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SHORT COMMUNICATION

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First records of *Gobio nigrescens* and *Gobio sibiricus* (Cypriniformes: Gobionidae) from the Amu Darya River basin, Uzbekistan

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1 | INTRODUCTION

The genus *Gobio* Cuvier, 1816 (Teleostei: Cypriniformes: Gobionidae) contains 46 valid species (Fricke, Eschmeyer, Van der, & Laan, 2019), and its members inhabit all types of waters, i.e., standing and flowing water, freshwater and, in some cases, salty water (Mendel et al., 2008). However, only one species of this genus, *G. lepidolaemus*, has been reported to occur in the natural waters of Uzbekistan (Mousavi-Sabet, Ganjbakhsh, Geiger, & Freyhof, 2016). *Gobio nigrescens* is found in the Hari River basin in Afghanistan, Iran, and Turkmenistan, and apparently in north-flowing streams of the Kopetdag in Turkmenistan (Coad, 2019; Esmaeili, Mehraban, Abbasi, Keivany, & Coad, 2017; Mousavi-Sabet et al., 2016). *Gobio sibiricus* occurs in the Selenge drainage in Mongolia, in the Nura drainage in Central Kazakhstan, and in the Ob and Yenisei drainages in Russia (Kottelat, 2006; Tagayev & Zhaparova, 2019). In the present study, we record for the first time, the occurrences of *G. nigrescens* and *G. sibiricus* in the Amu Darya River basin, Uzbekistan, as evidenced through both morphometric and genetic examinations.

2 | MATERIALS AND METHODS

Live specimens of *G. lepidolaemus* (n = 2), *G. nigrescens* (n = 8), and *G. sibiricus* (n = 2) were collected in July and August 2019 from the Kara Darya River (40°54′57.10″N, 71°50′52.93″E), Zeravshan River (40°08′44.58″N, 64°50′53.75″E), and Surkhan Darya River (38°17′00.13″N, 67°59′48.17″E), respectively (Figure 1). The pectoral fin was dissected from the right side of each fish specimen and stored in 99% ethanol at −20°C for molecular research, and the voucher specimens were fixed in 10% formalin for morphometric studies. One specimen from each newly recorded species was deposited in the collections of the Southwest University (SWU) School of Life Sciences with the accession numbers SWU02082019531 and SWU23072019373, respectively. The partial sequences of the cytochrome c oxidase subunit I (COI) gene was amplified to validate the morphological identification. Genomic DNA was extracted from fin tissue by proteinase K digestion followed by a standard phenol chloroform method (Sambrook & Russell, 2001). Approximately 650 bp were amplified from the 5′ region of the COI gene using, in all cases, the fish-specific primers described in Ivanova, Zemlak, Hanner, and Hebert (2007): VF2_t1 TGT AAA ACG ACG GCC AGT CAA CCA ACC ACA AAG ACA TTG GCA C and FR1d_t1 CAG GAA ACA GCT ATG ACA CCT CAG GGT GTC CGA ARA AYC ARA A. The PCR assay was performed in 25 μl final volume, containing 10 ng template DNA, 1 μl of each forward and reserve primer, 12.5 μl of 2× *Taq* Master Mix (Novoprotein), and double-distilled water. The thermal conditions consisted of an initial step of 3 min at 94°C followed by 35 cycles of 0.5 min at 94°C, 45 s at 54°C, and 1 min 10 s at 72°C; then, a final extension of 7 min at 72°C. Sequencing was carried out both in forward and reverse directions using primers VF2_t1 and FR1d_t1. *COI* sequences of other *Gobio* species were retrieved from the NCBI GenBank and *G. conocephalus* was selected as the outgroup. We computed genetic distances (K2P) and generated a maximum-likelihood (ML) tree, with 1,000 bootstrap replicates to explore phylogenetic affinities using MEGA 7 (Kumar, Stecher, & Tamura, 2016). Bayesian inference (BI) analysis was conducted using MrBayes 3.2 (Ronquist et al., 2012). Two million generations were conducted, and

every 1,000 generations were sampled with four Markov chains. The first 25% of the trees were discarded as burn-in, and a 50% majority consensus tree was calculated from the remaining samples.

3 | RESULTS

To the best of our knowledge, no previous study to date has reported on *Gobio nigrescens* and *G. sibiricus* in the Aral Sea basin (Berg, 1949; Mousavi-Sabet et al., 2016; Tagayev & Zhaparova, 2019; Tang et al., 2011; Turdakov, 1963); the results of the present study provide the first evidence of the occurrence of these species in the Amu Darya River basin (Figures 2 and 3). Comparison of morphometric measurements and meristic counts of *G. sibiricus* and *G. nigrescens* are presented in Table 1.

In the molecular analysis, the phylogenetic tree shows that *G. nigrescens* (GenBank accession numbers: MN810111, MN810112) from the Zeravshan River is nested with *G. nigrescens* (K2P distance 0.54%) from the Hari River, Iran, and *G. sibiricus* (GenBank accession number: MN810110) from the Surkhan Darya River is close to *G. sibiricus* (K2P distance 0.51%) from the Ob River (Russia), Irtysh River and Balkhash Lake (Kazakhstan). *Gobio sibiricus* and *G. lepidolaemus* (GenBank accession numbers: MN810113, MN810114) from the inland waters of Uzbekistan are very close to each other and distinguished by 0.72% K2P distance. The genetic differences between the COI sequences of *G. nigrescens* and *G. sibiricus*, compared with the COI sequence of *G. lepidolaemus*, were 6.34% and 7.01%, respectively (Figure 4).

4 | DISCUSSION

Molecular data collected in this study support our tissue samples belonging to *G. sibiricus* and *G. nigrescens*. These gobionid species were regarded as being native only to the Ob, Yenisei, Nura, and Hari rivers; however, the present study confirms their presence in the Amu Darya River basin also. *Gobio nigrescens*, originally *Bungia nigrescens*, is considered a synonym of *G. gobio* (Coad, 1981; Kottelat, 1997). Previous studies have suggested that *G. nigrescens* is an available name for the *Gobio* species of the Hari River (Mousavi-Sabet et al., 2016). Our molecular data also support the claim that *G. nigrescens* is not close to *G. gobio* (Figure 4). *Gobio nigrescens* is morphologically very similar to *G. lepidolaemus*, species that are native to the Syr Darya River basin; however, *G. nigrescens* can be distinguished from *G. lepidolaemus* by having a naked breast, scale rows on the caudal peduncle ½3/1/3½, usually 16 circumpeduncular scales, predorsal scales 16–18, scales between the lateral line and pelvic-fin origin 4½,

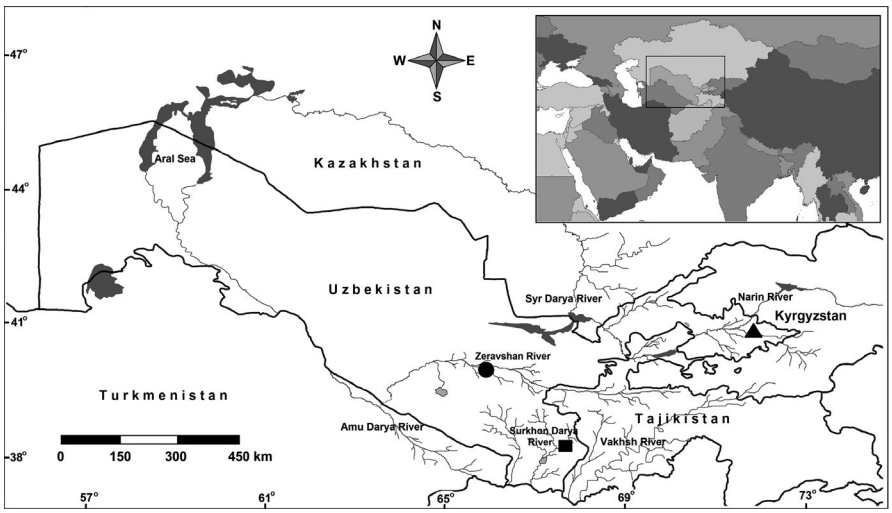

FIGURE 1 Map showing distribution of *Gobio nigrescens* (black circle), *G. sibiricus* (black square) and *G. lepidolaemus* (black triangle) in the natural water of Uzbekistan

FIGURE 2 *Gobio nigrescens*, SWU02082019531, 88 mm TL; Uzbekistan: Zeravshan River

FIGURE 3 *Gobio sibiricus*, SWU23072019373, 56 mm TL; Uzbekistan: Surkhan Darya River

TABLE 1 Comparison of morphometric measurements and meristic counts of *Gobio sibiricus* and *Gobio nigrescens* with the literary data on the same species

Morphometric characters	*G. sibiricus*		*G. nigrescens*	
	Tagayev & Zhaparova, 2019 (*n* = 22)	This study (*n* = 1)	Mousavi-Sabet et al., 2016 (*n* = 5)	This study (*n* = 4)
Total length (mm)	106.0–130.0	56.1	–	46.2–106.1
Standard length (mm)	89.0–108.0	44.2	65.3–95.2	37.9–84.3
In % of standard length				
Head length	24.5–27.7	27.4	22.3–25.8	26.3–29.0
Body depth	20.6–25.7	23.5	20.5–22.6	21.8–26.8
Body width	16.1–21.8	16.1	17.3–18.7	12.3–17.1
Predorsal length	47.4–51.7	52.5	43.3–45.4	50.0–51.0
Prepelvic length	48.4–54.5	55.4	46.4–50.7	51.1–55.4
Preanal length	70.1–75.3	74.4	67.1–69.1	71.3–75.2
Head depth	–	18.1	15.5–16.9	16.8–20.3
Caudal peduncle depth	9.4–10.9	11.3	8.3–9.3	10.7–12.9
Caudal peduncle length	16.8–20.4	19.2	21.0–24.0	19.3–24.3
Pectoral-pelvic distance	24.3–29.0	27.4	–	25.9–27.1
Pelvic-anal distance	18.8–23.0	20.1	–	20.6–22.8
Dorsal-fin length	17.9–21.2	23.7	19.9–20.6	20.8–24.3
Anal-fin length	13.1–16.2	18.6	16.4–17.0	15.8–17.7
Anal-fin depth	7.4–9.3	11.3	–	7.1–9.8
Pelvic-fin length	14.7–18.0	16.3	15.7–15.9	15.2–18.5
Pectoral-fin length	17.5–22.5	22.2	17.2–19.5	18.8–21.6
In % of head length				
Head depth	57.7–70.4	65.1	65.0–69.0	63.7–70.0
Eye diameter	16.2–20.8	24.4	22.0–28.0	21.0–27.3
Snout length	40.0–46.4	39.8	35.0–40.0	35.5–39.1
Interorbital width	28.0–34.8	33.3	34.0–41.0	32.3–38.0
Post orbital length	38.5–46.2	47.2	–	42.1–49.3
Dorsal-fin rays	–	iii, 6	iii, 7	iii, 7
Anal-fin rays	–	ii, 5	iii, 6	iii, 6
Pelvic-fin rays	–	12	14–17	14–15
Pectoral-fin rays	–	6	7	7

6–7 black or brown blotches on the back behind the dorsal fin, and a slender caudal peduncle (Mousavi-Sabet et al., 2016). The meristic counts of *G. nigrescens* collected from the Zeravshan River were the same as those reported by Mousavi-Sabet et al. (2016), with partial differences in some morphometric characters, which could partly be attributed to population-level variations.

Nikolski (1936) described *G. gobio sibiricus* from several localities in the Ob, Yenisei, and Nura drainages as a subspecies of *G. gobio*. Nevertheless, *G. g. sibiricus* had been considered a synonym of *G. g. cynocephalus* for a long time (Berg, 1949; Sideleva, 2003). Banarescu and Nalbant (1973) distinguished *G. g. sibiricus* from *G. g. cynocephalus* by lateral line scale count (40–44 vs. 43–48) and shape of the dorsal fin (distal edge more-or-less straight vs. distinctly 'notched' [concave]). The present study as well as previous studies (Kottelat, 2006; Mendel et al., 2008) provide evidence that *G. sibiricus* from *G. cynocephalus* are separated valid species. Our analysis further confirmed that *G. sibiricus* has a close relationship with *G. lepidolaemus*, concordant with Mousavi-Sabet et al. (2016).

It also has to be mentioned that the *Gobio* species of the Syr Darya River basin, as well as of part of the Fergana Valley, are incompletely studied; therefore, it is possible that *G. sibiricus* occurs in the Syr Darya River basin. In conclusion, *G. nigrescens* and *G. sibiricus* have been observed, identified, and reported to exist in the Amu Darya River basin on the basis of both morphological and molecular evidence. The results of this study will be useful for further research of the biology of these species, as well as the taxonomic and phylogenetic status of the gobionid fishes.

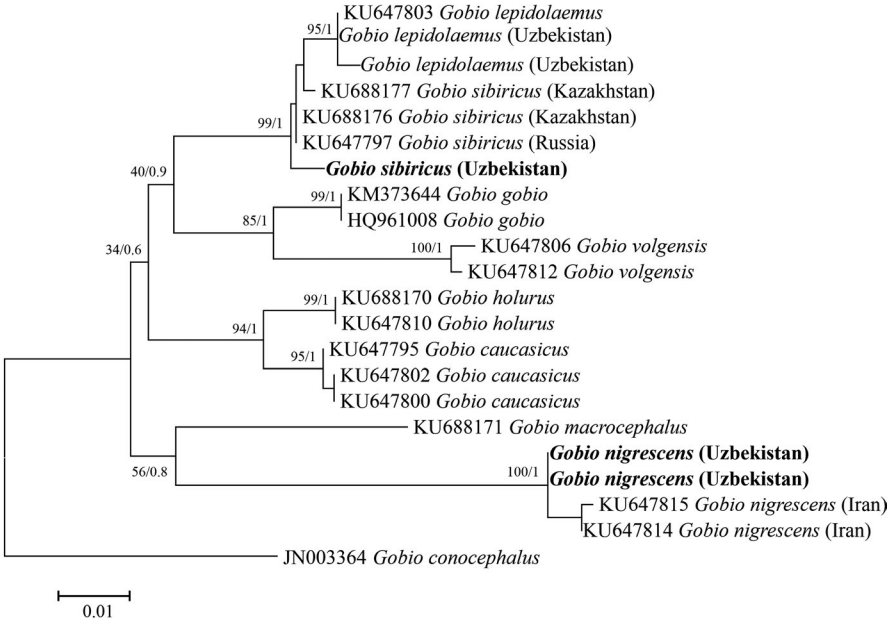

FIGURE 4 Maximum Likelihood (ML) estimation of the phylogenetic relationships of *Gobio sibiricus* and *G. nigrescens* with the other seven gobionid species based on the mitochondrial COI barcode region. The ML bootstrap value and Bayesian inference (BI) posterior probability are shown at the nodes

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

GenBank accession numbers for the new data have been presented in the manuscript.

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