., 2006; Poorbagher et al., 2017; Radkhah et al., 2017). In addition, the results revealed that *Sabanejewia* specimens of the Turkish parts of the Aras and Kura rivers are nested with other *S. aurata* DNA data available in the GenBank. The generated DNA sequences in the global database (GenBank) provide a trustworthy platform for speedy and correct species identification providing a better understanding of biodiversity (Shen et al., 2013).

The report of *S. caucasica* in the Turkish parts of the Kura-Aras river system by Kuru (1975) is apparently an error. In addition, solely *S. aurata* has been reported from the lower parts of the Çoruh (Rioni) River in Georgia, a river draining to the Black Sea (Ninua and Japoshvili, 2008), and not found in the Turkish part (Bayçelebi et al., 2015). According to Berg (1949), *S. caucasica* is distributed in the Terek, upper reaches of Kuban River. Furthermore, our sampling in the Kura and Aras rivers had not led of collecting of any *S. caspia* in Turkish parts of these rivers. *S. caspia* is found in fresh and brackish waters of the southern Caspian Sea basin, reported in the lower part of the Kura River, Novogolsk (Damakh), Kumashi, Lenkoran, Anzali Wetland and some rivers' estuaries in the Mazandaran Province of Iran (Berg, 1949; Coad, 2019). Therefore, *S. caucasica* and *S. caspia* should be excluded from Turkish fish fauna till providing further confirmation by specimens.

The results also revealed a high genetic distance i.e. 0.5-1.9%, between different populations of *S. aurata* in the Kura-Aras river system. This distance was lower between the populations of the same river (0.1-0.6%), however, there was no correlation between the sequence data and geographical distance of the studied *S. aurata* populations. One of the main difficulties regarding the identification of the ichthyofauna of the Kura-Aras river system can be inaccessibility to the whole river system. The Kura-Aras river basin is an internationally significant river system that covers almost all of Armenia and Azerbaijan, and a sizeable part of the populated and urbanized parts of Georgia. These countries together with Iran and Turkey rely heavily on the Kura-Aras river system as a principal source of water for all sectors and users including industry, agriculture, energy, and, residential. The Aras River has its source near Erzurum in Anatolia, and it is the longest river in the southern Caspian Sea and drains the south side of the Lesser Caucasus Mountains and then joins the Kura River which drains the north side of those mountains. Its total length is 1,072 km covering an area of 102,000 km². The lower parts of this river system form small lakes with associated marshes e.g. Akh Gol and even the main canal has 0.5-4.0 m depth, with an average of 2.5 m (Zakeri, 1997). Therefore, high genetic distances between the isolated populations of the *S. aurata* can be due to the above-mentioned barriers in the lower parts of the river system, leading to habitat fragmentations. The nocturnal *Sabanejewia* species inhabit faster water in the upper and middle reaches of rivers and they prefer shallow and clear waters (Coad, 2019). This non-migrant *S. aurata* prefers similar habitat, therefore, they show similar morphological characteristics despite having a high genetic distance (Fig. 3).



Figure 3: The Kura River at Ardahan, habitat of *Sabanejewia aurata*.

CONCLUSIONS

We examined the collected *Sabanejewia* specimens from Turkish parts of the Kura-Aras river system that were *Sabanejewia aurata*.

Based on our findings, the populations of the Kura and Aras rivers have similar morphology, however, they showed a genetic distance of 0.1-1.9%.

We could not find any evidence for the presence of *Sabanejewia caucasica* and *Sabanejewia caspia* in the Turkish part of this river system, and their presence needs to be confirmed.

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REFERENCES

1. Altschul S. F., Gish W., Miller W., Myers E. W. and Lipman D. J., 1990 – Basic local alignment search tool, *Journal of Molecular Biology*, 215, 3, 403-410.
2. Bayçelebi E., Turan D. and Japoshvili B., 2015 – Fish fauna of Çoruh River and two first records for Turkey, *Turkish Journal of Fisheries and Aquatic Sciences*, 15, 777-788, https://doi.org/10.4194/1303-2712-v15_4_01.
3. Berg L. S., 1949 – Freshwater fishes of the U.S.S.R. and adjacent countries, 4th edition, 2, English translation appeared in Israel Program of Scientific Translation, Jerusalem, 1964, 496.
4. Coad B. W., 2019 – Freshwater Fishes of Iran, retrieved from www.briancoad.com.
5. Çiçek E. and Sungur-Birecikligil S., 2016 – Ichthyofauna of the Turkish parts of Kura-Aras River Basin, *FishTaxa*, 1, 1, 14-26.
6. Çiçek E., Sungur-Birecikligil S. and Fricke R., 2015 – Freshwater fishes of Turkey: a revised and updated annotated checklist, *Biharean Biologist*, 9, 2, 141-157.
7. De Filippi F., 1863 – Nuove o poco note specie di animali vertebrati raccolte in un viaggio in Persia nell' estate dell' anno 1862, *Archivio per la Zoologia, l'Anatomia e la Fisiologia*, 2, 377-394. (in Italian)
8. Hoang D. T., Chernomor O., Von Haeseler A., Minh B. Q. and Vinh L. S., 2018 – UFBoot2: improving the ultrafast bootstrap approximation, *Molecular Biology and Evolution*, 35, 2, 518-522, <https://doi.org/10.1093/molbev/msx281>.
9. Kalyaanamoorthy S., Minh B. Q., Wong T. K. F., von Haeseler V. and Jermin L. S., 2017 – ModelFinder: fast model selection for accurate phylogenetic estimates, *Nature Methods*, 14, 587-589, <https://doi.org/10.1038/nmeth.4285>.
10. Kottelat M. and Freyhof J., 2007 – Handbook of European Freshwater Fishes, Cornol, Switzerland, 646.
11. Kumar S., Nei M., Dudley J. and Tamura K., 2008 – MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences, *Briefings in Bioinformatics*, 9, 299-306, <https://doi.org/10.1093/bib/bbn017>.
12. Kuru M., 1975 – Systematic and zoogeographic investigation of Pisces living in freshwater waters of Tigris-Euphrates, Kura-Aras, Van Lake and Black Sea Basin (Assoc. Prof. Thesis), Atatürk University, Erzurum, Turkey. (in Turkish)
13. Kuru M., Yerli S. V., Mangit F., Ünlü E. and Alp A., 2014 – Fish biodiversity in inland waters of Turkey, *Journal of Academic Documents for Fisheries and Aquaculture*, 3, 93-120.
14. Marcil J., Swain D. P. and Hutchings J. A., 2006 – Countergradient variation in body shape between two populations of Atlantic cod (*Gadus morhua*), *Proceeding Biological Science*, 22, 273, 1583, 217-223, <https://doi.org/10.1098/rspb.2005.3306>.
15. Ninua N. S. and Japoshvili B. O., 2008 – Check list of fishes of Georgia, *Proceedings of the Institute of Zoology*, XXIII, 163-176.
16. Poorbagher H., Eagderi S. and Pirbeigi A., 2017 – Temperature-induced phenotypic plasticity in *Aphanius arakensis* Teimori, Esmaeili, Gholami, Zarei and Reichenbacher, 2012 (Teleostei: Aphaniidae), *European Journal of Biology*, 76, 1, 1-6, <https://doi.org/10.5152/EurJBiol.2017.1701>.
17. Radkhah A. R., Poorbagher H. and Eagderi S., 2017 – Habitat effects on morphological plasticity of Saw-belly (*Hemiculter leuciscus*) in the Zarrineh River (Urmia Lake basin, Iran), *Journal of BioScience and Biotechnology*, 6, 1, 37-41.

18. Ronquist F., Teslenko M., van der Mark P., Ayres D. L., Darling A., Höhna S., Larget B., Liu L., Suchard M. A. and Huelsenbeck J. P., 2012 – MrBayes 3.2, Efficient bayesian phylogenetic inference and model choice across a large model space, *Bioinformatics*, 61, 539-542, <https://doi.org/10.1093/sysbio/sys029>.
19. Sambrook J., Fritsch E. F. and Maniatis T., 1989 – Molecular Cloning – A laboratory manual, 2nd edition, Cold Spring Harbor Press, Cold Spring Harbor, New York, 545.
20. Sayyadzadeh G., Abbasi K. and Esmaeili H. R., 2018 – Review and re-description of *Sabanejewia* species in Iran (Teleostei: Cobitidae), *Iranian Journal of Ichthyology*, 5, 4, 267-292, <https://doi.org/10.22034/iji.v5i4.322>.
21. Shen Y. Y., Chen X. and Murphy R. W., 2013 – Assessing DNA barcoding as a tool for species identification and data quality control, *PLoS ONE*, 8, 2, e57125, <https://doi.org/10.1371/journal.pone.0057125>.
22. van der Laan R., 2019 – Freshwater Fish List (Online), 27th edition, Richard van der Laan, Almere, the Netherlands, 1086.
23. Ward R. D., Zemlak T. S., Innes B. H., Last P. R. and Hebert P. D., 2005 – DNA barcoding Australia's fish species, *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 360, 1462, 1847-857, <https://doi.org/10.1098/rstb.2005.1716>.
24. Zakeri H., 1997 – Water catchment area of the Caspian Sea, Abangan, Student Quarterly of the Water Engineering Faculty of Khajeh Nassir ud-Din Tousi, 1997, 12, www.netiran.com/Htdocs/Clippings/Social/970700XXSO02.html.