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MORPHOMETRIC FEATURES AND PHYLOGENETIC POSITION OF TURKESTAN GUDGEON *GOBIO LEPIDOLAEMUS* KESSLER, 1872 (CYPRINIFORMES: GOBIONIDAE) IN THE SYR DARYA RIVER BASIN, UZBEKISTAN

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Turkestan gudgeon (Gobio lepidolaemus) is endemic in the Aral Sea Basin and found in many rivers in the region. Morphological features of Gobio lepidolaemus differ in various water bodies. In this article, we investigate the morphological characteristics of the Turkestan gudgeon found in the upper reaches of the Syr Darya River, as well as in the middle and lower reaches of the Chirchik River in Uzbekistan, in comparison with other species of gudgeon and the position in the phylogenetic tree based on the mitochondrial cytochrome oxidase I barcode region.

Key words: *gudgeon, morphology, molecular phylogeny, DNA barcoding, cytochrome oxidase I, fish standard length, ichthyofauna.*

Туркестанский пескарь *(Gobio lepidolaemus)* является эндемичным видом в бассейне Аральского моря и встречается во многих реках региона. Морфологические особенности различаются в разных водоемах. В статье, сообщается о морфологических характеристиках туркестанского пескаря, обитающего в верховьях Сырдарьи*, а также в среднем и нижнем течении реки Чирчик в Узбекистане в сравнении с другими видами пескаря;* его положение в филогенетическом дереве на основе нуклеотидов гена митохондриальной цитохромоксидазы I.

Ключевые слова: *пескарь, морфология, молекулярная филогения, ДНК-штрихкодирование, цитохром оксидаза 1,* стандартная длина рыбы*, ихтиофауна.*

INTRODUCTION

Gobio is a genus of Gobionidae family, mainly Eurasian freshwater fishes distributed from the Iberian Peninsula in the west to China, Mongolia, and eastern Siberia in the east [22] and contains 47 valid species [5]. Its representatives live in all types of waters, i.e., in standing and flowing waters, in freshwaters and in some cases found in brackish waters [11]. Three species of this genus, *G. lepidolaemus, G. nigrescens* and *G. sibiricus*, have been reported to occur in the inland waters of Uzbekistan [17].

Gobio lepidolaemus was described by Kessler (1872) based on about 50 specimens collected from the Zeravshan drainage, Uzbekistan and the Syr Darya drainage, Tajikistan. For a long time, *G. lepidolaemus* was known a subspecies of *G. gobio* [2, 13,

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19]. Nearly within past two decade, it is considered a valid species [4, 14, 20]. *Gobio lepidolaemus* is found in the Amu Darya River basin in Afghanistan [4], Tajikistan [12], Turkmenistan [18] and Uzbekistan [3], and in the Syr Darya River basin in south Kazakhstan [2, 13], Kyrgyzstan [10, 19] and Uzbekistan [2, 17]. The increase in species of genus *Gobio* in the region [17], as well as the introduction of *Abbottina rivularis* from the Far Eastern rivers, as an alien gudgeon species [10], require careful study of the morphological characteristics of *G. lepidolaemus* in order to distinguish it from above-mentioned species.

In the present study, we aimed to investigate the morphometric characteristics of *G. lepidolaemus* and explore the phylogenetic position of *G. lepidolaemus* in the phylogenetic tree of the genus *Gobio* based on the mitochondrial COI barcode region of 32 gudgeon fishes.

MATERIALS AND METHODS

Live specimens of *Gobio lepidolaemus* were collected in July and August 2019 from the Kara Darya River (n=3; 40°54′57.10″N, 71°50′52.93″E) and Chirchik River (n=5; 41°18′26.06″N, 69°25′51.05″E) (Figure 1). After anesthesia, fish were fixed and stored in 10% formalin. Muscle tissue samples and each individual's right pectoral fin were directly fixed and stored in 99% ethanol at -20 °C for subsequent DNA isolation. Body measurements were performed using a digital caliper, and numbers were rounded to the nearest 0.01 mm. All measurements were made from point to point and not by projection. Methods for counts and measurements followed Kottelat & Freyhof (2007) [8]. Standard length (SL) was measured from the tip of the snout to the end of the hypural complex. Length of the caudal peduncle was measured from behind the base of the last ray of the anal fin to the end of the hypural complex at mid-height of the base of the caudal fin. The last two branched rays articulating on a single pterygiophore in the dorsal and anal fins were counted as one ray. Scales in a lateral line were counted from immediately behind the pectoral girdle to the last scale on the base of the caudal fin. All the specimens were stored at Museum of the Key Laboratory of Freshwater Fish Reproduction and Development (Ministry of Education), Southwest University (SWU), China.

DNA extraction and PCR. DNA was extracted from muscle or fin tissue using proteinase K digestion followed by a standard phenol chloroform method [16]. A 652 bp fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene was PCR-amplified using the primers FishF1 (5´-TCA ACC AAC CAC AAA GAC ATT GGC AC-3´) and FishR1 (5´-TAG ACT TCT GGG TGG CCA AAG AAT CA-3´) [21]. PCR amplifications were performed on 25 μL total volume as follows: 14.8 μL dd H_2O, 2.0 μL DNA template, 1.0 μL each primer, 2.5 μL 10 x PCR buffer, 1.5 μL of 25 mM $MgCl_2$, 2.0 μL of 2.5 mM dNTPs, 0.2 μL rTaq DNA polymerase (TaKaRa; Dalian, China). Thermal cycling consisted of an initial step at 95 °C for 2 min, 35 cycles of 94 °C for 30 sec, 54 °C for 30 sec, and 72 °C for 1 min 10 sec, and a final extension step at 72 °C for 10 min. The reactions were performed using a thermocycler (Veriti™ 96-Well thermal Cycler; Applied Biosystems, Singapore), and PCR products were visualized using electrophoresis with a 1% agarose gel stained with Gel Doc™ XR (Bio-Rad Laboratories, Inc., USA). PCR products were sequenced using the Applied Biosystems 3730 DNA analyzer (Thermo Fisher Scientific Inc., USA). Sense and antisense strands of the PCR products were sequenced using either the FishF1 or the FishR1 primer.

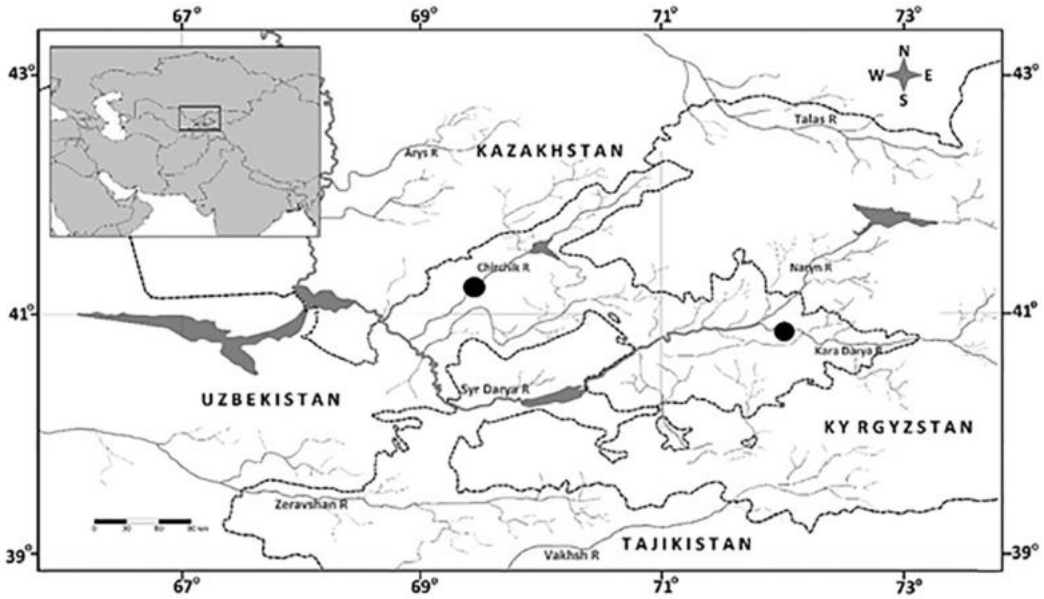


Figure 1. Collection locality of *Gobio lepidolaemus* (black circle) from Kara Darya and Chirchik rivers, Uzbekistan.

Table 1. List of COI-sequences of genus *Gobio* downloaded from NCBI.

No.	Species	GenBank Acc. No.	No.	Species	GenBank Acc. No.
1.	*G. acutipinnatus*	MT632635	17.	*G. kizilirmakensis*	MH319417
2.	*G. artvinicus*	MH319434	18.	*G. kovatschevi*	MH319433
3.	*G. balcanicus*	KJ553332	19.	*G. lepidolaemus*	KU647808
4.	*G. baliki*	MH319427	20.	*G. lozanoi*	KJ533453
5.	*G. battalgilae*	MH319414	21.	*G. macrocephalus*	MT632636
6.	*G. bulgaricus*	MH319428	22.	*G. maeandricus*	MH319424
7.	*G. caucasicus*	KU647799	23.	*G. microlepidotus*	MH319413
8.	*G. coriparoides*	MN864250	24.	*G. nigrescens*	KU647815
9.	*G. cynocephalus*	KU314700	25.	*G. obtusrostris*	HQ960938
10.	*G. feraeensis*	KJ553486	26.	*G. occitaniae*	KJ553646
11.	*G. gobio*	AB239596	27.	*G. ohridanus*	MT522028
12.	*G. gymnostethus*	MH319416	28.	*G. sakaryaensis*	MH319419
13.	*G. hettitorum*	MH319415	29.	*G. sibiricus*	KU688178
14.	*G. holurus*	KU688170	30.	*G. skadarensis*	KJ553601
15.	*G. insuyanus*	MH319422	31.	*G. soldatovi*	KU688172
16.	*G. intermedius*	MH319423	32.	*G. volgensis*	KU688174

Molecular data analyses. Sequences were compared to published *Gobio* sequences using the BLASTn algorithm [1]. Sequences of other members of the genus *Gobio* retrieved from the NCBI GenBank database following BLAST searches are shown in Table 1. The COI fragment sequences were aligned using the Clustal_W algorithm in MEGA7 [9], and inconsistencies were checked by eye. For phylogenetic reconstructions, the datasets were analyzed using a neighbor-joining (NJ) method of MEGA7 [9] and Bayesian inference (BI) using MrBayes 3.2 [15]. MrBayes was run with 6 substitution types (nst=6) and considered gamma-distributed rate variation and the proportion of invariable positions (GTR+G+I) for the COI datasets. For BI, we ran four simultaneous Monte Carlo Markov chains for 500,000 generations with sampling every 1,000 generations and 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) using a Kimura 2-parameter (K2P) model. Genetic distances were investigated based on K2P distances [7] for the COI datasets. The phylogenetic trees were visualized and edited using FigTree 1.4.2. We used *Romanogobio tenuicorpus*, as an outgroup (GenBank accession number: KF647873).

Results

Gobio lepidolaemus Kessler, 1872 (Fig. 2)

Gobio fluviatilis var. *lepidolaemus* Kessler, 1872:15



Figure 2. Lateral view of *Gobio lepidolaemus*, uncatalogued, 84.5 mm SL, Kara Darya, Andijan Region, Uzbekistan

Description. All morphometric characteristics of *G. lepidolaemus* found from Kara Darya, Chirchik, Chu and Sarisu rivers are given in Table 2. Moderately deep body, laterally compressed, maximum body width 15.9–18.1% of standard length. Greatest body depth at dorsal-fin origin, body depth 22.1–24.6% of standard length, decreasing towards caudal-fin base. Head laterally compressed, maximum width almost equal to depth at nape. Snout length 24.9–35.7% of lateral head length. Snout length shorter than postorbital length, snout length 1.2–1.5 times in postorbital length. Anterior and posterior nostril adjacent and anterior nostril in very short tube with elongated barbel-like tip, tip of the nostril appendage not reaching anterior margin of eye. Eyes normal, horizontal diameter 19.3–22.4% of lateral head length. Mouth inferior and mouth corner situated below anterior nostril. Lips thin and without furrows and papillae. Pair of barbels, reaching to posterior eye margin, barbel length 25.1–33.9% of lateral head length.

Dorsal fin with 3 simple and 7 branched rays in all samples found in Kara Darya and Chirchik rivers. Margin of dorsal fin concave, origin anterior to pelvic-fin insertion, situated slightly anterior to midpoint between tip of snout and caudal-fin base, first branched ray longest, dorsal fin length 17.7–22.2% of standard length and 1.2–1.4 times in lateral head length. Anal fin with 2 simple and 6 branched rays, anal fin length 1.7–1.9 times in its base length. Pectoral fin with 12 (1), 13 (3), 14 (3) or 15 (1) branched rays. Pectoral fins developed, 61.4–81.4%

of distance between pectoral-fin and pelvic-fin origin. Pelvic fin with seven branched rays and tips of depressed pelvic fins reaching anus, pelvic fin length 66.8–85.8% of distance between pelvic-fin and anal-fin origin. Anus close to anal fin, vent-anal fin origin distance 4.8–7.6% of standard length. Caudal fin forked; with 9+9 (6) or 8+9 (2) branched rays. Body totally covered by scales, breast completely scaled in all samples found in Kara Darya and Chirchik rivers. Lateral line complete, with 39-40 scales on body, reaching caudal-fin base. 5–5½ scales between lateral line and dorsal-fin origin, 3½–4½ scales between lateral line and pelvic-fin origin and 15–16 circumpedencular scales. 3–4 scales between anus and anal fin origin. Pharyngeal teeth in two rows, 2,5–5,2 or 3,5–5,3, slightly hooked at tip [13].

Colouration. Head and body from pale silvery to dark grey with many small brown spots. Laterally 10–11 roundish, dark blotches. Lateral blotches situated slightly above lateral line until vertical of dorsal-fin base and then along lateral line. Fins also with small faint dark spots. Ventral body from whitish to light grey.

Phylogenetic position. COI barcode region sequences were analysed in 32 *Gobio* species. Two individuals were sequenced by this study and 32 sequences obtain from Genbank (Table 1). *Gobio* species were divided into five main clades in the phylogenetic analysis. The second clade consisted of *G. nigrescens*, *G. holurus*, *G. caucasicus*, *G. lepidolaemus*, *G. sibiricus*, *G. acutipinnatus* and *G. artvinicus*. *Gobio lepidolaemus* constituted a highly supported clade sister to *G. sibiricus*, concordant with Mousavi-Sabet et al. (2016) and Sheraliev et al. (2020) (Figure 3). Intrageneric K2P distances between species ranged from 0.2% (*G. microlepidotus* and *G. battalgilae*) to 11.1% (*G. coriparoides* and *G. nigrescens*). K2P distance is 0.6% between *G. lepidolaemus* and closest relative *G. sibiricus* and 4.3% between *G. lepidolaemus* and *G. holurus*.

DISCUSSION

There are three species of *Gobio* (*G. lepidolaemus, G. nigrescens* and *G. sibiricus*) in the inland waters of Uzbekistan. *Gobio lepidolaemus* is distinguished from *G. nigrescens* by the following characteristics: breast covered by scales (vs. naked); less predorsal scales (14–16 vs. 16–18); less or absent irregularly shaped,

Table 2. Comparison of morphometric measurements of *Gobio lepidolaemus* with the literary data on the same species

Characteristics	Kara Darya (n=3)		Chirchik River (n=5)		Chu River (n=25) [13]		Sarisu River (n=15) [13]	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
Total length (TL)	70.66–9 9.07		72.38–84.63		–		–	
Standard length (mm) (SL)	57.93–84.45		60.05–70.28		–		–	
In % of SL								
Lateral head length (HL)	25.40–27.42	26.23±1.06	25.24–27.48	26.22±1.00	23.0–29.0	25.88±0.30	25.0–30.0	27.32±0.31
Body depth at dorsal fin origin	23.15–24.57	23.91±0.72	22.12–24.17	23.50±0.82	19.0–26.0	21.94±0.35	16.0–23.0	19.92±0.42
Body width maximum	15.93–16.90	16.35±0.50	16.85–18.05	17.16±0.51	–	–	–	–
Predorsal length	48.63–49.39	48.89±0.43	49.40–50.76	49.95±0.65	45.0–54.0	49.73±0.46	45.0–54.0	49.72±0.61
Postdorsal length	40.19–41.83	40.89±0.84	38.33–43.57	41.39±2.03	34.0–44.0	41.45±0.37	35.0–43.0	39.38±0.62
Prepelvic length	50.38–51.10	50.68±0.37	48.56–52.83	50.51±1.87	–	–	–	–
Preanal length	69.04–70.86	70.21±1.01	67.18–71.41	69.77±1.74	–	–	–	–
Preanus length	63.42–65.67	64.29±1.21	61.44–65.07	63.64±1.80	–	–	–	–
Dorsal-fin length	17.70–22.23	20.05±2.27	18.80–20.24	19.51±0.52	16.0–26.0	21.24±0.43	18.0–23.0	21.85±0.43
Dorsal fin base length	13.06–14.26	13.85±0.68	12.81–14.33	13.31±0.60	10.0–16.0	13.44±0.28	11.0–16.0	13.13±0.36
Anal-fin length	13.80–15.90	14.99±1.08	13.77–16.50	15.66±1.15	10.0–17.0	14.81±0.37	14.0–20.0	16.98±0.36
Anal fin base length	8.31–8.68	8.49±0.19	8.12–9.21	8.63±0.41	7.0–11.0	8.63±0.19	6.0–11.0	9.10±0.33
Pectoral-fin length	15.64–20.90	18.80±2.79	17.70–19.90	18.60±0.92	16.0–23.0	19.94±0.36	16.0–20.0	18.25±0.34
Pelvic-fin length	13.66–17.49	15.58±1.91	14.84–16.55	15.43±0.66	12.0–19.0	15.45±0.30	14.0–16.0	15.52±0.23

Caudal-fin length	17.06–22.41	19.50±2.70	17.52–21.55	19.15±1.54	17.0–25.0	21.57±0.41	–	–
Caudal-peduncle length	20.25–23.08	22.12±1.62	23.05–24.75	23.69±0.65	14.0–24.0	20.14±0.58	19.0–24.0	22.32±0.38
Caudal-peduncle depth	10.98–11.60	11.22±0.33	10.19–11.64	11.04±0.60	8.0–10.0	9.38±0.10	7.0–10.0	8.98±0.22
Caudal peduncle width	6.00–8.24	7.43±1.24	7.12–8.94	7.88±0.67	–	–	–	–
Pectoral–pelvic distance	24.40–26.64	25.50±1.12	24.34–27.70	25.71±1.28	20.0–33.0	26.27±0.69	16.0–26.0	21.58±0.69
Pelvic–anal distance	20.39–20.63	20.49±0.13	19.70–21.06	20.64±0.57	14.0–24.0	18.53±0.53	14.0–20.0	17.92±0.59
Vent – anal fin origin distance	4.80–6.97	5.92±1.09	5.76–7.64	6.68±0.77	–	–	–	–
In % of HL (* in % of SL)								
Head depth at nape	65.81–71.75	68.75±2.97	61.37–71.41	66.87±4.04	–	–	14.0–18.0*	16.85±0.24
Head width	67.56–72.30	69.77±2.38	62.28–70.87	66.84±3.44	–	–	–	–
Snout length	29.44–35.71	32.09±3.25	32.65–35.16	33.69±1.15	8.0–13.0*	10.79±0.24	–	–
Eye diameter	19.27–22.28	20.93±1.53	20.15–22.42	20.89±0.89	5.0–7.5*	6.47±0.13	–	–
Interorbital width	32.39–37.72	34.99±2.67	29.41–37.00	33.72±2.79	6.0–8.5*	7.37±0.15	–	–
Postorbital head length	42.30–45.53	43.96±1.61	40.72–45.16	43.08±1.65	–	–	–	–
Maxillary barbel length	26.60–33.71	30.72±3.69	25.11–33.87	28.35±3.30	2.5–10.0*	6.58±0.44	7.0–10.0*	8.45±0.21
CPL/CPD	1.84–2.08	1.97±0.12	2.01–2.29	2.15±0.12	–	–	–	–

black or brown blotches on back behind dorsal-fin base (2–4 vs. 6–7); barbel reaching to posterior eye margin (vs. reaching vertical through middle of eye) and less slender caudal peduncle (caudal peduncle depth 1.8–2.3 times in its length vs. 2.4–2.9). *Gobio lepidolaemus* can be distinguished from *G. sibiricus* by the following characteristics: 7 branched dorsal-fin rays (vs. 6); 6 branched anal-fin rays (vs. 5) and caudal peduncle length 20.2–24.7% of standard length (vs. 16.8–20.4%).

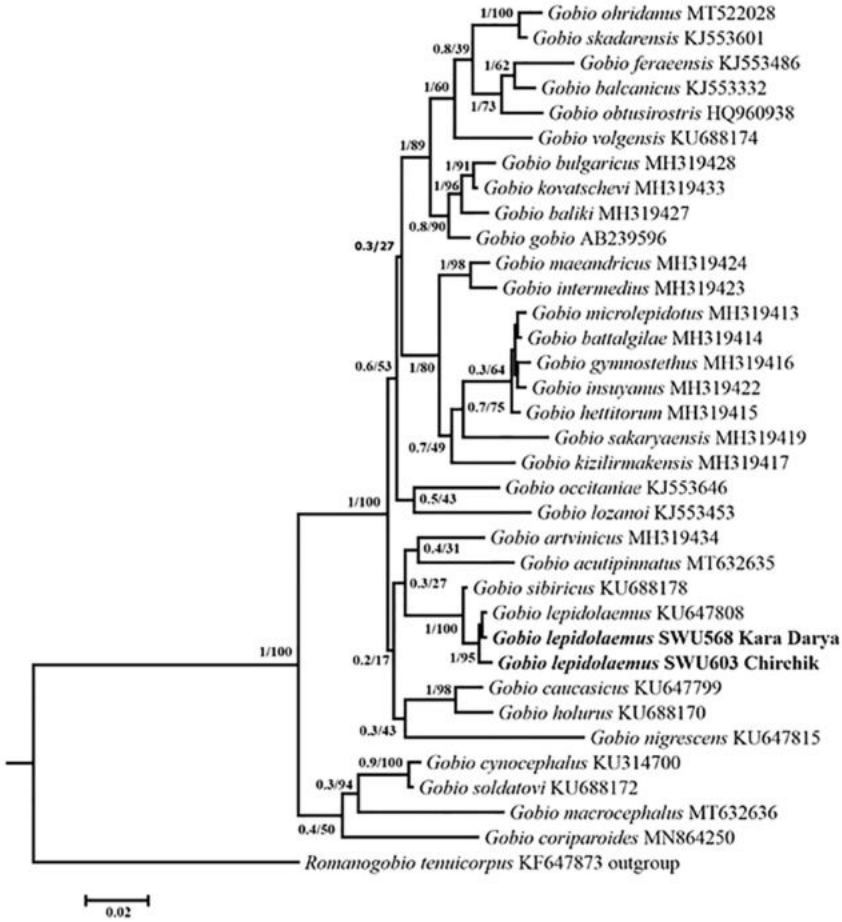


Figure 3. Bayesian inference (BI) estimation of the phylogenetic relationships of *Gobio lepidolaemus* with the other 31 gudgeon species based on the mitochondrial COI barcode region. The BI posterior probability and Neighbor-joining (NJ) bootstrap value are shown at the nodes.

Gobio lepidolaemus can be distinguished from other *Gobio* species by the following characteristics: barbel reaching to posterior eye margin (vs. not reaching to posterior eye margin in *G. holurus*), body depth 4.1–4.5 times in standard length (3.8–3.9 times in *G. soldatovi*), caudal peduncle depth 1.8–2.3 times in its length (vs. more than 3 times in *G. uranoscopus*), breast covered by scales (vs. naked in *G. kessleri*), 5–5½ scales between lateral line and dorsal-fin origin (vs. 6½–7½ in *G. delyamurei*); the stouter caudal peduncle (its depth 1.8–2.3 times in its length, vs. 2.4–2.6 in *G. brevicirris*), 39–40 scales in lateral line (vs. 40–45 in *G. albipinnatus*), vent-anal fin origin distance 23.4–34.5% of pelvic-anal fins distance (vs. more than 60% in *G. cynocephalus*), barbels long, length 7.8–8.7 times in standard length (vs. about 7 times in *G. gobio*) and maximum body depth 22.1–24.6% of standard length (vs. 17.8–19.3% in *G. acutipinnatus*).

Gobio lepidolaemus constituted a highly supported clade sister to *G. sibiricus*, concordant with Mousavi-Sabet et al. (2016) and Sheraliev et al. (2020) [14, 17].

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СИРДАРЁ ҲАВЗАСИДА УЧРАЙДИГАН ТУРКИСТОН ҚУМБАЛИҒИ *Gobio lepidolaemus* Kessler, 1872 (Cypriniformes: Gobionidae)НИНГ МОРФОМЕТРИК ХУСУСИЯТЛАРИ ВА ФИЛОГЕНЕТИК ҲОЛАТИ

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Калит сўзлар*: қумбалиқ, морфология, молекуляр филогения, ДНК-штрих кодлаштириш, цитохром оксидаза 1, балиқнинг стандарт узунлиги, ихтиофауна.*

Қумбалиқлар (*Gobio*) Gobionidae оиласининг Евроосиё қитъасидаги сувларда учрайдиган уруғи бўлиб, 47 турни ўз ичига олади. Бугунги кунда Ўзбекистон ички сув ҳавзаларида ушбу уруғнинг учта: Туркистон қумбалиғи (*G. lepidolaemus*), қора доғли қумбалиқ (*G. nigrescens*) ва Сибир қумбалиғи (*G. sibiricus*) каби турлари учраши қайд этилган.

Туркистон қумбалиғи К.Кесслер (1872) томонидан Зарафшон ва Сирдарё ҳавзасидан олинган намуналар асосида кашф қилинган. Кашф қилинганидан сўнг узоқ вақт мобайнида оддий қумбалиқ (*G. gobio*)нинг кенжа тури сифатида кўрилган ва сўнгги йигирма йилликда ёзилган тадқиқот ишларида мустақил тур сифатда қайд этила бошланди. Ушбу тур айни вақтда Амударё ва Сирдарё ҳавзасидаги кўплаб катта ва кичик дарёларда учрайди. Минтақа ҳудудида қумбалиқлар уруғига мансуб турлар сонининг ортиши, шу билан бирга ташқи томондан қумбалиқларга жуда ўхшаш бўлган Амур сохта қумбалиғи (*Abbottina rivularis*)нинг минтақа сувларига иқлимлаштирилгани, шу сабабли турларни ўзаро фарқлашда Туркистон қумбалиғининг морфометрик кўрсаткичларини чуқур тадқиқ этиш муҳим таксономик аҳамиятга эга ҳисобланади.

Тадқиқот давомида Сирдарё ҳавзасига мансуб Қорадарё ва Чирчиқ дарёларидан тутилган намуналар тадқиқ этилди, шу билан биргаликда Чу ва Сарису дарёларидан олинган намуналарнинг морфометрик кўрсаткичларидан фойдаланилди. Туркистон қумбалиғининг бошқа қумбалиқлар орасидаги филогенетик ўрни молекуляр тадқиқот асосида ўрганилди.

Туркистон қумбалиғи ўзининг бошқа турдошларидан танасининг баландлиги (стандарт узунлигининг 22.1–24.6% и), бошининг икки ён томондан сиқилганлиги, мўйловларининг узун бўлиши (бош узунлигининг 25.1–33.9% и), кўкрак соҳасини тангачалар қоплаганлиги билан фарқланади. Унинг орқа сузгич қанотида 7 та, анал сузгич қанотида 6 та, кўкрак сузгич қанотида 12–15 та, қорин сузгич қанотида 7 та ва дум сузгич қанотида 17–18 та шохланган нурлар бўлади. Ён чизиғи тўлиқ, ўртача 39-40 та тангачадан иборат. Балиқнинг ён томонида 10–11 та айлана шаклдаги, тўқ рангли доғлари мавжуд. Туркистон қумбалиғи мамлакатимиз жанубий сувларида учрайдиган қора доғли қумбалиқдан кўкрак соҳасининг тангачалар билан қопланганлиги, мўйловининг учи кўзининг орқа чегарасигача етиши ва дум бандининг баланд бўлиши билан фарқланади. Сибир қумбалиғидан эса орқа ва анал сузгич қанотларидаги шохланган нурлар сонининг кўплиги ва дум бандининг узун бўлиши би-

лан фарқланади.

Филогенетик тадқиқот натижаларига кўра, Туркистон қумбалиғи юқоридаги икки тур билан бир клададан жой олган. У турдошлари ичида Сибир қумбалиғига энг яқин жойлашган бўлиб, улар орасидаги генетик масофа (K2P) 0.6% ни ташкил этади.

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