

Research Article

Seminemacheilus dursunavsari, a new nemacheilid species (Teleostei: Nemacheilidae) from Turkey

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Abstract: *Seminemacheilus dursunavsari*, new species, is described from the Goksu River drainage, Eastern Mediterranean Basin, Konya Province, Turkey. This species is distinguished from the other members of the genus by a combination of the following characters: having irregular molted dark black spots and blotches on flanks, lacking blotches and spots on dorsal faces of head and ventral part of body, possessing fused black spots at base of caudal fin, a saddle-like black spot on the dorsal-fin origin, and blunt snout. *Seminemacheilus dursunavsari* n. sp. is also diagnosed from *S. lendlii*, *S. ispartensis* and *S. ahmeti* by 13 fixed, diagnostic nucleotide substitutions in the mtDNA COI barcode region, and a K2P genetic distance of 5.8, 5.6 and 4.7%, respectively.

Keywords: Loaches, Inland water, Molecular analysis, Taxonomy.

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Introduction

The genus *Seminemacheilus* Bănărescu & Nalbant (1995) is endemic to Turkish inland waters with three species viz. *S. lendlii* (Hanko, 1925), *S. ispartensis* Erk'akan, Nalbant & Özeren, 2007, and *S. ahmeti*, Sungur, Jalili, Eagderi & Çiçek, 2018 described from the Eskişehir (Sakarya Basin), Isparta (Antalya Basin) and Sultan Marshes (Kayseri, Kızılırmak Basin), respectively (Banărescu & Nalbant 1995; Kuru 2004; Erk'akan et al. 2007; Çiçek et al. 2015, 2018; Sunger et al. 2018). The members of this genus are distinguished by having a deep and moderately compressed body, short lateral line, blunt snout, small eyes, and adipose crest on the caudal peduncle (Bănărescu & Nalbant 1995). A morphological and molecular comparison of the collected specimens of the genus *Seminemacheilus* from the Alanözü Dam Lake, Goksu River drainage, Eastern Mediterranean Basin, Konya Province, revealed that this population could not be identified as any described species. Hence, this study aimed to describe this new species

of the genus *Seminemacheilus* based on differences found.

Materials and Methods

Sampling and morphological study: The specimens were collected by electrofishing device. After anesthesia, fish were fixed in 5% buffered formaldehyde or directly fixed in 96% ethanol for molecular work and then those formaldehyde fixed specimens were stored in 70% ethanol. Measurements were made using a digital caliper to the nearest 0.1 mm. All measurements were made point to point, never projections. Methods for counts and measurements follow Kottelat & Freyhof (2007). Standard length (SL) is measured from the tip of the snout to the end of the hypural complex. The length of the caudal peduncle is measured from behind the base of the last anal-fin ray to the end of the hypural complex, at mid-height of the caudal-fin base. The last two branched rays articulating on a single pterygiophore in the dorsal and anal fins are counted

as "1½".

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'-TAGACTTCTGGGTGGCCAAAG AATCA-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 H₂O. 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 purification Kit (Bioneer, Inc, Daejeon, Korea). The PCR products were sequenced using Sanger method by a robotic ABI-3130xl sequencer using manufacturer's protocol (Macrogen, Inc, Daejeon, Korea). The forward and reverse primers were used to single strand sequencing.

Molecular data analysis: We newly generated six DNA barcodes and the sequences were compared to the published *Seminemacheilus* sequences using basic local alignment search tool (BLASTn) (Altschul et al. 1990). The retrieved sequences of the other members of the genus *Seminemacheilus* 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 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 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 6 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, four simultaneous Monte Carlo

Markov Chains were ran for 10000000 generations, sample frequency every 1000 generations, chain temperature 0.2. Log-likelihood stability was attained after 10000 generations, and the first 1000 trees were excluded 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 K2P+G4 model. The genetic distances were investigated based on Kimura two-parameter (K2P) distances (Kumar et al. 2008). As outgroups, *Oxyemacheilus namiri* and *O. atili* (accession numbers: KJ553891 and KJ553726, respectively) were retrieved from GenBank.

Results

Based on the results, the Bayesian and ML analyses yielded similar topologies, therefore one tree was presented (Fig. 1). The results revealed that those specimens of *S. lendlii* from the Sakarya Basin are placed in the same clad with that *S. ispartensis* from its type locality (Antalya Basin) as well as the Baysehir and Aksehir Lakes (Konya Endorheic and Akarçay basins, respectively). In addition, *S. lendlii* shows a K2P genetic distance of 0.2% with *S. ispartensis*, suggesting *S. ispartensis* as a junior synonym of *S. lendlii*. *Seminemacheilus dursunavsari* n. sp. is placed as sister taxon of *S. ahmeti* with K2P genetic distance of 4.7% and these two share the same ancestor with *S. lendlii* + *S. ispartensis*, with K2P genetic distance of 5.6-5.8 showing well-supported species. Furthermore, *S. dursunavsari* new species. possesses 13 fixed, diagnostic nucleotide substitutions in the mtDNA COI barcode region.

Seminemacheilus dursunavsari, new species

(Figs. 2–6, Tables 2, 3)

Holotype: NUIC-1811, male, 53.0 mm SL, Turkey: Konya prov.: Input of Alanözü Dam Lake, Goksu River drainage, Eastern Mediterranean Basin, 37°07'48.8"N, 32°42'19.3"E, 19 August 2018.

Paratypes: NUIC-1812 (1-20), 20, 52.4-76.3 mm SL;

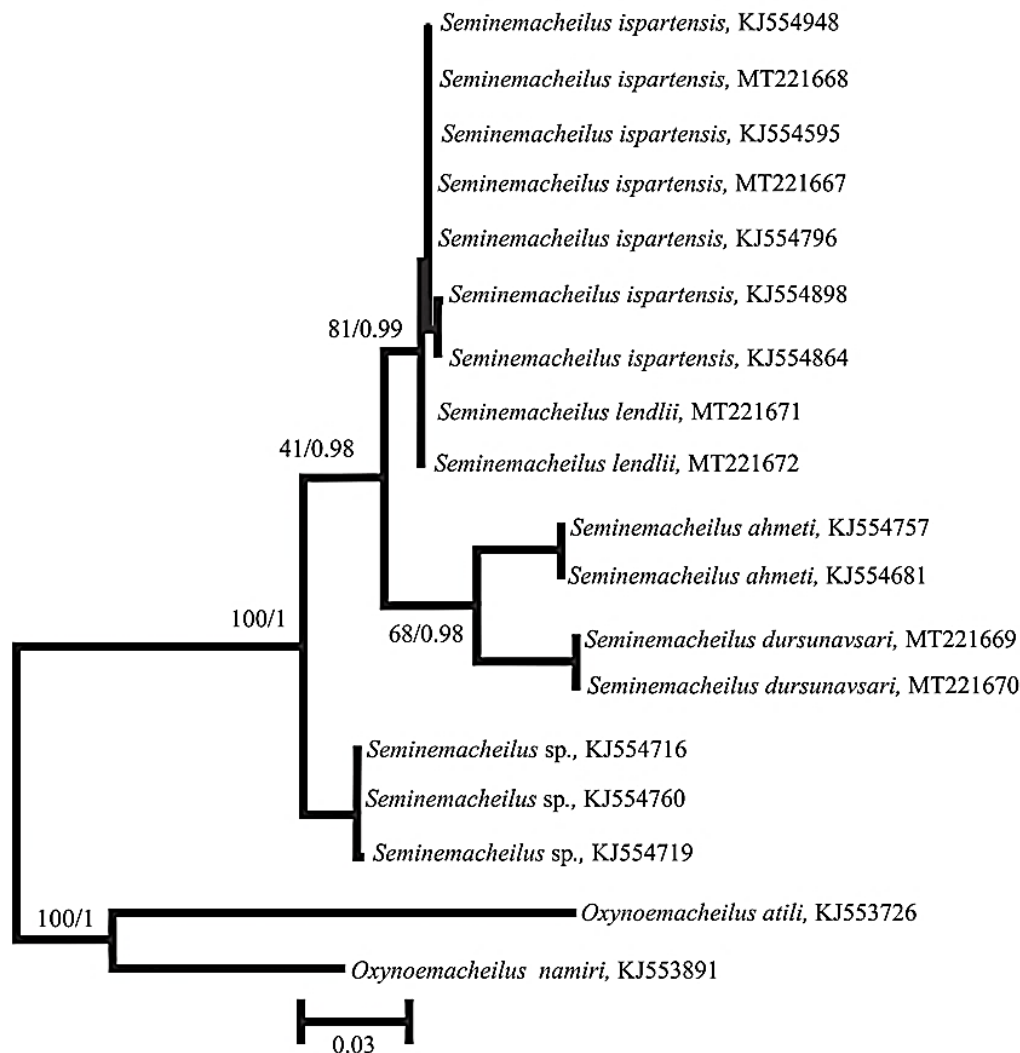


Fig.1. Maximum likelihood estimation of the phylogenetic relationships based on the mitochondrial COI barcode region. Values at nodes correspond to ML bootstrap / BI posterior probability.

data same as holotype.

Diagnosis: *Seminemacheilus dursunavsari* n. sp. is distinguished from *S. lendlii* and *S. ispartensis* by having irregular molted dark black blotches on flanks (vs. possessing a clear strip on mid-lateral of flank with-irregular spots its above and below), a saddle-like spot at base of dorsal-fin origin (vs. absent), blunt snout (vs. pointed snout i.e. tapering toward its anterior end), fused spots at base of caudal fin (vs. absent), 13 fixed, diagnostic nucleotide substitutions in mtDNA COI barcode region, and a K2P genetic distance of 5.6-5.8%.

Seminemacheilus dursunavsari n. sp. is distinguished from *S. ahmeti* by lacking spots and

blotches on dorsal face of head or having small spots (vs. having large dark black spots and blotches), pale ventral part of body without colour pattern (vs. having many irregularly scattered spots), wider caudal peduncle (5.5-7.1 vs. 2.2-4.3 %SL), 13 fixed, diagnostic nucleotide substitutions in mtDNA COI barcode region, and a K2P genetic distance of 4.7%.

Description: See Figures 2-6 for general body appearance and Tables 2 and 3 for morphometric and meristic data. Body stout, deep and short with broad head and blunt snout in dorsal and lateral views. A little hump at nape as deepest part of body; eye medium in size; mouth arched, a slight indication of processus dentiformis, upper lip smooth, thick lower



Fig.2. Live picture of *Seminemacheilus dursunavsari* new species, Uncatalogued, Turkey: Konya prov.: Input of Alanözü Dam Lake, Goksu River drainage, Eastern Mediterranean Basin.



Fig.3. *Seminemacheilus dursunavsari* new species, NUIC-1811, male, holotype, 53.0mm SL, Turkey: Konya prov.: Input of Alanözü Dam Lake, Goksu River drainage, Eastern Mediterranean Basin.

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

No	Species	Accession no.
1	<i>Seminemacheilus ispartensis</i>	KJ554948
2	<i>Seminemacheilus ispartensis</i>	KJ554864
3	<i>Seminemacheilus ispartensis</i>	KJ554796
4	<i>Seminemacheilus ispartensis</i>	KJ554898
5	<i>Seminemacheilus ispartensis</i>	KJ554595
6	<i>Seminemacheilus</i> sp.	KJ554816
7	<i>Seminemacheilus</i> sp.	KJ554719
8	<i>Seminemacheilus</i> sp.	KJ554960
9	<i>Seminemacheilus ahmeti</i>	KJ554681
10	<i>Seminemacheilus ahmeti</i>	KJ554757
11	<i>Oxynoemacheilus atili</i>	KJ553726
12	<i>Oxynoemacheilus namiri</i>	KJ553891

Table 2. Morphometric and meristic data of *Seminemacheilus dursunavsari* n. sp. (Holotype, NUIC-1811; paratypes, NUIC-1811, 20 specimens).

Morphometric characters	Holotype	Holotype+Paratype	
		range	mean± SD
Standard length (mm)	53.0	52.4-76.3	63.0±6.3
In percent of standard length			
Body depth maximal	18.8	16.7-20.0	18.2±0.9
Caudal peduncle depth	14.7	13.0-15.7	13.8±0.6
Predorsal length	54.1	49.5-54.7	51.9±1.3
Postdorsal length	36.4	35.8-40.2	38.1±1.2
Prepelvic length	56.6	52.0-58.0	54.4±1.4
Preanal length	80.0	72.0-87.5	77.1±2.9
Caudal peduncle length	12.7	14.2-17.8	15.7±0.9
Dorsal-fin base length	11.2	10.3-13.8	12.2±0.8
Dorsal-fin depth	23.8	18.9-25.0	20.3±1.5
Anal-fin base length	11.2	8.3-11.1	9.5±0.6
Anal-fin depth	20.6	15.9-19.3	17.3±1.0
Pectoral-fin length	28.5	15.3-28.9	21.4±3.8
Pelvic-fin length	14.9	11.0-14.6	12.1±0.9
Pectoral – pelvic-fin origin distance	30.3	28.5-32.3	30.7±1.3
Pelvic – anal-fin origin distance	21.6	20.0-23.7	21.9±1.0
Caudal-fin length	24.5	18.3-22.9	21.2±1.1
Body width	13.1	13.1-16.0	14.8±0.8
Caudal peduncle width maximum	5.7	5.5-7.1	6.4±0.5
Head length	24.6	23.0-26.1	24.1±0.8
In percent of head length			
Snout length	37.2	33.8-44.2	40.0±2.6
Eye horizontal diameter	16.4	13.8-17.7	15.7±1.1
Postorbital distance	45.9	40.0-50.8	48.2±2.4
Head depth at nape	67.9	52.6-71.4	65.1±4.5
Head width	64.1	49.4-70.5	64.8±4.5
Inter Orbital	34.7	32.0-42.3	38.2±2.6
Inter nasal	23.8	24.7-32.3	29.3±2.3
Mouth width	35.9	30.1-41.5	38.0±2.6
Inner rostral barbel	30.4	23.9-38.5	30.0±3.4
Outer rostral barbel	41.0	30.1-43.2	36.7±3.6
Maxillary barbel	39.8	32.8-45.6	38.3±3.3



Fig.4. *Seminemacheilus dursunavsari* new species, paratypes (above) NUIC–1812-2, 56.8mm SL, and (below) NUIC–1812-7, 67.9mm SL, Turkey: Konya prov.: Input of Alanözü Dam Lake, Goksu River drainage, Eastern Mediterranean Basin.



Fig.5. *Seminemacheilus dursunavsari* new species, paratypes (above) NUIC–1812-2, 56.8mm SL, and (below) NUIC–1812-7, 67.9mm SL, Turkey: Konya prov.: Input of Alanözü Dam Lake, Goksu River drainage, Eastern Mediterranean Basin.



Fig.6. *Seminemacheilus dursunavsari* new species, paratypes (above) NUIC–1812-2, 56.8mm SL, and (below) NUIC–1812-7, 67.9mm SL, Turkey: Konya prov.: Input of Alanözü Dam Lake, Goksu River drainage, Eastern Mediterranean Basin.

Table 3. Meristic data of *Seminemacheilus dursunavsari* new species, Goksu River drainage, Eastern Mediterranean Basin (Holotype, NUIC-1811; paratypes, NUIC-1811, 20 specimens).

Branched dorsal-fin rays				
N	8½	9½	mode	
20	8	12	9	
Branched anal-fin rays				
N	6½	7½	mode	
20	15	5	6	
Pelvic-fin rays				
N	6	7	mode	
20	13	7	6	
Pectoral-fin rays				
N	11	12	mode	
20	17	3	11	
Caudal-fin rays				
N	17	18	19	mode
20	2	7	11	19

**Fig.7.** Type locality and natural habitat of *Seminemacheilus dursunavsari* new species, Turkey: Konya prov.: Input of Alanözü Dam Lake, Goksu River drainage, Eastern Mediterranean Basin.

lip markedly furrowed with a deep median incision; barbels long, outer rostral barbel reaching to origin of maxillary barbel or vertical to middle of eye, inner one not reaching to anterior margin of eye, and maxillary barbel reaching behind vertical of posterior margin of eye. Anterior nostril opening flap-like tube

and posterior one close to anterior margin of eye.

Pectoral fin pointed reaching approximately 0.60-0.85% of distance between pectoral and pelvic fins' origins passing origin of dorsal-fin in males; pelvic-fin origin below 3th or 4th branched rays of dorsal fin, usually not reaching to anus, without an axillary

lobe; anus about eye diameter in front of anal-fin origin; Anal- fin origin vertical to anterior half between dorsal-fin insertion and caudal-fin dorsal origin and not reaching caudal fin origin; distal edge of dorsal and anal fins convex; caudal-fin almost truncate; dorsal fin with 3-4 unbranched and 8½-9½ branched rays, anal fin with 4 unbranched and 6½-7½ branched rays, pectoral fin with 11-12 and pelvic-fin with 6-7 branched rays, caudal fin with 8+8 or 9+9 rays. Caudal peduncle length 0.95-1.35 times of its depth with short to moderate dorsal and ventral adipose crest; dorsal crest reaching to vertical anal-fin insertion; males with a longer pectoral fin; lateral line not complete, reaching to anterior one-third of pectoral fin length (with 5-8 pores); body scaleless.

Coloration: Body light grey in dorsal part and yellowish ventrally. Head in dorsal part without spots and blotches, or having tiny spots and yellowish plain ventrally. Dorsum plain grey without any colour pattern in midline, and with a large saddle-like black spot at dorsal-fin origin. Irregular molted dark black blotches on flanks, smaller and densely covered in anterior part and larger fused sometimes on caudal peduncle area. Ventral part of body plain yellowish without colour pattern, with some tiny spot around base of anal fin. Caudal fin with 3-4 dark black bands on rays, pelvic fin hyaline without colour pattern, pectoral and anal fins with small spots mostly laterally and dorsally, respectively, and anal fin with tiny spots on its base. Barbels grey with tiny spots close to their bases.

Sexual dimorphism: Males possess longer pectoral fin.

Distribution and Habitat: *Seminemacheilus dursunavsari* new species is known from input of the Alanözü Dam Lake, Goksu River drainage, Eastern Mediterranean Basin, Konya Province, Turkey. This species inhabits in small stream with sandy and clay bottoms covered by dense vegetation (Fig. 7).

Etymology: The new species is named after Prof. Dr. Dursun Avsar (Cukurova University, Adana) for his supports as my supervisor.

Collection codes: NUIC, Ichthyology Collections of

Nevşehir Hacı Bektaş Veli University, Nevşehir, Turkey.

Comparative materials for morphological analyses: All from Turkey. *Seminemacheilus lendlii*: NUIC 1814, 15, 43.7-60.7mm SL; Afyonkarahisar prov.: A spring canals at Hacibeyli Village, Sakarya Basin, 39°03'20.3"N 30°16'49.2"E, 18 August 2018.

Seminemacheilus ahmeti: NHVUIC 1990-01-16, 21, 40.1-53.2 mm SL; Kayseri prov.: Sultan Marshes near Yeşilova Village, Kızılırmak Basin, 38°12'05.26"N 35°13'19.76"E; 12 March 2017. — IMNRFI-UT-3016, 5, 43.0-51.2 mm SL; data same as NHVUIC 1990-01-16.

Seminemacheilus ispartensis: NUIC 1813, 10, 36.1–67.6 mm SL; Isparta prov.: Sevinçbey village, Eğirdir-Isparta road, Antalya Basin, 37°52'22"N 030°46'47"E, 18 August 2018. — NUIC 1815, 4, 43.5-54.6mm SL; Isparta prov.: Akkeçili village, Input of Eğirdir Lake, Konya Basin, 38°08'47"N 030°44'11"E, 18 August 2018.

Materials used for molecular analyses: All from Turkey: *Seminemacheilus ispartensis*: NUIC 1813-2f, Isparta prov.: Sevinçbey village, Eğirdir-Isparta road, Antalya Basin, 37°52'22"N 030°46'47"E, 18 August 2018. GeneBank Accession number (MT221668). — NUIC 1815-1f, Isparta prov.: Akkeçili village, Input of Eğirdir Lake, Konya Basin, 38°08'47"N 030°44'11"E, 18 August 2018. GeneBank Accession number (MT221667).

Seminemacheilus lendlii: NUIC 1814-2f; Afyonkarahisar prov.: A spring canals at Hacibeyli Village, Sakarya Basin, 39°03'20.3"N 30°16'49.2"E, 18 August 2018. GeneBank Accession number (MT221671, MT221672).

Seminemacheilus dursunavsari n. sp. Konya prov.: Input of Alanözü Dam lake, Goksu River drainage, Eastern Mediterranean Basin, 37°07'48.8"N, 32°42'19.3"E, 19 August 2018. GeneBank Accession number (MT221669, MT221670).

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Correction: During proof editing, the collection code subtitle was missed by IJI, and later based on the journal policy under authors' responsibilities ([Publication Ethics](#)); after notifying by author, it was inseted.

مقاله پژوهشی

Seminemacheilus dursunavsari (ماهیان استخوانی عالی: ماهیان بدون خار جویباری)

یک گونه جویبارماهی جدید از ترکیه

اردوغان چیچک

گروه زیست‌شناسی، دانشکده هنر و علوم، دانشگاه حاجی بکتاش ولی نوشهیر، نوشهیر، ترکیه.

چکیده: گونه *Seminemacheilus dursunavsari* به عنوان یک گونه جدید از رودخانه گوک‌سو، حوضه آبریز مدیترانه شرقی، استان قونیه ترکیه در این مقاله توصیف می‌گردد. این گونه از دیگر اعضای این جنس به واسطه داشتن ترکیبی از صفات زیر قابل تشخیص می‌باشد: داشتن لکه‌ها و نقاط سیاه نامنظم شکسته بر روی پهلوها، فقدان لکه و نقاط بر روی سطح پشتی سر و سطح شکم، داشتن نقاط سیاه چسبیده در پایه باله دم، یک لکه سیاه زین-مانند در منشاء باله سینه‌ای و یک پوزه کند. جویبارماهی دورسون‌اوشاری همچنین از گونه‌های *S. lendlia*، *S. ispartensis* و *S. ahmeti* به واسطه داشتن ۱۳ نوکلئوتید جایگزین شده تشخیصی ثابت در ناحیه ژن COI میتوکندریایی و فاصله ژنتیکی به ترتیب ۵/۸، ۵/۶ و ۴/۷ درصد قابل تشخیص است.

کلمات کلیدی: جویبارماهیان، آب‌های داخلی، آنالیز مولکولی، آرایه‌شناسی.