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A New Species of Golden Loach (genus *Sabanejewia*, Cobitidae) from the Sea of Azov Basin

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Abstract—A new species of golden loach is described from the Don River basin. This species is distinguished from all other *Sabanejewia* species by the following set of characters: a midlateral row of relatively large 9–16 (more often 13–14) dark blotches along flank usually well developed; no black line along flank; interspaces between midlateral blotches usually with dark pigmentation; some individuals with uniform dark lateral coloration; dorsal fin origin usually in front of pelvic fin origin; body relatively low: body depth noticeably less than the length of the caudal peduncle, usually does not reach 17% standard length—*SL*, it is contained more than 6 times in *SL*; dorsal adipose crest on caudal peduncle very weakly developed, ventral crest poorly visible; two black spots at caudal fin base usually merge with each other. Previously, this species was considered conspecific for *S. baltica* distributed in the Black and Baltic Sea basins, but recent phylogenetic studies have shown that they belong to separate mtDNA phylogenetic lineages.

Keywords: new species, freshwater fishes, Don River, *Sabanejewia*

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INTRODUCTION

Species of the genus *Sabanejewia,* described by Vladykov (1929), are small bottom freshwater fishes, very similar in morphology to spined loaches from the closely related genus *Cobitis* Linnaeus, 1758. They differ from *Cobitis* species in the presence of usually 12 branched caudal fin rays (versus 14 rays), the absence of a specific secondary male character of *Cobitis* (ossified plate-like extensions of the pectoral fin rays—"lamina circularis"), instead of which males in the reproductive period usually develop noticeable swellings on the body sides, and a specific color pattern (Economidis and Nalbant, 1996). Unlike *Cobitis,* which includes 117 considered valid species, the total range of which covers most of Europe (from northern France, including the British Isles and Scandinavia, to the south, including North Africa) and most of Asia, with the exception of the northernmost regions of Siberia, golden loaches of *Sabanejewia* are distributed over a relatively small area of water bodies in the basins of the Mediterranean, Baltic, Black, Caspian, and Aral Seas, with only ten species accepted (Kottelat and Freyhof, 2007; Kottelat, 2012; *Eschmeyer's Catalog...*, 2022). Of these, *S. baltica* Witkowski, 1994 was the last to be described (as a Baltic subspecies of *S. aurata* (De Filippi, 1863)). Type specimens of this taxon were fish from the Odra River basin. Witkowski (1994) suggested that it is probably distributed in the cleanest lowland rivers of the Vistula, Neman and Odra basins. The generally accepted contemporary range of *S. baltica* includes the Baltic Sea basin in the Vistula and Odra drainages, as well as the Black Sea basin in the Dniester, Dnieper, and Don drainages (Kottelat and Freyhof, 2007).

It should be noted that the modern fauna of the Baltic freshwater fish is relatively young. The depression of the Baltic Sea was covered with ice during the last glacial advance in the Pleistocene and filled with fresh water at the end of the Quaternary after the retreat of the ice cover, started about 13 thousand years ago (Reid and Orlova, 2002). Berg (1949) associated the appearance of southern forms of fish in the Baltic zoogeographic province with their dispersal from the south after the retreat of the ice. However, Reid and Orlova (2002) concluded that much of the current biodiversity in the Baltic Sea reflects relatively recent colonization of non-native species, intentionally or unintentionally introduced by human activities. Indeed, the Dnieper–Bug (former Royal) Canal, an important part of the transport artery connecting the Baltic and Black Seas, was built around 1775–1783, and the first publications about golden loaches from the Baltic Sea basin appeared at the beginning of the second half of the 20th century (Oliva, 1960; Rolik, 1960, 1971; Surdacki, 1965; Penczak, 1969), while single findings of the species have been known since 1931 in the Neman

and Vistula (Surdacki, 1965). Both proposed entry routes seem likely, but both have yet to be confirmed. The last of these was previously proposed by Surdacki (1965).

In any case, the origin of the Baltic populations of *S. baltica* from the Black Sea basin populations is confirmed by phylogenetic studies (Perdices et al., 2003), which showed the monophyly of *S. baltica*, represented by samples from the Vistula and Dniester drainages. However, recent phylogenetic results (Vasil'eva et al., 2022) revealed the existence of two differentiated mtDNA lineages of *S. baltica*: (1) samples from the Baltic Sea basin and the Dniester River system and (2) samples from the Don River drainage. (These lineages were also more or less separated within the RAG-1 mixed group, which included all available nominal *Sabanejewia* species except *S. caspia* (Eichwald, 1838), *S. larvata* (De Filippi, 1859), and *S. romanica* (Băcescu, 1943).) The first of them should be attributed to *S. baltica* s. stricto, while the second one is considered as a new cryptic species of *Sabanejewia* (Vasil'eva et al., 2022).

The existence of an independent evolutionary unit of *Sabanejewia*, formed in the Don River basin as a result of long-term isolation, is consistent with the observed differences between the ichthyofauna of the Don basin and the Dnieper and Dniester systems. The following freshwater endemics of the Don are currently accepted: *Gobio brevicirris* Fowler, 1976, *Romanogobio tanaiticus* Naseka, 2001, *Alburnus leobergi* Freyhof et Kottelat, 2007, *Leuciscus danilewskii* (Kessler, 1877); ecologically close species distributed in the Dnieper and Dniester are also absent in the Don: *Gobio sarmaticus* Berg, 1949, *Romanogobio belingi* (Slastenenko, 1934), *Alburnus sarmaticus* Freyhof et Kottelat, 2007, *Leuciscus leuciscus* (Linnaeus, 1758). Some other species inhabiting the Dnieper or Dniester rivers are also not recorded in the Don system (*Gymnocephalus baloni* Holčík et Hensel, 1974, species of the genus *Zingel*, etc.). These differences correspond to the recent discovery of a long-term refugium in the rivers of the Sea of Azov, confirmed by molecular studies of the freshwater duck mussel *Anodonta anatina* (Tomilova et al., 2020). According to the data obtained, the isolation of the Azov lineage of this species is estimated from the middle of the Pliocene (1.85–5.73 MYA).

The aim of this study was to identify the morphological diagnostic features of the golden loach living in the Don River system and to describe it as a new species of *Sabanejewia*.

MATERIALS AND METHODS

Morphometric characters commonly studied in *Sabanejewia* species (Banărescu et al., 1972; Vasil'eva and Poznyak, 1986; Vasil'eva and Vasil'ev, 2019), as well as color pattern, the shape of the lower lip, and suborbital spine, the relative barbel length, and development of swellings on the body sides of males were studied in total in 28 golden loaches in the materials of Zoological Museum of Moscow State University (ZMMU), collected in the Don River basin and presented below in the description of a new species, and in 31 specimens of *S. baltica* s. stricto, collected from five localities:

P-21220, 2 specimens total length—*TL* 66.5–76.7 mm, standard length—*SL* 55.0–63.5 mm, Vistula River, Poland, collector J. Kotusz; P-23074, 10 specimens *TL* 82.0–99.0 mm, *SL* 70.8–85.2 mm, Dniester River at Soroca city, Moldova, collector A. Moshu; P-23739, 9 specimens *TL* 55.0–81.0 mm, *SL* 46.2–69.0 mm, Dnieper River at Bilino village, Smolensk District, Russia, 03.07.2014, collector V. Vasil'ev; P-24011, 5 specimens *TL* 67.5–81.2 mm, *SL* 56.5–69.0 mm, Dnieper River at Bilino village, 10.07.2005, collector V. Vasil'ev; P-24223, 4 specimens *TL* 58.5–90.2 mm, *SL* 49.0–77.0 mm, Busha River, Dniester River basin, Vinnitsa Region, Ukraine, 16.04.1989, collector V. Vasil'ev; P-24227, 1 specimen *TL* 72.0 mm, *SL* 61.3 mm, Teterev River, Dnieper River basin, Ukraine, 23.06.1998, collector A. Moshu.

All measurements were carried out with a vernier caliper from point to point and recorded with an accuracy of 0.1 mm. Methods for counts and measurements follow Kottelat and Freyhof (2007). The last two branched rays articulating on a single pterygiophore in the dorsal and anal fins are counted as "1½". Similarly, the last normal branched and closely related undeveloped unbranched ray in the pectoral and pelvic fins are counted as "1½". For statistical analysis of features, standard univariate methods and the coefficient of difference *CD* were used.

Characters that showed significant differences between the studied *S. baltica* and golden loaches from the Don River basin were also analyzed in 12 females *TL* 85.5–98.0 mm, *SL* 71.7–84.3 mm and 10 males *TL* 79.0–85.2 mm, *SL* 66.7–72.0 mm from the sample ZMMU P-21485 from the Don basin to confirm the stability of diagnostic features. In addition, to test the diagnostic value of the selected features, we studied them on another 135 specimens (males, females, and juveniles) from museum samples collected in the Don basin and presented below in additional materials.

For comparative morphological analysis, we also used data on *S. aurata* (De Filippi, 1863), *S. balcanica* (Karaman, 1922), *S. kubanica* Vasil'eva et Vasil'ev, 1988, *S. caucasica* (Berg, 1906), *S. caspia, S. larvata, S. bulgarica* (Drensky, 1928), *S. vallachica* (Nalbant, 1957) and *S. romanica* (Băcescu, 1943) from previously studied ZMMU collections and presented in our publications (Vasil'eva and Poznyak, 1986; Vasil'eva and Vasil'ev, 1988, 2019; Vasil'eva and Ráb, 1992).

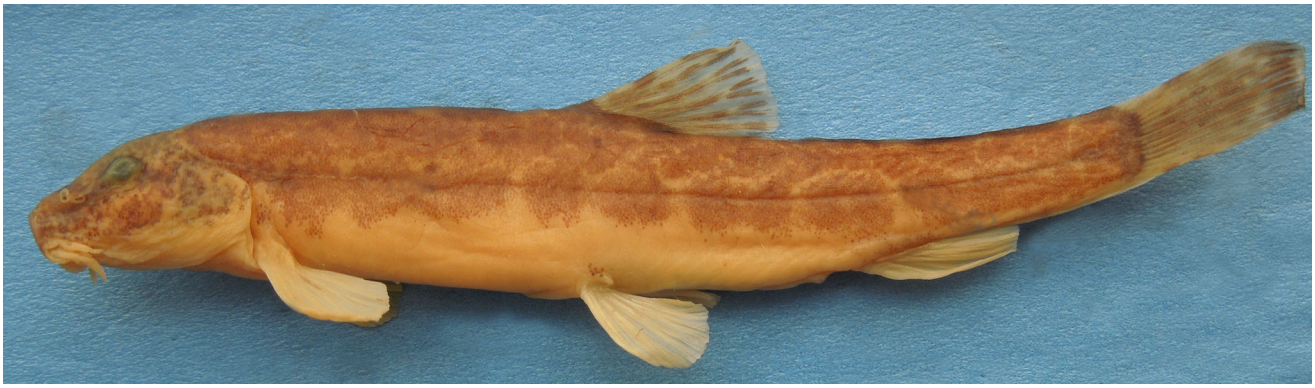

Fig. 1. *Sabanejewia maeotica* sp. nov., holotype ZMMU P-24519 78.5 mm *SL*, female.

RESULTS AND DISCUSSION

A comparative study of the external morphology of the golden loach from the Don basin and other species of the genus *Sabanejewia* made it possible to identify diagnostic characters and prepare a description of the new species, which is given below.

Sabanejewia maeotica sp. nova—common name (proposed in this study): Azov golden loach

Fig. 1, Table 1

Cobitis aurata (non De Filippi, 1863): Rubtsov, 1939. P. 67: Voronezh River; upper Don system; Berg, 1949. P. 894—partim: upper Don River basin.

Cobitis aurata balcanica (non Karaman, 1922): Movchan, 1982. P. 32—partim: Seversky Donets; Movchan, 1988. P. 28—partim: Seversky Donets.

Sabanejewia aurata aurata (non De Filippi, 1863): Pushkin, 1988. P. 82: Khoper River.

Sabanejewia aurata (non De Filippi, 1863): Vasil'eva, 1998. P. 102—partim: Don River basin.

Sabanejewia baltica (non Witkowski, 1994): Kottelat, Freyhof, 2007. P. 322—partim: Don drainage; Shandikov, Goncharov, 2008. P. 82—partim: Seversky Donets; Boldyrev et al., 2021. P. 83, 84: upper section of the Tsimlyansk Reservoir and its tributaries Chir and Ilovlya; Ivanchev et al., 2013. Pp. 32, 105: upper Don system.

Holotype. ZMMU P-24519, *TL* 92.0 mm, *SL* 78.5 mm, Don River at Donskoye, Lipetsk region, Russia, 28.05.2005, collectors V. Vasil'ev and E. Vasil'eva.

Paratypes. ZMMU P-21830, 11 specimens *TL* 50.7–90.3 mm, *SL* 42.7–77.6 mm, collected together with the holotype.

Non-type specimens. P-24060, 4 specimens *TL* 66.0–88.3 mm, *SL* 55.0–75.0 mm, Stanovaya Ryasa River at Blagie city, 19.08.2011, collector V. Sarychev (vouchers for DNA study in Vasil'eva et al., 2022); P-24061, 6 specimens *TL* 51.5–63.2 mm, *SL* 43.8–54.8 mm, Moskovaya Ryasa River, 02–06.08.2011, collector V. Sarychev (vouchers for DNA study in Vasil'eva et al., 2022); P-24062, 6 specimens *TL* 58.8–76.5 mm, *SL* 49.0–65.0 mm, Stanovaya Ryasa River at Shilkino village, 18.08.2011, collector V. Sarychev (vouchers for DNA study in Vasil'eva et al., 2022).

Additional materials (partially studied, only some characters were analyzed): P-21419, 87 spec., *TL* 58.0–94.5 mm, *SL* 48.5–80.5 mm, Don River at Donskoye, Lipetsk region, Russia, 20–30.08.2004, collector V. Babenko; P-21485, 45 spec., *TL* 79.0–98.0 mm, *SL* 66.7–84.3 mm, Sosna River at Yelets city, Lipetsk region, 27.05.2005, collector V. Vasil'ev; P-21514, 25 spec., *TL* 43.5–70.0 mm, *SL* 36.0–60.0 mm, the estuary of the Seversky Donets, 05.10.2005, collector V. Luzhnyak; P-22693, 1 spec., *TL* 94.0 mm, *SL* 80.2 mm, Khupta River, Volga River system et Miloslavskoye village, Ryazan region, 28.06.2007, collector E. Ivancheva.

Diagnosis. A species of *Sabanejewia* s. stricto. A midlateral row of relatively large 9–16 (more often 13–14) dark blotches along flank usually well developed; no black line along flank; interspaces between midlateral blotches usually with dark pigmentation; some individuals with uniform dark lateral coloration; dorsal fin origin usually in front of pelvic origin; body relatively low: body depth noticeably less than the length of the caudal peduncle, usually does not reach 17% *SL,* it is contained more than 6 times in *SL* (6.2–8.2); dorsal adipose crest on caudal peduncle very weakly developed, ventral crest poorly visible; two black spots at caudal fin base usually merge with each other.

Description. *D* III 6½–7½ (usually 6½–7), *A* III 5–6 (usually 5½), *P* I 7½–8½ (usually 7½–8), *V* I 5½–6½ (usually 6), *C* I 11–12(13) I (usually 12); vertebrates (according to Rubtsov, 1939 and Pushkin, 1988) 39–42, in average 39.1– 0.8; sp. br. (8–12) (Movchan, 1982; Pushkin, 1988; this study). Morphometric characters are presented in Tables 1, 2. Body elongated, laterally compressed, not deep. Dorsal fin origin usu-

Table 1. Morphometric data of *Sabanejewia maeotica* sp. nov. (holotype, holotype together with paratypes, and non-type materials) and *Sabanejewia baltica* of similar body length

Character	*S. maeotica*			*S. baltica*	
	holotype	types ($n = 11$)*	non-types ($n = 16$)**	large specimens ($n = 10$)***	small specimens ($n = 15$)****
Total body length (*TL*, mm)	92.0	69.0–92.0 84.1 + 1.93	51.5 – 88.3 65.9 + 2.28	82.0–97.0 90.2 + 1.90	58.5 – 81.2 74.8 + 1.67
Standard body length (*SL*, mm)	78.5	48.0 – 78.5 71.4 + 1.71	43.8 – 75.0 55.6 + 1.95	70.8–85.2 77.3 + 1.69	49.0–69.0 62.9 + 1.46
			In % *SL*		
Predorsal length (*aD*)	48.4	46.5–49.1 47.7 + 0.28	44.2–49.2 47.0 + 0.40	46.2–52.3 49.2 + 0.54	44.4–51.9 49.1 + 0.56
Postdorsal length (*pD*)	40.6	40.6–45.9 44.0 + 0.47	41.7–46.2 43.8 + 0.37	38.8–43.6 41.1 + 0.47	37.9–45.7 42.3 + 0.57
Preventral length (*aV*)	47.1	45.3–48.9 47.5 + 0.35	43.4–51.5 47.8 + 0.55	48.6–51.0 49.9 + 0.28	45.8–52.0 49.1 + 0.47
Preanal length (*aA*)	72.7	69.1–73.9 72.1 + 0.45	64.9–74.5 71.3 + 0.57	71.6–75.7 73.8 + 0.40	70.7–79.1 73.9 + 0.51
Length of caudal peduncle (*pc*)	18.9	17.8–20.9 19.4 + 0.31	17.4–21.5 19.6 + 0.28	16.8–17.8 17.5 + 0.10	15.2–20.2 17.6 + 0.57
Body depth at dorsal fin origin (*H*)	15.5	12.2–16.1 14.4 + 0.34	11.0–16.6 13.9 + 0.39	17.0–20.9 19.0 + 0.47	12.0–15.8 14.3 + 0.31
Depth of caudal peduncle (*h*)	9.2	7.9–11.1 9.1 + 0.26	7.8–10.0 8.8 + 0.18	9.6–12.6 11.1 + 0.33	7.6–10.2 9.0 + 0.20
Length from pectoral fin base to ventral fin base (*P–V*)	28.3	26.2–30.5 28.3 + 0.40	24.3–30.1 28.4 + 0.47	30.1–38.8 32.6 + 0.81	26.8–33.1 29.5 + 0.46
Length of pectoral fin (*lP*)	14.3	13.9–16.4 15.2 + 0.26	15.0–18.6 16.8 + 0.27	14.1–16.9 15.5 + 0.37	14.4–18.4 15.9 + 0.34
Length of pelvic fin (*lV*)	13.0	11.9–14.4 13.5 + 0.23	11.3–16.3 14.4 + 0.35	11.5–14.1 12.8 + 0.31	12.2–16.7 13.8 + 0.28
Length of dorsal fin base (*lD*)	9.2	7.6–10.1 9.1 + 0.21	8.2–11.1 9.4 + 0.22	9.2–11.1 10.4 + 0.19	9.0–11.6 10.3 + 0.24
Depth of dorsal fin (*hD*)	15.5	13.5–15.5 14.6 + 0.23	13.9–16.4 14.9 + 0.21	13.3–15.5 14.5 + 0.21	11.2–18.4 14.7 + 0.38

Table 1. (Contd.)

Character	*S. maeotica*			*S. baltica*	
	holotype	types (n = 11)*	non-types (n = 16)**	large specimens (n = 10)***	small specimens (n = 15)****
Length of anal fin base (*lA*)	7.9	7.3–9.2 8.4 + 0.16	7.3–8.6 7.8 + 0.14	7.2–9.3 8.1 + 0.23	6.6–9.4 8.0 + 0.21
Depth of anal fin (*hA*)	14.3	10.4–14.3 11.8 + 0.34	10.4–13.8 12.1 + 0.21	9.9–12.8 11.8 + 0.33	10.0–13.6 12.2 + 0.25
Caudal fin length (*lC*)	18.7	13.7–20.4 18.0 + 0.58	18.0–21.6 19.1 + 0.22	15.6–18.1 16.9 + 0.24	17.3–20.8 19.4 + 0.28
Body width before dorsal fin (*w*)	7.9	5.0–9.2 7.2 + 0.34	3.8–6.9 5.3 + 0.23	9.9–13.3 11.7 + 0.41	3.8–11.9 7.1 + 0.51
Head length (*HL*)	19.8	18.8–21.5 20.2 + 0.24	18.3–22.2 19.7 + 0.40	18.6–21.4 19.7 + 0.27	17.2–22.3 19.8 + 0.36
			In % *HL*		
Snout length (*ao*)	37.4	33.3–43.5 37.8 + 0.88	32.2–52.2 39.9 + 1.26	37.5–44.9 42.0 + 0.88	36.9–46.9 40.9 + 0.65
Eye diameter (*o*)	21.9	19.1–22.4 20.9 + 0.40	16.9–28.3 20.9 + 0.70	16.4–22.2 19.4 + 0.56	17.4–21.9 19.5 + 0.35
Postorbital length (*po*)	55.5	44.1–56.5 50.8 + 1.17	37.1–59.8 50.0 + 1.37	47.6–51.8 49.8 + 0.43	43.0–58.3 48.8 + 0.97
Length of mandibular barbel (*lB*)	22.6	17.0–24.8 19.9 + 0.98	18.3–25.6 21.9 + 0.55	19.2–29.8 24.9 + 1.04	15.7–26.1 19.5 + 0.86
Head depth (*hc*)	65.8	54.1–69.5 60.8 + 1.28	49.5–76.1 60.0 + 1.79	60.2–73.5 66.5 + 1.45	54.5–70.3 61.9 + 1.09
Interorbital distance (*io*)	13.1	9.8–16.8 13.0 + 0.62	8.9–13.0 10.7 + 0.29	13.1–18.0 15.6 + 0.45	7.8–14.4 11.9 + 0.54
lC, in % *pc*	99.3	76.8–113.6 95.1 + 2.98	89.8–114.8 97.7 + 1.65	88.7–106.7 96.2 + 1.67	86.2–165.0 112.5 + 4.96
h, in % *pc*	48.7	42.3–56.2 47.1 + 1.31	38.4–53.8 44.8 + 1.20	55.6–70.8 63.0 + 1.82	37.8–77.5 52.2 + 2.79
SL, in % *TL*	85.3	83.4–85.9 84.9 + 0.23	82.8–86.7 84.3 + 0.23	84.5–86.3 85.8 + 0.21	82.7–85.5 84.1 + 0.25

Mean values with errors below line and ranges—above line; n, number of specimens; * the smallest specimen with *TL* 50.7 mm is not included in the table; ** specimens from samples ZMMU P-24060–24062; *** specimens from sample ZMMU P-23074; **** specimens from samples ZMMU P-21220, P-23739, P-24011, P-24223, P-24227.

Table 2. Data on some morphometric characters in large females and males of *Sabanejewia maeotica* sp. nov. from the sample P-21485 (mean values with errors and ranges, $M \pm m$) and from the Upper Don system (mean values with standard deviation and range, $M \pm \sigma$)

Character*	P-21485, Sosna River				Upper Don system ($n = 36$) from Ivanchev et al., 2013	
	Females ($n = 12$)		Males ($n = 10$)			
	range	$M \pm m$	range	$M \pm m$	range	$M \pm \sigma$
TL, mm	85.5–98.0	90.0 ± 1.01	79.0–85.2	82.2 ± 0.69	79.0–101.5	88.0 ± 5.64
SL, mm	71.7–84.3	76.7 ± 0.99	67.5–72.0	69.6 ± 0.57	68.5–88.5	75.9 ± 4.93
			In % *SL*			
pc	17.8–20.2	19.2 ± 0.22	18.6–20.7	19.7 ± 0.24	18.3–24.1	20.7 ± 1.19
H	13.8–16.0	14.6 ± 0.20	13.0–16.8	15.1 ± 0.33	13.3–17.1	15.4 ± 1.06
h	8.4–9.7	8.9 ± 0.12	8.9–10.8	10.0 ± 0.18	6.7–9.2	8.1 ± 0.74
lC	16.0–19.8	17.7 + 0.35	17.3–19.7	18.6 ± 0.23		
h, in % *pc*	43.2–52.4	46.4 ± 0.87	43.4–55.6	50.8 + 1.01		

* Character designation as in Table 1.

ally in front of pelvic origin (57.7%), rarely at the same level (27.0%) or slightly behind pelvic fin origin. Caudal peduncle elongated, well compressed, about the same length as the length of the head; its depth is usually less than 50% of its length; in most individuals the depth of the caudal peduncle is contained in the length of the caudal peduncle more than twice (1.8–2.6). Head relatively short (about 20% *SL*), compressed, with upper profile convex. Eyes moderate in size, superiorly located; interorbital space narrow, convex. Snout relatively long: its length usually about 40% of the head length. Suborbital spine bifid, slightly curved, its anterior branch shorter than posterior one: anterior branch more often reaching center of eye (53.6%), rarely not reaching, and posterior branch usually reaching the posterior edge of pupil (42.9%), or reaching up to the end of eye (17.9%), rarely ends in front of the posterior edge of pupil (17.9%). Mouth small, inferior, with fleshy lips; the lower lip is divided into two well-developed parts, its mental lobes are short, deeply folded, without any barbel-like outgrowths (Fig. 2). Three pairs of short barbels: rostral, maxillary, and mandibular; mandibular barbel rarely reaching center of eye (17.9%), in some individuals not reaching anterior edge of eye (10.7%). Dorsal adipose crest on the caudal peduncle is very weakly developed, ventral crest poorly visible, only before caudal fin base. The caudal fin slightly notched. Body covered with very small scales; scales at dorsal-fin base elongated, with a large not displaced focal zone and small number of transverse grooves. According to our data, adult females and males can reach 84.3 and 72.0 mm *SL* and 98.0 and 85.2 mm *TL*, respectively (Table 2). The largest fish from the Upper Don system collected by Ivanchev et al. (2013) had 101.5 mm *TL* and 88.5 mm *SL*.

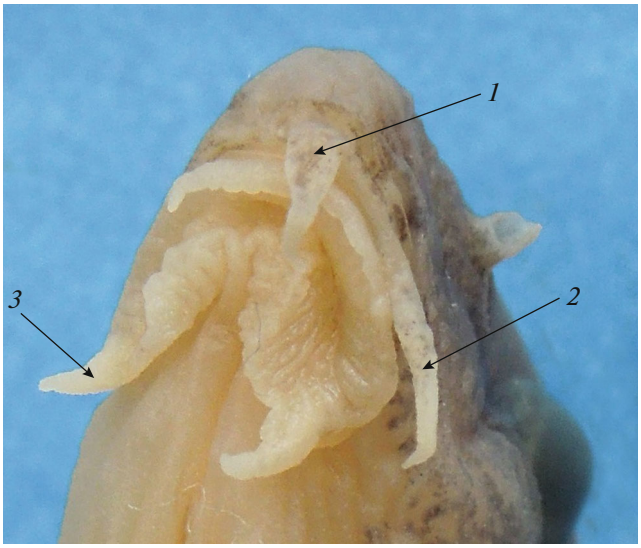

Fig. 2. *Sabanejewia maeotica* sp. nov., head of female ZMMU P-21485 84.3 mm *SL*, ventral view: *1*, rostral barbel; *2*, maxillary barbel; *3*, mandibular barbel.

Coloration in alcohol. The background coloration is light beige in preserved individuals. Head with small dark brown speckles on the back and sides; a narrow whitish saddle behind the head usually with dark brown spots. On the body, small dark brown speckles are usually organized in one dorsal and two lateral longitudinal zones. The dorsal zone is usually represented by 10–15 (more often 13) mid-dorsal blotches separated by lighter interspaces formed by rarer dots; in some specimens, the dorsal blotches have partially or completely merged. The upper lateral zone between the midlateral blotches and the dorsal zone consists of numerous small dots, forming a marbled or more or less uniform dark pigmentation. In some individuals, both lateral zones are combined into a uniform dark lateral coloration. Two narrow bracket-like spots at the caudal fin base usually merge with

each other. Dorsal and caudal fins with numerous brown dots along rays; similar small dots are sporadically found on the rays of other fins.

Sexual dimorphism. Females are somewhat larger than males; adult males have vertical swellings of the flanks in front of dorsal fin origin (Fig. 3).

Distribution. *S. maeotica* occurs in the Don River basin, including its tributaries: the Seversky Donets, Khoper, Sosna, and Voronezh; it is widespread in small rivers of the Upper Don basin (according to Ivanchev et al., 2013, it is common in the Moskovaya Ryasa). Unlike the freshwater duck mussel (another supposed invader from the Pliocene Azov refugium), *S. maeotica* did not inhabit Prikuban lowland, including the Kuban River basin. *S. kubanica*, common in these water bodies, differs well from *S. maeotica* both in morphology and genetic features. Probably, the settlement of the ancient Don lineage of *Sabanejewia* from the Azov refugium in the Prikuban region was limited by the earlier colonization of this region by *S. kubanica* or by geographical barriers between river systems.

It should be noted that *S. baltica* was recorded in the Khupta River (Ivancheva, 2008), a tributary of the Oka River (Volga River system). The author believed that the spined loach could get into the Volga basin from the Don basin through a system of canals or during floods (the tributaries of the Don and Oka are located in close proximity). The present analysis of the specimen from the Khupta River (P-22693) confirms that it belongs to *S. maeotica*. This female corresponds to its species diagnosis and has a poorly developed dorsal adipose crest, 14 large spots in midlateral row, body depth 15.0% *SL*, caudal peduncle length 19.8% *SL*, etc. Probably as a result of the observed invasion, *S. maeotica* may be found in the future in other parts of the Volga system.

Etymology. The species name *maeotica* is derived from the ancient Latin name Maeotis of the modern Sea of Azov.

Comparative remarks. The new species differs from *S. caspia* and *S. larvata,* identified as a separate subgenus *Andrzewia* Vasil'eva, Solovyeva et Vasil'ev, 2022, by a longer caudal peduncle, a longer snout and usually developed large dark blotches on the sides of the body: both mentioned species have a short caudal peduncle (its length does not exceed 18% *SL*), a short snout (usually less than 40% of the head length) and a continuous longitudinal dark stripe along the midline of the flanks, formed by small dark speckles or numerous merged small spots (Vasil'eva et al., 2022). The species *S. caucasica* differs from *S. maeotica* in its specific color pattern with marbled pigmentation along flanks, while *S. romanica* is distinguished by the presence of a fine black line along the flank through midlateral blotches, and *S. aurata* by the origin of dorsal fin behind the origin of pelvic fins (Vasil'eva and Poznyak, 1986; Kottelat and Freyhof, 2007; Vasil'eva and Vasil'ev, 2019). In contrast to *S. maeotica,* in *S. vallachica* the two black spots at the caudal fin base are well separated and the distance between them is greater than their diameter, while *S. kubanica* and *S. aralensis* (Kessler, 1877) have interspaces between midlateral blotches with very few or no dark speckles. Moreover in *S. kubanica* mandibular barbel is long (about 30% of head length), often reaching posterior margin of eye or further (21.9%), dorsal adipose crest on caudal peduncle is well developed, and the mental lobes of the lower lip are not folded, and in *S. aralensis* black spots at the caudal fin base are often separated (Vasil'eva and Vasil'ev, 1988; Kottelat and Freyhof, 2007; this study).

S. bulgarica differs from the new species in a smaller number of midlateral blotches (4–9, rarely up to 10) and dorsal spots (5–8, rarely up to 10), a deeper body (16.5–20.0% *SL*) and specific coloration of the body above the midlateral blotches, with few dark spots or unpigmented, in contrast to the marbled or more or less uniform dark pigmented zone in *S. maeotica*. In *S. balcanica* interspaces between midlateral blotches without dark pigmentation, the body above midlateral blotches is brown with whitish to yellowish marbling or vermiculation, and the dorsal adipose crest on caudal peduncle is well developed (Bănărescu et al., 1972; Vasil'eva and Vasil'ev, 1988; Kottelat and Freyhof, 2007). In addition to these morphological differences, *S. maeotica* is well separated from most *Sabanejewia* species (with the exception of *S. aurata* and *S. caucasica*) in a phylogenetic tree based on cyt *b* sequences (Vasil'eva et al., 2022).

The most important is the comparative analysis of *S. maeotica* and *S. baltica,* since these allopatrically distributed species were previously considered conspecific. According to this study, the genetic divergence of *S. maeotica* is accompanied by the following set of morphological differences. Unlike *S. maeotica*, in *S. baltica* the dorsal adipose crest on the caudal peduncle is well developed (Rolik, 1960, 1967; Penczak, 1969; Movchan, 1982; Witkowski, 1994; present study). The larger individuals of *S. baltica* with *SL* > 70 mm have a noticeably deep body (Fig. 4): the body depth before the dorsal fin origin usually exceeds the caudal peduncle length and 17% *SL* and is contained less than 6 times in *SL* (4.8–5.8). The *CD* between type specimens of *S. maeotica* and large specimens of *S. baltica* (Table 1) for *H* in %*SL* is 1.75. Unfortunately, in various previous publications, the studied individuals of *S. baltica* were small, very rarely exceeding 75 mm *SL*, while their *H* varied from 13.2 to 17.9% *SL* (average 14.8–15.9) in different populations of the Black Sea basin (Rolik, 1967; Movchan, 1988 (as *C. aurata balcanica*)) and from 12.0 to 17.9 (average 13.5–14.7) in the Baltic Sea basin (Rolik, 1960; Frankiewicz, 1985; Witkowski, 1994). While even in the largest specimens of *S. maeotica* the only individual had *H* 17.1% *SL*, and the average values never

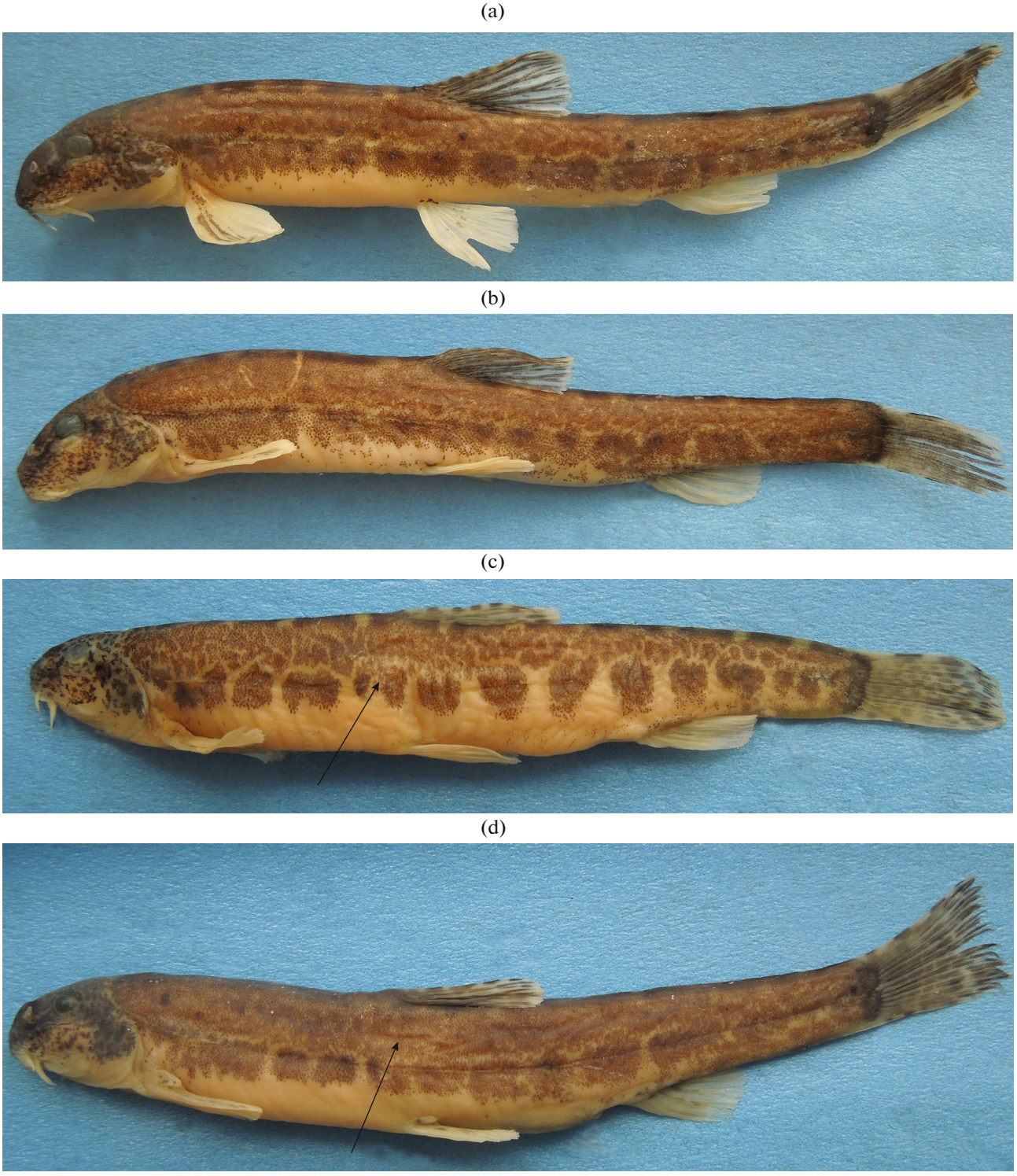

Fig. 3. *Sabanejewia maeotica* sp. nov., large adults ZMMU P-21485: (a, b) females 90.2 and 91.0 mm *TL*, respectively; (c, d) males 78.0 and 82.5 mm *TL*, respectively, with marked swellings of the flanks (→).

reach 15.5 (Tables 1, 2). The specimens of *S. baltica* from our materials also had a shorter and deeper caudal peduncle (Fig. 4, Table 1): *CD* between type specimens of *S. maeotica* and large individuals of *S. baltica* was 1.48 and 1.57, respectively. But these last characters are not confirmed as diagnostic according to the data of previous studies (Rolik, 1960, 1967; Frankiewicz, 1985; Movchan, 1988; Witkowski, 1994).

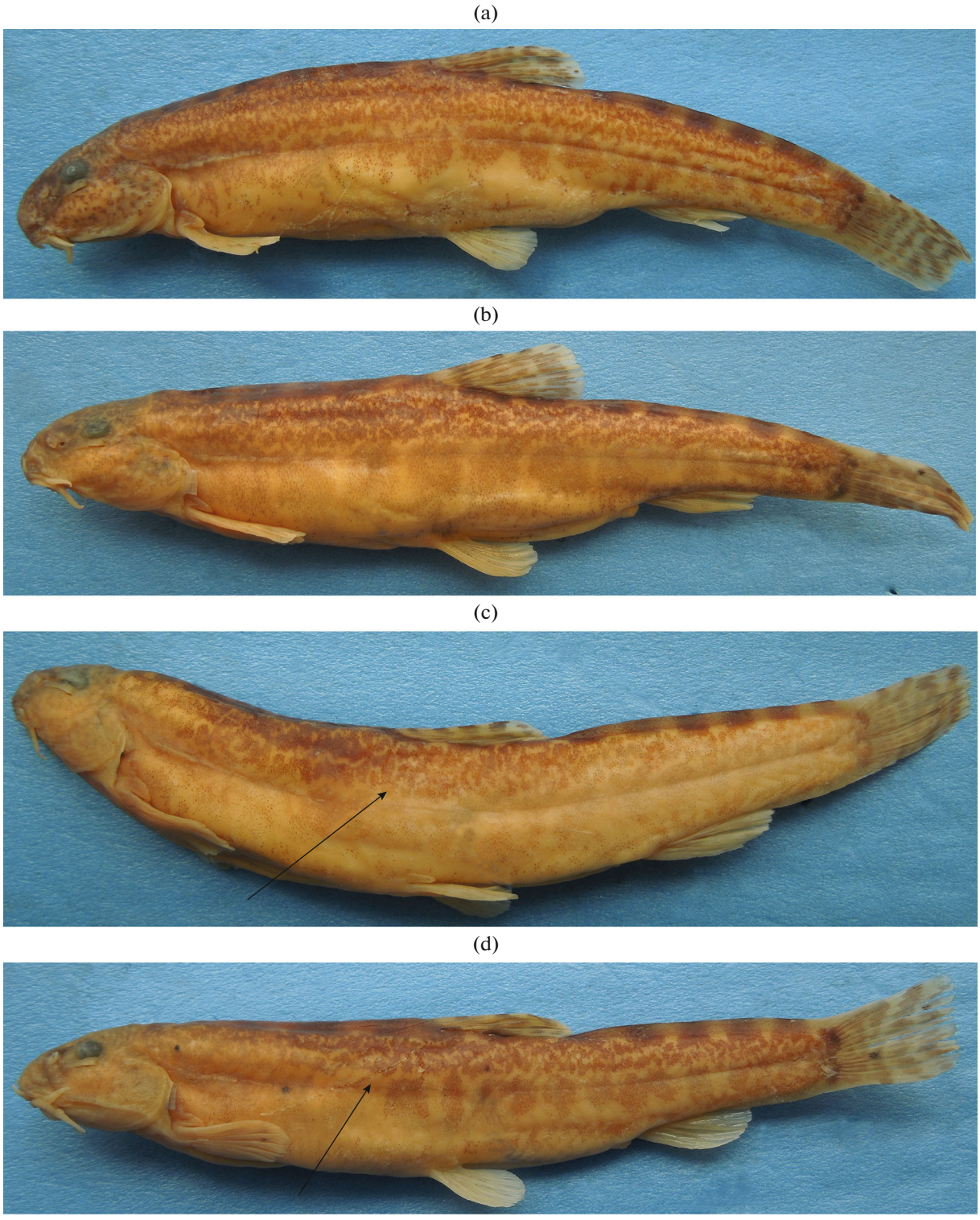

Fig. 4. *Sabanejewia baltica,* large adults ZMMU P-23074: (a, b) females, 90.0 and 95.3 mm *TL*, respectively; (c, d) males, 89.3 and 83.3 mm *TL*, respectively, with marked swellings of the flanks (→).

In conclusion, we would like to emphasize once again that *S. maeotica* and *S. baltica* are allopatric species. Their reproductive isolation is maintained by geographic barriers and therefore does not require deep morphological differentiation in addition to the developed genetic divergence, which is detected both by mtDNA markers and by RAG-1 (Vasil'eva et al., 2022). Nevertheless, the identified interspecies mor-

phological differences are quite sufficient for their diagnosis.

It would be logical enough to complete this work with an identification key for species of the genus *Sabanejewia*. However, up today, diagnostic characters of nominal species from the phylogenetic Danube-Balkan group (Perdices et al., 2003; Vasil'eva et al., 2022), which includes such species as *S. radnensis* (Bănărescu, Müller et Nalbant, 1960), *S. bulgarica, S. montana* (Vladykov, 1925), *S. doiranica* Economidis et Nalbant, 1996, and *S. thrakica* Economidis et Nalbant, 1996 combined with *S. balcanica* whose haplotypes are widespread among haplotypes of other nominal taxa in Central and Southern Europe (Ludwig et al., 2000; Perdices et al., 2003; Bartoňovà et al., 2008; Buj et al., 2008; Križek et al., 2020; Vasil'eva et al., 2022) seem not successfully developed. Moreover, our preliminary analysis suggests that morphological and genetic variability of the species identified as *S. aurata* and *S. aralensis*, as well as the synonymy of *S. aurata,* requires further detailed study. We therefore present below a provisional key for most (but not all accepted) *Sabanejewia* species based on the key developed by Kottelat and Freyhof (2007) with our latest modifications.

PROVISIONAL KEY TO SPECIES OF *SABANEJEWIA* (EXCLUDING THE DANUBE-BALKAN SPECIES GROUP)

1a. Marbled pigmentation or lines along flanks, not forming midlateral row of dark blotches **2**

1b. A midlateral row of dark blotches along flanks..... **3**

2a. A narrow continuous dark brown stripe along flank; no large dorsal blotches; lower lips with two barbel-like processes on inside of each lobe ..... ***S. caspia***

2b. Marbled pigmentation along flank not forming a stripe; dark blotches on the back, often fused; lower lips without two barbel-like processes on inside of each lobe ..... ***S. caucasica***

3a. A fine black line along flank, running through a longitudinal row of narrow, vertically elongated midlateral blotches ..... ***S. romanica***

3b. No black line along flank **4**

4a. A row of 15–27 small rounded midlateral blotches, often fused into an irregular stripe; ventral and dorsal adipose crests on caudal peduncle similarly developed; a dark spot on lower lip; at least one row of spots directly below midlateral blotches; caudal peduncle short, less than 18% *SL*..... ***S. larvata***

4b. A row of 7–23 midlateral blotches; ventral adipose crest on caudal peduncle absent or less developed than dorsal one; no dark spot on lower lip; no row of small spots directly below midlateral blotches; caudal peduncle relatively long, usually exceeding 18% *SL* **5**

5a. Dorsal fin origin usually behind pelvic origin..... ***S. aurata***

5b. Dorsal fin origin usually in front or above pelvic origin..... **6**

6a. Distance between two black spots at caudal fin base greater than their diameter..... ***S. vallachica***

6b. Distance between two black spots at caudal fin base much less than their diameter or these spots merge with each other **7**

7a. Interspaces between midlateral blotches usually with dark pigmentation; some individuals with uniform dark lateral coloration..... **8**

7b. Interspaces between midlateral blotches usually with very little or no dark pigmentation..... **9**

8a. Dorsal adipose crest well developed; maximum body depth usually exceeds caudal peduncle length and 17% *SL* (contained less than 6 times in *SL*) in individuals with *SL* > 70 mm..... ***S. baltica***

8b. Dorsal adipose crest very weakly developed; maximum body depth less than caudal peduncle length, usually does not reach 17% *SL* (contained more than 6 times in *SL*) ..... ***S. maeotica***

9a. Usually 7½ branched dorsal and 7½ branched pectoral fin rays; two black spots at caudal fin base usually merge with each other; Kuban River basin ..... ***S. kubanica***

9b. Usually 6½ branched dorsal and 6½–7½ branched pectoral fin rays; two black spots at caudal fin base often separated with a gape of 1–2 rays; Aral Sea basin .....***S. aralensis***

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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