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Turcinoemacheilus ekmekciae, a new dwarf loach from upper Tigris and Euphrates (Teleostei: Nemacheilidae)

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Abstract

Turcinoemacheilus ekmekciae, new species, from upper Euphrates and Tigris drainages is distinguished from other species of *Turcinoemacheilus* in Western Asia by having a dark stripe broader than the eye diameter along the lateral line, rarely possessing roundish blotches, 5–6 mandibular pores in mandibular canal, a comperatvely smaller head, a deeper body, and a greater pre-pelvic distance. Our specimens collected from the upper Great Zab, near the type locality of *Turcinoemacheilus kosswigi*, showed notable genetic divergence (a minimum K2P of 3.3%) from sequences reported as *T. kosswigi* in previous studies. Despite morphological similarities, this molecular difference suggests that the populations analysed in previous studies may represent a potential new species of *Turcinoemacheilus*, which we tentatively named as *Turcinoemacheius* cf. *kosswigi*. Molecular data also suggest that *T. ekmekciae* is characterized by a minimum K2P distance of 3.5% from *Turcinoemacheilus minimus* and *T.* cf. *kosswigi*. The three methods for species delimitation (assemble species by automatic partitioning [ASAP], Poisson tree processes [PTP], and multi-rate PTP [mPTP]) that were utilized for testing species assignments consistently identified our test group as a distinct species.

KEYWORDS
cytochrome c oxidase I, freshwater fish, taxonomy, Western Asia

1 | INTRODUCTION

The family Nemacheilidae, comprising more than 600 freshwater fish species inhabiting rivers and streams throughout Asia, Europe, and Ethiopia, exemplifies the high biodiversity of Cypriniformes (Kottelat, 2012). Being the second-largest family within this order, it represents a significant proportion of the Western Palaearctic's freshwater fauna. It is interesting to note that almost a quarter of nemacheilid species, or unique loaches, are situated in Western Asia, thus highlighting the taxonomic wealth of this geographic area.

Until the early 2010s, Nemacheilidae was one of the most taxonomically challenging groups. The taxonomic problems associated with the groups belonging to the family have been largely resolved with the studies carried out in recent years. Recent molecular (Bektaş et al., 2022; Geiger et al., 2014) and morphological (Kottelat, 2012) studies, such as generic revisions (Esmaeili et al., 2014; Freyhof et al., 2014, 2015; Yoğurtçuoğlu et al., 2020), identification of new taxa (e.g., Freyhof

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et al., 2016; Freyhof et al., 2019; Freyhof, Kaya, et al., 2021; Freyhof, Yoğurtçuoğlu, & Kaya, 2021; Kaya et al., 2021; Kaya, Turan, Bayçelebi, et al., 2020; Kaya, Turan, Kalayci, et al., 2020; Yoğurtçuoğlu, Kaya, & Freyhof, 2021; Yoğurtçuoğlu, Kaya, Özuluğ, & Freyhof, 2021; Yoğurtuoğlu et al., 2022), and taxonomic re-evaluations (Freyhof et al., 2012) have supported the resolution of these problems.

However, despite these advancements, it is widely recognized that the taxonomic classification of this family remains incomplete, particularly regarding the less-studied genera such as *Turcinoemacheilus*. These diminutive fish are challenging to come across during field samplings due to their small size, cryptic colouration, and their preference for the swiftest parts of rivers (Breil & Bohlen, 2001). Nevertheless, once captured, they are readily distinguishable by their slender body and the unique anterior placement of their anus. Historically, this genus, first described by Banarescu and Nalbant (1964), was initially thought to be monotypic, with the sole representative being the Zagros dwarf loach (*Turcinoemacheilus kosswigi*). The discovery of an additional species from this genus in the Himalayas suggests that their distribution may be wider than previously thought. Consequently, further discoveries enhanced our understanding, and it is now acknowledged as a polytypic genus, by the addition of *Turcinoemacheilus hafezi* (Golzarianpour et al., 2013) described from Karoun and Dez River drainages (Iran), *Turcinoemacheilus bahaii* described from Zayandeh River, an endorheic river in Central Iran, *Turcinoemacheilus saadii* from tributaries of Karkheh (Iran), and *Turcinoemacheilus minimus*, from the upper Euphrates drainage in Türkiye (Esmaeili et al., 2014). Over the course of a decade, these findings have revealed a previously undiscovered diversity within this genus, emphasizing the importance of a more thorough exploration of its taxonomic richness. This can be achieved through integrative taxonomy, which employs multiple lines of evidence, including newly established genetic approaches that investigate gene variability and morphological examination. To achieve this objective, our explorations in the upper streams of the Tigris and Euphrates led us to uncover previously unknown populations of *Turcinoemacheilus*. Therefore, employing an integrative approach formulated earlier, we aimed to investigate whether these newly found populations of *Turcinoemacheilus* in the upper Tigris and Euphrates drainages might represent a previously undescribed species. During our expeditions in search of comparative material, we discovered additional populations from the Kahta stream in the upper Euphrates. Given their morphological similarity, we hypothesized that they belonged to *T. minimus*. Therefore, our secondary objective was to test whether the new population we collected from the Kahta stream matched both morphologically and genetically to *T. minimus* from the upper Euphrates. Given the long history and evolutionary complexity of the Nemacheilidae family, this study holds immense promise in advancing our comprehension of this captivating group of organisms.

2 | MATERIALS AND METHODS

The care and use of experimental animals was in accordance with Republic of Türkiye animal welfare laws, guidelines, and policies as approved by the Animal Experiments Local Ethics Committee of RTE University (2020/04).

Following anesthesia, fish specimens were fixed in 5% formaldehyde and stored in 70% ethanol. Alternatively, to examine DNA material, specimens were directly preserved using 99% ethanol. Measurements were made using a dial caliper and recorded to an accuracy of 0.1 mm. All measurements were made point-to-point, never through projections. Methods for counts and measurements adhere to the established protocols outlined in Kottelat and Freyhof (2007), further elaborated by Freyhof et al. (2019), and the nomenclature of head pores followed Kottelat (1990). Standard length was measured from the tip of the snout to the posterior extremity of the hypural complex. The length of the caudal peduncle was measured from the posterior part of the base of the last anal-fin ray extending to the posterior extremity of the hypural complex. This was measured at the mid-height of the caudal-fin base. The final pair of branched rays articulating on a single pterygiophore in the dorsal and anal fins was counted as "1½". Simple rays of dorsal- and anal fins were not counted as they were deeply embedded.

Morphological data from Golzarianpour et al. (2013) and Esmaeili et al. (2014) were used to compare *Turcinoemacheilus bahaii*, *Turcinoemacheilus hafezi*, and *T. saadii*.

2.1 | Abbreviations used

SL, standard length; HL, head length; K2P, Kimura 2-parameter. Collection codes: FFR, Recep Tayyip Erdogan University Zoology Collection of the Faculty of Fisheries, Rize.

2.2 | DNA extraction, PCR, and sequencing

Genomic DNA was extracted from muscle tissue following the instructions provided by the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) protocol. The amplification of the standard vertebrate DNA barcode region, cytochrome c oxidase subunit 1 (COI), was performed using forward primer FishF1 (5′-TCAACCAACCACAAAGACATTGGCAC-3′; Ward et al., 2005) and the reverse primer FishR1 (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′; Ward et al., 2005). PCR protocol was performed using Qiagen Multiplex as follows: 33.8 µL of sterile double-distilled water (ddH2O), 1 µL of each primer (10 pmol/µL), 6 µL of deoxyribonucleoside triphosphate (dNTP) (10 mM), 5 µL of 10× PCR buffer with Mg^{2+} (100 mM Tris–HCl, pH 8.3, 500 mM KCl), 0.2 µL of Taq polymerase (5 U/µL), and 3.0 µL (50 ng/mL) of DNA template. PCR was carried out as follows: thermal cycling conditions with a first denaturation of 94°C for 4 min, followed by 30 cycles of 94°C for 1 min, 61°C for 30s, 72°C for 1 min, and a final extension of 72°C for 7 min. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). The bidirectional sequencing of the purified PCR products was performed with FishF1 and FishR1 primers used in amplification according to the Sanger method with an ABI PRISM 3730 × 1 Genetic Analyser using a BigDye Terminator 3.1 cycle sequencing ready reaction kit (Applied Biosystem) at Macrogen Europe.

2.3 | Molecular analyses

To perform molecular analysis, we used 13 newly generated DNA barcodes and 23 existing ones from the NCBI GenBank database (Table 1). Following sequencing, the chromatograms of the raw COI sequences were thoroughly inspected using the Bioedit 7.2.5 (Hall, 1999) software. Any anomalies or errors recognized in this process were manually rectified. The next step involved initial comparisons with established reference data catalogued within a global database, which was accomplished by employing nBLAST (Basic Local Alignment Search Tool) on GenBank. The dataset included all COI sequences corresponding to the genus *Turcinoemacheilus* listed in the aforementioned database. After the alignment of the final data file by the Clustal W method (Thompson et al., 1994), sequences were trimmed from both the forward and reverse ends to ensure a consistent length across all sequences.

To establish the root of the phylogenetic hypothesis, we incorporated the DNA barcodes from individual specimens of *Seminemacheilus tubae* (MT077010), *Oxynoemacheilus nasreddini* (MW916396), *Sasanidus kermanshahensis* (KU928288), and *Paracobitis malapterura* (KJ723511) as out-groups. To determine the most suitable evolutionary model for our data and reconstruct the mitochondrial relationships among the taxa under investigation, we employed the sequence evolution model test present within the MEGA X software (Kumar et al., 2018). We followed the principle that the model with the lowest BIC (Bayesian information criterion) scores usually represents the substitution pattern most accurately. The K2P + G model was found to be the most appropriate for nucleotide substitution in COI (5 categories [+G, parameter = 0.1859]). Phylogenetic trees were constructed using neighbor-joining (Saitou & Nei, 1987), maximum parsimony (Swofford, 2003, with PAUP4b), and maximum likelihood (ML) methods with 1000 bootstrap replicates in MEGA X (Kumar et al., 2018) to investigate mitochondrial lineage relationships. During this process, we ensured that any positions exhibiting less than 98% coverage were excluded. We estimated evolutionary divergence between sequences using the Kimura 2-parameter (K2P) model to produce comparable data with other studies that use standard DNA barcoding metrics. Moreover, for the purpose of investigating species boundaries through an integrative approach, we employed several species delimitation techniques, including two tree-based methodologies: The Poisson tree processes (PTP) and its enhanced version, the multi-rate PTP (mPTP). Both methods relied on a tree topology that was constructed using an ML framework. In addition, we adopted a distance-based method namely the Assemble of Species by Automatic Partitioning (ASAP) to augment our investigation into the delineation of species boundaries. As per PTP and mPTP, the objective was to identify a delimitation for a group that optimizes the likelihood of the partition of branch lengths in PTP, using a uniform evolutionary rate (lambda) and different rates for each group (species) in the mPTP model. The AIC is utilized in place of the *p*-value test to determine the number of groups that best fit the given topology and branch lengths, to avoid excessive over-splitting into multiple groups (Kapli et al., 2017; Zhang et al., 2013, http://mptp.h-its.org/#/tree [accessed May 17, 2023]). ASAP presents a novel approach that defines species partitions by utilizing a hierarchal clustering algorithm that is grounded on pair-wise genetic distances that are derived from single-locus sequence alignments (Puillandre et al., 2021). This particular process employs the pair-wise genetic distances to formulate a comprehensive list of partitions that are subsequently ranked by a score. The score is determined through the probabilities of groups being panmictic species. Therefore, ASAP provides a neutral mechanism for outlining pertinent species hypotheses and serves as the preliminary stage in the process of integrative taxonomy. This method mitigates the Automatic Barcode Gap Discovery (ABGD)'s constraints by not needing a preset intraspecific diversity limit and by offering users an ASAP score per partition to aid in species delimitation choice.

3 | RESULTS

3.1 | Key for the genus *Turcinoemacheilus*

1a. Anus situated behind middle between pelvic-fin and anal-fin origins.

.....2

TABLE 1 Matrix displaying the minimum interspecific genetic distances for the genus *Turcinoemacheilus* using the Kimura 2-parameter model, with gray shaded boxes representing the maximum intraspecies genetic variability for each species.

Species	*T. ekmekciae*	*T. minimus*	*T. kosswigi*	*T.* cf. *kosswigi*	*T. saadii*	*T. bahaii*	*T. hafezi*
T. ekmekciae	0.60						
T. minimus	3.52	0.60					
T. kosswigi	4.20	5.06	0.20				
T. cf. *kosswigi*	3.51	3.94	3.31	1.62			
T. saadii	5.96	6.65	5.97	5.92	1.01		
T. bahaii	11.12	11.12	12.15	10.41	11.42	0.00	
T. hafezi	11.40	12.17	12.69	11.91	11.96	3.50	0.40

1b. Anus situated at or in front of middle between pelvic-fin and anal-fin origins.

.....3

2a. An elongated, irregularly shaped dark blotch on sides of anal-fin base; anal-fin origin situated at vertical of tip of dorsal fin when adpressed to body.

.....*T. bahaii*.

2b. No dark blotch on side of anal-fin base; anal-fin origin situated behind vertical of tip of dorsal fin when adpressed to body.

.....*T. hafezi*.

3a. Lateral stripe or row of blotches absent along lateral midline, 7–9 distinct dark saddles on body.

.....*T. saadii*.

3b. Prominent row of dark brown blotches along lateral midline, usually fused into a lateral stripe.

.....4

4a. Caudal peduncle length 6–7% SL; 4–5 mandibular pores in mandibular canal.

.....*T. minimus*.

4b. Caudal peduncle length 7%–9% SL; 5–7 mandibular pores in mandibular canal.

.....5

5a. A dark mid-lateral stripe narrower than eye diameter throughout lateral line; pre-pelvic distance 47%–50% SL; upper caudal fin lobe 18%–21% SL; maxillary barbel 21%–26% HL.....*T. kosswigi*.

5b. A mid-lateral stripe thicker than eye diameter present throughout lateral line, rarely possessing roundish blotches; prepelvic distance 50%–53% SL; upper caudal fin lobe 14%–18% SL; maxillary barbel 15%–21% HL.

.....*T. ekmekciae*.

3.2 | *Turcinoemacheilus ekmekciae*, new species

3.2.1 | Holotype

FFR 3608, 36 mm SL; Türkiye: Muş Prov.: stream Kaynarca at Kalecik, Murat drainage, 39.1519N 41.3534E.

3.2.2 | Paratypes

FFR 3624, 7, 31–37 mm SL; FFR 3615, 14, 31–46 mm SL; FFR 3617, 5, 27–33 mm SL: same data as holotype.—FFR 3603, 7, 48–54 mm SL: Türkiye: Bitlis Prov.: stream Çıratan at 3 km southwest of Üçadım, Yanarsu drainage, 38.3547N 41.7814E.—FFR 3604, 1, 48 mm SL; FFR 3611, 7, 35–47 mm SL; FFR 3616, 5, 36–45 mm SL; Türkiye: Şırnak Prov.: stream Nerduş about 7 km south-west of Şırnak, 37.4755N 42.3739E.—FFR 3610, 1, 29 mm SL; FFR 3618, 3, 29–30 mm SL; Türkiye: Batman Prov.: stream Sason (Han) at Cevizli, Batman drainage, 38.2885N 41.2879E.—FFR 3613, 4, 29–50 mm SL; Türkiye: Bitlis Prov.: stream Destumi at Tanrıyar, Botan drainage, 38.2258N 41.8825E.

3.2.3 | Additional material

FFR 3602, 2, 52–55 mm SL; Türkiye: Bitlis Prov.: stream Oraniz at Ekinli, 38.1386N 42.4303E.—FFR 3609, 1, 55 mm SL; Türkiye: Bitlis Prov.: stream Oraniz at Dönertaş, Botan drainage, 38.3158N 42.5653E.

3.2.4 | Genetic material

FFR DNA-Tur1; Türkiye: Bitlis Prov.: stream Çıratan at 3 km south-west of Üçadım, Yanarsu drainage, 38.3547N 41.7814E (GenBank accession number: OQ758274). FFR DNA-Tur7, 9; Türkiye: Bitlis Prov.: stream Destumi at Tanrıyar, Botan drainage, 38.2258N 41.8825E (GenBank accession numbers: OQ758275, OQ758279). FFR DNA-Tur18, 19; Türkiye: Şırnak Prov.: stream Nerduş about 7 km south-west of Şırnak, 37.4755N 42.3739E (GenBank accession numbers: OQ758276, OQ758283). FFR DNA-Tur11, 38; Türkiye: Batman Prov.: stream Sason (Han) at Cevizli, Batman drainage, 38.2885N 41.2879E (GenBank accession numbers: OQ758278, OQ758282). FFR DNA-Tur4, 5, 6; Türkiye: Muş Prov.: stream Kaynarca at Kalecik, Murat drainage, 39.1519N 41.3534E (GenBank accession numbers: OQ758280, OQ758281, OQ758277).

3.2.5 | Diagnosis

Turcinoemacheilus ekmekciae is distinguished from its congeners from upper Tigris and Euphrates drainages by a combination of characters. The new species is distinguished from *T. minimus* from Göksu and Kahta drainages by having a deeper caudal peduncle (7%–9% SL *v.* 6–7), and 5–6 mandibular pores in mandibular canal (*v.* 4–5). *T. ekmekciae* is distinguished from *T. kosswigi* from upper Greater Zab by having a greater pre-pelvic distance (50%–53% SL *v.* 47–50), a shorter upper caudal fin lobe (14%–18% SL *v.* 18–21), and a shorter maxillary barbel (15%–21% HL *v.* 21–26). The new species is further distinguished from *T. kosswigi* by having a dark stripe broader than the eye diameter along the lateral line, only in one population possessing roundish blotches (*v.* a dark-brown mid-lateral stripe narrower than the eye diameter).

Turcinoemacheilus ekmekciae is distinguished from the two Iranian *Turcinoemacheilus*, *T. bahaii* and *T. hafezi*, by the position of the anus, which is situated in the middle or in front of midpoint between pelvic and anal fins (*v.* situated behind the midpoint between pelvic and anal origins). The new species is further differentiated from *T. bahaii* by the lack of dark-brown blotch on each side of the anal-fin base (*v.* present). *T. ekmekciae* is further distinguished from *T. hafezi* by having a smaller head (17%–20% SL, *v.* 20–23), a slenderer body (body depth at dorsal-fin origin 11%–14% SL, *v.* 15–17), and a shorter and slenderer caudal peduncle (its length 16%–19% SL, *v.* 19–23; its depth 7%–9% SL, *v.* 9–11).

The new species is distinguished from *T. saadi* by having a dark stripe broader than the eye diameter along the lateral line, only in one population possessing roundish blotches (*v.* 7–9 distinct saddles on

flank, lateral stripe, or row of blotches absent along lateral midline). It further differs from *T. saadi* by having a longer pre-dorsal length (55%–59% SL, *v.* 52–55), a shorter post-dorsal length (32%–37% SL, *v.* 37–40), and a longer pre-anal length (75%–78% SL, *v.* 70–75).

3.2.6 | Description

For general appearance see Figures 1–4; morphometric data are provided in Table 2. Small-sized and slender species. Head short, body depth at dorsal-fin origin 1.3–1.7 times in HL. Pre-dorsal profile slightly convex, pre-pelvic profile straight. Body deepest and widest at mid-point of pre-dorsal distance, depth decreasing toward caudal-fin base. No hump at nape. Section of head roundish, flattened on ventral surface, straight or slightly convex in interorbital space, distinctly convex on snout. Snout pointed. Caudal peduncle compressed laterally, 1.8–2.7 times longer than deep. Pelvic axillary lobe present, its tip not attached to body. Pelvic-fin origin in front of dorsal-fin origin. Pectoral fin reaching 41%–55% of distance from pectoral-fin origin to pelvic-fin origin. Pelvic fin reaching beyond anus. Distance from anus to anal-fin origin 0.4–0.5 times in distance from pelvic-fin to anal-fin origins. Anal-fin origin posterior to vertical to tip of dorsal fin when folded backwards. Anal fin not reaching to middle of caudal peduncle. No adipose crest on caudal peduncle. Margin of dorsal fin straight. Caudal fin slightly emarginate. Largest known specimen 55 mm SL.

One central and one lateral pores on each side of supratemporal canal, 6–8 pores in anterior infraorbital canal, 3–4 pores in posterior infraorbital canal, 7–9 pores in supraorbital canal, and 5–6 pores in mandibular canal. No suborbital flap or groove in male.

Dorsal fin with 6–7½ (usually 6½) branched rays. Anal fin with 5½ branched rays. Caudal fin with 8 + 8 or 8 + 7 branched rays. Pectoral fin with 8(9) and pelvic fin with seven branched rays. Body without scales. Lateral line incomplete, with 18–28 pores, usually extends to the midpoint of area between tip of pectoral fin and dorsal-fin origin.

FIGURE 1 *Turcinoemacheilus ekmekciae*, FFR 3608, holotype, 36 mm standard length (SL); Kaynarca stream.

Anterior nostril opening at end of a pointed flap-like tube. Posterior nostril oval, posterior tip of anterior nostril not, or just overlapping posterior nostril when folded backwards. Mouth small, slightly arched (Figure 1). Lips moderately thick. A median interruption in lower lip. Upper lip without median incision. Processus dentiformis small and blunt. No median notch in lower jaw. Barbels short, inner rostral barbel not reaching base of maxillary rostral barbel; outer one reaching to base of maxillary barbel. Maxillary barbel reaching vertical of anterior part of eye. No external sexual dimorphism observed.

3.2.7 | Colouration

Yellowish or cream background in life and formalin preserved individuals. In Yanarsu population, a row of large, irregular, brown blotches along the lateral line and is not in form of a stripe, often fused into a prominent irregular lateral stripe, in other populations, a stripe broader than the eye diameter along the mid-lateral line. Generally, there are no saddles on the back. If present, they are large and brown,

FIGURE 2 *Turcinoemacheilus ekmekciae*, from the top, FFR 3608, holotype, 36 mm standard length (SL); Kaynarca stream, Euphrates: FFR 3616, paratype, 41 mm SL, Nerduş stream, Tigris: Not preserved, ~35 mm SL; not preserved, ~40 mm SL; not preserved, ~40 mm SL; Kaynarca stream, Euphrates.

FIGURE 3 *Turcinoemacheilus ekmekciae*, FFR 3603, paratypes, from the top, 54 mm standard length (SL); 51 mm SL; 51 mm SL; 48 mm SL; Çıratan stream.

FIGURE 4 *Turcinoemacheilus ekmekciae*, paratypes; FFR 3616, from the top, 45 mm standard length (SL); 39 mm SL, Nerduş stream: FFR 3618, 30 mm SL; 30 mm SL; Sason stream.

connected to lateral blotches along the body. On the pre-dorsal back and upper part of the flank sometimes completely covered by pigments and no saddles appear. Flank below lateral stripe without pigmentation. An irregularly shaped, dark-brown or black bar at caudal-fin base.

Dorsal and caudal fins hyaline, with elongated spots on rays, forming one wide row, approximately in median part of the rays. Last unbranched dorsal-fin ray hyaline at base, black at anterior half and hyaline on posterior half. Caudal, anal, and anterior pectoral fins are yellowish or hyaline. Proximal and median part of the rays of caudal and anal fins, and sometimes anterior half of pectoral fin with dark-brown pigments.

Cheeks and ventral surface of head cream or yellow, head above cheeks plain brown to brown.

3.2.8 | Distribution

The new species is currently known from Kaynarca Stream (upper Murat River drainage) as well as from Yanarsu, Botan, Nerduş, Batman River drainages (all upper drainages of the Tigris River). It usually prefers fast-flowing, shallow and clean streams with stone or pebble substrate (Figure 5).

3.2.9 | Etymology

The species is named for Fitnat Güler Ekmekçi for her contribution to the knowledge of the ichthyofauna of Türkiye. A noun in genitive, indeclinable.

3.3 | Phylogenetic relationships

Upon examining the COI sequence data, we found that the *Turcinoemacheilus* populations incorporated in our study into roughly seven taxonomic clusters (Figure 6). These phylogenetic assemblages align with six acknowledged species, thereby indicating a taxonomic complexity within the genus. Phylogenetic analysis was conducted employing three distinct methods (Neighbour-joining [NJ], Maximum likelihood [ML], Maximum Parsimony [MP]), with branch validity assessed through bootstrap values. The tree topologies generated by all three methods were nearly identical (ML-based topology is depicted in Figure 6). Bootstrap test support values yielded moderate to maximum possible support (95–100) for four species-level nodes, yet support was generally lower when it came to interspecies relationships.

The specimens we collected from Eziki Stream (Hakkari, Figure 7), in close proximity to the presumed type locality of *T. kosswigi*, exhibited a significant genetic divergence from those previously sequenced as *T. kosswigi* by Esmaeili et al. (2014) (a minimum of 3.3% K2P distance). Our findings revealed considerable, though not complete, morphological consistency between our sample and the original taxonomic description of *T. kosswigi*. This provided sufficient grounds for us to tentatively identify our Hakkari samples as *T. kosswigi*. On the contrary, the substantial molecular discrepancy observed in the samples examined by Esmaeili et al. (2014) suggested the existence of a yet-undescribed *Turcinoemacheilus* species, which we have tentatively designated as *Turcinoemacheius* cf. *kosswigi*.

TABLE 2 Morphometric data of *Turcinoemacheilus ekmekciae* (holotype FFR 3608 and paratypes FFR 3624, $n = 4$; FFR 3603, $n = 6$; FFR 3615, $n = 5$; FFR 3611, $n = 5$).

		Holotype + paratypes			
	Holotype	Min	Max	Mean	S.D.
Standard length (mm)	36	33	54	43.2	6.3
In percentage of standard length					
Head length	19.1	17.3	20.5	18.8	0.9
Body depth at dorsal-fin origin	14.0	10.8	14.0	12.7	0.9
Pre-dorsal length	56.5	54.6	59.1	56.8	1.3
Postdorsal length	36.3	32.2	36.8	34.8	1.3
Prepelvic length	49.7	49.7	52.8	50.8	1.0
Pre-anal length	75.0	72.7	77.9	75.5	1.3
Distance between pectoral and anal-fin origins	57.8	54.6	59.5	57.5	1.5
Distance between pectoral and pelvic-fin origins	30.6	28.9	35.6	32.6	1.9
Distance between pelvic and anal-fin origins	24.9	22.6	26.6	25.1	1.0
Distance between pelvic-fin origin and vent	9.3	9.3	14.4	11.6	1.6
Distance between vent and anal-fin origin	11.7	9.2	13.4	11.5	1.2
Dorsal-fin height	12.8	12.8	15.7	14.3	0.9
Anal-fin height	12.0	12.0	15.2	13.2	0.9
Pectoral-fin length	14.4	13.4	17.7	15.4	1.2
Pelvic-fin length	12.0	11.5	14.7	12.9	0.9
Length of upper caudal fin lobe	15.0	14.4	17.6	16.3	1.0
Length of middle caudal fin ray	13.7	12.4	15.8	14.0	0.8
Length of caudal peduncle	18.4	15.5	19.2	16.8	1.0
Depth of caudal peduncle	8.1	6.5	9.0	7.8	0.7
In percentage of head length					
Head depth at eye	48	44	50	46.9	2.1
Maximum head width	65	52	66	59.7	4.0
Snout length	45	41	47	43.7	1.5
Eye diameter	18	15	19	17.1	0.9
Postorbital distance	48	43	49	46.2	1.7
Interorbital width	32	23	32	28.3	2.4
Length of inner rostral barbel	16	14	21	16.8	2.1
Length of outer rostral barbel	18	17	23	19.50	1.7
Length of maxillary barbel	20	15	21	19.1	1.5

Our study group, described here as *T. ekmekciae* sp. nov., emerged as a distinct lineage within the broader assemblage of species found in the Upper Tigris and Euphrates drainages. With a minimum genetic divergence of 3.5% from both *T. minimus* and *T.* cf. *kosswigi*, *T. ekmekciae* establishes its unique position within the genus *Turcinoemacheilus*. The minimum COI distance in our dataset ranged from 3.3% (between *T. kosswigi* and *T.* cf. *kosswigi*) to 12.7% (between *T. kosswigi* and *T. hafezi*) (Table 1).

Our additional test group from the Kahta River (Figure 5), located in the Upper Euphrates drainage, yielded identical results in the COI barcode region as *T. minimus* (Figure 8). Furthermore, the morphological characteristics of this species matched, leading to the discovery of a new record of *T. minimus* (Figure 7). This finding has expanded our understanding of its distribution range, extending toward the far eastern region of the Upper Euphrates.

The application of PTP using the ML topology for species delimitation on the studied dataset resulted in disparate estimates of the total number of species present. When the PTP model was employed, it identified seven entities ($p = 0.001$, null-model score: 88.149668, best score for single coalescent rate: 104.923507). Conversely, the utilization of the mPTP model generated a more conservative approximation, recognizing six entities as putative species (wherein *T. bahai* and *T. hafezi* were classified as a single species). The null-model score was determined to be 78.641775, which is identical to the best score for multi coalescent rate, indicating no significant difference between the null and alternative hypotheses in this context. The optimal partition of ASAP, which obtained a score of 2.0, was realized with a K80 Kimura threshold of 0.03%. This partition proposed six entities (Figure 6), exhibiting significant alignment with the results obtained from the PTP model. An exception was noted in the

FIGURE 5 Sampling sites where *Turcinoemacheilus* specimens found in upper Tigris-Euphrates: Habitat of *T. kosswigi*, (a) Eziki stream: Habitats of *T. minimus*, (b) Göksu River, (c) Kahta stream: Habitats of *T. ekmekciae*, (d) Çıratan stream, (e) Destumi stream, (f) Nerduş stream, (g) Sason stream, (h) Kesan stream, and (i) Kaynarca stream.

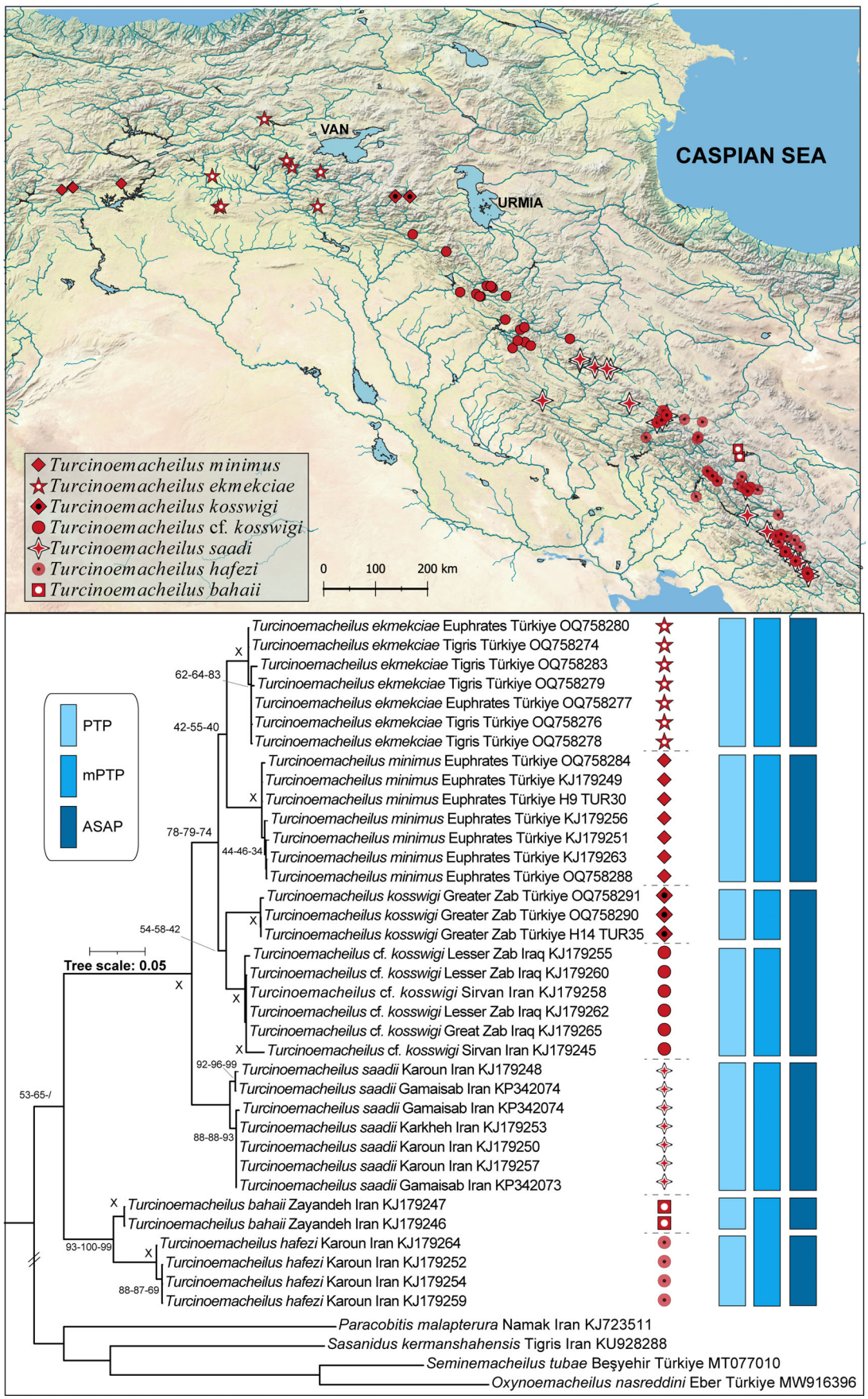

FIGURE 6 Legend on next page.

FIGURE 7 *Turcinoemacheilus kosswigi*, from the top, FFR 3600, 60 mm standard length (SL); Dilektaşı stream: FFR 3601, 53 mm SL; 53 mm SL; 49 mm SL; Eziki stream.

classification of *T. kosswigi* and *T.* cf. *kosswigi*, which ASAP lumped them into one entity. Yet, the subsequent optimal partition from ASAP (score = 2.5) yielded an identical partitioning result to that of the PTP model (Figure 6). Therefore, the congruence of PTP and the subsequent optimal ASAP suggests a closer alignment with morphology-based specimen identification, thus providing robust support for all *a priori* identified species. *Turcinoemacheilus ekmekciae* was identified as a distinct entity through the utilization of all three species delimitation methods.

4 | DISCUSSION

We employed molecular techniques to establish the validity of the recently discovered *T. ekmekciae*. Our investigation thoroughly examined samples from five distinct populations, namely streams of Çıratan, Destumi, Kaynarca, Sason, and Nerduş from the Upper Tigris and Euphrates drainages. We also analysed samples from recognized species within the genus *Turcinoemacheilus*, specifically *T. minimus* and *T. kosswigi*, which were procured explicitly for this study. Moreover, we took into account previously collected data from other valid species, including *T. minimus*, *T. saadii*, *T. hafezi*, *T. bahaii*, and *T. kosswigi*.

FIGURE 8 *Turcinoemacheilus minimus*, from the top, FFR 3607, 46 mm standard length (SL); Göksu River; FFR 3605, 31 mm SL; Kahta stream.

To aid in species identification, we utilized the COI barcode marker, a tool widely acknowledged as effective in species differentiation that has also proven to be particularly useful in assessing members of this genus in previous studies (Esmaeili et al., 2014). Our findings have confirmed that the use of COI barcode marker is an effective tool in distinguishing among *Turcinoemacheilus* species. We also employed the K2P model for calculating the genetic distances between postulated species. Recent literature has presented arguments for and against the utilization of model-corrected genetic distance measures, such as K2P (Srivathsan & Meier, 2012). Specifically, these arguments highlight the potential limitations of such models when applied to closely related COI sequences and in the context of neighbor-joining trees and distance-based identification methods. Our dataset, however, covers a diverse range of interspecific distances, ranging from 3.3% to 12.7% which, may benefit from the more refined evolutionary modelling provided by the K2P distance measure with an ML approach. To assess the robustness of our findings, a parallel analysis was conducted using the uncorrected *P* distance measure. The results indicated strong concordance between the K2P and uncorrected *P* measures. Given these considerations, we concluded that the K2P distance measure is both suitable and informative for this study.

We conducted a collaborative effort to acquire comparative material by locating *T. kosswigi* in its type locality, with the aim of verifying the distinctiveness of our newly discovered population from it. A significant amount of effort was expended in searching *Turcinoemacheilus* specimens from Hakkari, which is believed to be the genuine type locality of *T. kosswigi*. It is worth noting that Esmaeili et al. (2014) conducted a comprehensive review of the *Turcinoemacheilus* genus, utilizing both morphological and molecular data. They identified populations from three locations—the Little Zab, Great Zab,

FIGURE 6 The distribution range of *Turcinoemacheilus* spp. in Western Asia (top). Maximum likelihood (ML) estimation of the phylogenetic relationships (bottom) based on the mitochondrial cytochrome c oxidase subunit 1 (COI) barcode region (Kimura 2-parameter (K2P) model, discrete gamma distribution for rate differences with five categories +G parameter = 0.1859). Nucleotide positions with less than 98% site coverage were eliminated, resulting in 540 analysed positions. Numbers of major nodes indicate bootstrap values of 1000 pseudoreplicates from the ML, MP, and NJ methods. "X" indicates the highest possible support from all three methods (>95). Blue bars to the right of the specimen labels indicate species boundary outcomes from the Poisson tree processes (PTP) followed by the results of the multi-rate PTP (mPTP) approach and the assemble species by automatic partitioning (ASAP) method at the rightmost.

and Sirvan, as *T. kosswigi*. However, a comparative analysis between these populations and the original description of *T. kosswigi* has not been provided, thus offering an opportunity for further investigation. The specimen from the Great Zab region, specifically from the Chami Rean River in Iraq, sampled by Esmaeili et al. (2014) is of great note. It is important to note that this site is located approximately 80 km southeast of the type locality of *T. kosswigi*, which is Kapozik Kadun located in Hakkari (southeastern Türkiye). It seems that Esmaeili et al. (2014) overlooked the possibility that a species found in a different drainage of the same river system and at such close range might not actually be *T. kosswigi*. However, our molecular analysis and morphological investigation provided clear insight into this matter. We found that the population from the upper Great Zab region significantly diverged by the COI sequence data from the material identified as *T. kosswigi* by Esmaeili et al. (2014). Alongside our molecular findings, we conducted a detailed morphological examination of our *T. kosswigi* specimens to further support our hypothesis. Our investigation revealed that most of the morphological characters extracted from the original description of *T. kosswigi* generally agreed with our measurements of the same species, demonstrating a subtle discrepancy. Specifically, the pre-anal distance and the caudal peduncle length of our specimens differed slightly from those detailed in the original description. In our specimens, the pre-anal distance was 74%–78% of SL in contrast to the original description's range of 73%–74%. Likewise, the caudal peduncle length in our sample was 14.2%–17.0% of SL *v.* 18.6%–20.7% in the original description (Table 3). The observed

TABLE 3 Morphometric data of *Turcinoemacheilus kosswigi* (FFR 3600, $n = 1$; FFR 3601, $n = 3$; FFR 3614, $n = 4$) and *Turcinoemacheilus minimus* (FFR 3607 $n = 5$; FFR 3605, $n = 1$).

	T. kosswigi				*T. minimus*			
	Min	Max	Mean	S.D.	Min	Max	Mean	S.D.
Standard length (mm)	48	54	50.6	1.7	29	46	33.9	6.1
In percentage of standard length								
Head length	17.3	18.6	18.0	0.4	18.0	21.3	19.5	1.1
Body depth at dorsal-fin origin	9.3	12.5	11.4	1.0	10.2	12.5	11.4	0.7
Pre-dorsal length	54.1	56.7	55.3	1.0	55.8	60.3	58.1	1.8
Postdorsal length	35.1	37.5	36.3	0.8	32.5	34.8	34.0	0.8
Prepelvic length	47.2	50.4	48.7	1.0	47.1	52.6	51.3	2.2
Pre-anal length	73.7	78.1	76.0	1.4	72.4	79.5	75.5	2.5
Distance between pectoral and anal-fin origins	55.9	60.6	59.5	1.5	56.0	60.9	57.3	1.9
Distance between pectoral and pelvic-fin origins	28.8	32.6	31.8	1.3	30.9	34.8	33.2	1.4
Distance between pelvic and anal-fin origins	24.4	27.6	26.8	1.0	21.5	25.2	23.7	1.4
Distance between pelvic-fin origin and vent	9.9	16.1	12.8	2.3	9.5	12.7	10.5	1.4
Distance between vent and anal-fin origin	10,3	15.2	12.6	1.5	9.4	11.5	10.8	0.8
Dorsal-fin height	14.7	15.6	15.2	0.3	15.5	16.9	16.2	0.9
Anal-fin height	12.0	15.4	14.0	1.2	13.5	15.9	14.4	0.8
Pectoral-fin length	15.5	18.3	16.8	1.0	13.9	17.7	15.7	1.4
Pelvic-fin length	13.9	16.1	15.0	0.7	12.5	15.4	13.9	1.0
Length of upper caudal fin lobe	18.1	20.5	19.1	0.9	15.8	17.9	16.8	0.7
Length of middle caudal fin ray	14.9	16.6	15.8	0.6	13.7	15.6	14.8	0.7
Length of caudal peduncle	14.2	17.0	15.8	0.9	15.3	18.7	16.8	1.2
Depth of caudal peduncle	7.3	8.5	8.0	0.5	5.9	7.4	6.6	0.8
In percentage of head length								
Head depth at eye	37	49	42.7	3.7	37	47	42.7	3.9
Maximum head width	57	60	58.8	1.2	45	63	57.3	6.7
Snout length	39	47	43.0	2.4	37	48	42.7	3.9
Eye diameter	14	17	15.7	1.4	15	21	17.7	2.6
Postorbital distance	42	48	45.2	1.7	43	56	47.4	5.0
Interorbital width	22	28	25.3	1.8	20	30	23.6	3.6
Length of inner rostral barbel	15	21	17.9	2.2	14	16	15.0	0.9
Length of outer rostral barbel	16	26	22.0	3.0	18	22	19.7	1.5
Length of maxillary barbel	21	26	22.8	1.6	18	24	21.0	2.1

slight differences can be attributed to a few factors. It should be noted that these are small-bodied fish and the measurement of certain body parts can be inherently variable, especially if the reference points for measurement are not standardized across studies. Additionally, a larger sample size could yield a broader range of morphometric values, which could account for the slightly expanded ranges observed in our study. Consequently, morphological variations mentioned above, combined with our molecular results, further strengthen the argument that the population from the upper Great Zab region identified by Esmaeili et al. (2014) may represent a distinct species from *T. kosswigi*.

4.1 | Comparative material

Turcinoemacheilus kosswigi FFR 3600, 1, 60 mm SL; Türkiye: Hakkari Prov.: Dilektaşı Stream, 37.6664 N 44.1393E. —FFR 3601, 3, 50–53 mm SL; Türkiye: Hakkari Prov.: Eziki Stream at 6 km northeast Konak, 37.6717 N 43.8628E. —FFR 3614, 5, 50–54 mm SL; Türkiye: Hakkari Prov.: Eziki Stream at 3 km southwest of Akbulut, 37.6884 N 43.8538E.

Turcinoemacheilus minimus FFR 3605, 1, 32 mm SL; Türkiye: Adıyaman Prov.: Gendere stream at 15 km north of Kahta, 37.9173 N 38.6109E. —FFR 3619, 3, 27–31 mm SL; Türkiye: Adıyaman Prov.: Gendere stream at Burmapınar, 37.9331 N, 38.6091E.—FFR 3607, 13, 28–48 mm SL; Türkiye: Adıyman Prov.: Göksu River at 7 km north of Gölbaşı, 37.8443 N 37.6703E. —FFR 3612, 4, 29–41 mm SL; Türkiye: Kahramanmaraş Prov.: Göksu River at Düzbağ, 37.7953 N 37.4704E.

4.2 | Materials used in molecular genetic analysis

Turcinoemacheilus kosswigi FFR DNA-Tur36, 37; Türkiye: Hakkari Prov.: Ezikistream at 3 km southwest of Akbulut, 37.6884 N 43.8538E (GenBank accession numbers: OQ758290, OQ758291; this study). Iraq: Great Zab drainages (GenBank accession number: KJ179265; Esmaeili et al., 2014). Iraq: Little Zab drainages (GenBank accession numbers: KJ179262, KJ179260, KJ179255; Esmaeili et al., 2014). Iran: Sirvan drainage (GenBank accession number: KJ179258; Esmaeili et al., 2014).

Turcinoemacheilus minimus FFR DNA-Tur14, 15; Türkiye: Adıyaman Prov.: Gendere stream at 15 km north of Kahta, 37.9173 N 38.6109E (GenBank accession numbers: OQ758284, OQ758286; this study). FFR DNA-Tur23, 25, 29, 30; Türkiye: Kahramanmaraş Prov.: Göksu River at Düzbağ, 37.7953 N, 37.4704E (GenBank accession numbers: OQ758289, OQ758285, OQ758288, OQ758287; this study). Türkiye: Euphrates River (GenBank accession numbers: KJ179263, KJ179256, KJ179251, KJ179249; Esmaeili et al., 2014).

Turcinoemacheilus saadii Iran: Karoun drainage (GenBank accession numbers: KJ179261, KJ179250, KJ179248, KJ179257; Esmaeili et al., 2014). Iran: Karkheh drainage (GenBank accession number: KJ179253; Esmaeili et al., 2014). Iran: Gamasiab drainage (GenBank accession numbers: KP342073, KP342074; Paknejad et al., 2019).

Turcinoemacheilus hafezi Iran: Karoun drainage (GenBank accession numbers: KJ179252, KJ179254, KJ179259, KJ179264; Esmaeili et al., 2014).

Turcinoemacheilus bahaii Iran: Zayandeh drainage (GenBank accession numbers: KJ179246, KJ179247; Esmaeili et al., 2014).

AUTHOR CONTRIBUTIONS

C.K. conceived and designed the study, contributed fieldwork and laboratory studies, developed figures, drafted the manuscript. B.Y. conceived and designed the study, contributed fieldwork, data analyses and writing the manuscript. İ.A. contributed laboratory studies of the genetic part of the study. E.B. contributed laboratory studies and fieldworks. D.T. conceived and designed the study, contributed fieldwork. All authors read and approved the final version of the manuscript.

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