



OPEN

New insights into the phylogeny of *Carasobarbus* Karaman, 1971 (Actinopterygii, Cyprinidae) with the description of three new species

Arash Jouladeh-Roudbar¹, Cüneyt Kaya², Saber Vatandoust³ & Hamid Reza Ghanavi⁴✉

Fishes from the genus *Carasobarbus*, widely distributed throughout the river systems of North Africa and West Asia, are commonly referred to as Himris. In the Persian Gulf basin, they are widespread and are also found in fast-flowing rivers or the deeper regions of lakes. In this region, representation of these fishes in scientific collections is scarce, and except for *C. luteus*, the other species are very poorly documented and frequently misidentified due to their similarities. In this study we analysed the relationships among *Carasobarbus* species using mitochondrial genes (Cyt *b*, COI) and present morphological characters based on examinations. Our results revealed three new species which we describe here. *Carasobarbus doadrioi*, new species, is distinguished by 40–44 scales on the lateral line and a prominent black blotch on end of caudal peduncle in specimens < 85 mm SL. *Carasobarbus hajhosseini*, new species is distinguished by 32–34 scales on the lateral line and long head length (20–24% SL). *Carasobarbus saadatii*, new species, is distinguished by 38–40 scales on the lateral line and short head length (19–20% HL). In the Persian Gulf basin, *Carasobarbus* species exhibit uncorrected genetic distances of 1.6 to 5.5% in the COI barcode region and 2.6% to 9.9% in the Cyt *b* gene. This study highlights the importance of investigating the unexplored diversity that exists within poorly sampled and understudied freshwater fish group. Such investigations are essential for developing a comprehensive understanding of the true extent of biodiversity, which is critical for informing effective conservation and protection strategies.

Keywords Himri, Freshwater fish, Morphology, Integrative taxonomy, Western Asia, Phylogeny

Abbreviations

| | |
|--------|---|
| SL | Standard length |
| HL | Lateral head length |
| BIAUBM | Babol Islamic Azad University Biological Museum, Babol, Iran |
| AJRPC | A. Jouladeh-Roudbar personal fish collection, Tehran, Iran |
| VPFC | S. Vatandoust Personal Fish collection, Qaem Shahr, Iran |
| FFR | Faculty of Fisheries, Recep Tayyip Erdogan University, Rize, Turkey |

Carasobarbus Karaman, 1971 is a small genus of Cyprinidae comprising 10 valid species distributed across Southwest Asia and Northwest Africa^{1–3}. These fishes known as Himris and characterized by large scales and special forms of the lips^{4,5}. Three species of Himris are currently known from the Persian Gulf basin: *Carasobarbus luteus* (Heckel, 1843), *C. kosswigi* (Ladiges, 1960), and *C. sublimus* (Coad & Najafpour, 1997), with the latter considered to be endemic to Iran. Initially, *C. kosswigi* was described as *Cyclocheilichthys* Bleeker, 1859, *C. sublimus* as *Barbus* Daudin 1805, and *C. luteus* as *Systomus* McClelland 1838. Bianco and Bănărescu⁶ considered *luteus* as *Carasobarbus* validating the genus. Karaman⁷ erected *Kosswigobarbus* and placed *kosswigi* in it.

¹Museum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, 10115 Berlin, Germany. ²Faculty of Fisheries, Recep Tayyip Erdogan University, Rize, Turkey. ³Department of Fisheries, Babol Branch, Islamic Azad University, Babol, Iran. ⁴Department of Biology, Lund University, Lund, Sweden. ✉email: hamid.ghanavi@biol.lu.se

However, Borkenhagen et al.⁸ synonymized this genus with *Carasobarbus*. In the following, Borkenhagen and Krupp² conducted a comprehensive taxonomic revision of the genus *Carasobarbus*, revealing three valid species inhabiting Iran: *C. luteus*, *C. kosswigi*, and *C. sublimus*. They mentioned that *C. sublimus* is present in Zohreh and Karkheh drainages, and *C. kosswigi* is found in Karun and Tigris drainages.

The elusive nature of *Carasobarbus* species and the challenges associated with sampling them have rendered the study of these fishes extremely difficult. This is especially accentuated because some species are rare and easily misidentified with other species inhabiting the same habitats. After approximately 15 years of field expeditions across Iran, Iraq, and Türkiye, during which *Carasobarbus* specimens were collected from the type localities of *C. kosswigi* and *C. sublimus*, as well as other populations from the Tigris to Zohreh drainages, a comprehensive examination revealed significant morphological and genetic differences among them. Our findings provide evidence supporting the existence of three undescribed species in Iran, which we describe based on a combination of morphological and molecular genetic characters.

Materials and methods

Fish sampling and preservation

All fish specimens used in this study were sampled following local guidelines and rules. All experimental protocols are approved routine procedures by ethics committee in Lund University. All methods were carried out in accordance with relevant guidelines and regulations, and all methods are reported in accordance with ARRIVE guidelines. The sampling permits were issued by the local environment department. Fish were euthanized with an overdose of clove oil, fixed in 10% formalin for 24 h, and preserved in ethanol 70%. The samples used in molecular analyses were fixed in 99% EtOH (whole body or a fin clip).

Morphological examination

Measurements were made point-to-point with a digital calliper and recorded to 0.1 mm. Counts and measurements were made on the left side of specimens whenever possible, following Kottelat & Freyhof⁹. Head length and measurements of body parts are given as proportions of standard length (SL). Subunits of the head are presented as proportions of head length (HL). Standard length (SL) was measured from the tip of the snout to the posterior extremity of the hypural complex. The skin fold at the posterior part of the gill cover was included in the measurement of HL. The length of the caudal peduncle was measured from behind the base of the posterior anal-fin ray to the posterior extremity 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 noted as "11/2". The distribution map (Fig. 1) was created with QGIS v.3.18 software (<http://qgis.org>). In addition to examined specimens of *C. sublimus*, morphometric data were obtained from Coad and Najafpour¹.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted using Macherey & Nagel NucleoSpin[®] Tissue kits following the provided protocol. The barcode region of the COI (cytochrome c oxidase subunit 1) gene was amplified using FishF1-5'TCAACC AACCACAAAGACATTGGCAC3' and FishR1-5'TAGACTTCTGGGTGGCCAAAGAATCA3'¹⁰, and the Cyt *b* genetic marker using GluF-5'AACCACCGTTGTATTCAACTACAA3' and ThrR5' ACCTCCGATCTTCGG ATTACAAGACCG3'¹¹. The T7Promoter (5'TAATACGACTCACTATAGGG3') and T3 (5'ATTAACCCTCAC TAAAGGG3') standard sequences were added to the sequence of forward and reverse primers respectively, to simplify the sequencing of different PCR products on the same plate. Sequencing of the PCR products was performed at an external sequencing service provider.

Molecular data analysis

The obtained sequences and the ones downloaded from GenBank (Tables 1, 2), were aligned using MAFFT^{12,13} as implemented in Geneious v. 10.0.2 (Biomatters, <http://www.geneious.com/>). The obtained datasets were concatenated in Geneious to create three different datasets: COI dataset, Cyt *b* dataset, and the concatenated dataset. In the case of the concatenated dataset, in the ingroup, we only kept the samples with both genetic markers amplified from the same specimen. This was not possible for the outgroups as none of the sequences in Genbank, used for outgroups, came from the same specimen for both genes. In these cases, sequences from unrelated specimens were concatenated together. This does not affect the phylogenetic results of the ingroup. To determine intraspecific species uncorrected pairwise genetic distances (p-distances) (Tables 3, 4), we employed Mega 6¹⁴.

Both maximum likelihood (ML) and Bayesian (BI) methods have been used to construct phylogenetic relationships of the group. In the case of ML approach, IQ-TREE 1.6.12^{15,16} were used. In this case, the optimal substitution model and the best partitioning scheme based on the codon information, was investigated using ModelFinder¹⁷ with the Bayesian information criterion (BIC). In the case of single marker datasets, the codon position information was provided, and in the concatenated dataset both codon position and gene separation were provided to the program. The bootstrap (– b 500) approximations was used to calculate support values¹⁸. FigTree 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>) was used to visualize the resulting trees. In the case of the BI approach, MrBayes 3.2.7¹⁹ were used with two parallel simultaneous analyses for 2×10^7 generations, each with four MCMC chains, and sampling every 2000 generations. The initial 25% of generations were discarded as the burn-in. An rjMCMC²⁰ approach was implemented using the nst = mixed command. The proper convergence of the runs was verified using Tracer 1.7²¹.

Three distance-based molecular species delimitation methods were used: automatic barcode gap discovery (ABGD)²², assemble species by automatic partitioning (ASAP)²³, and Bayesian Poisson Tree Processes model (bPTP)²⁴. The ABGD analysis were performed on its online webserver (<https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html>), exploring a range of ABGD settings with a parameter range of Pmin = 0.001, Pmax = 0.1, and a

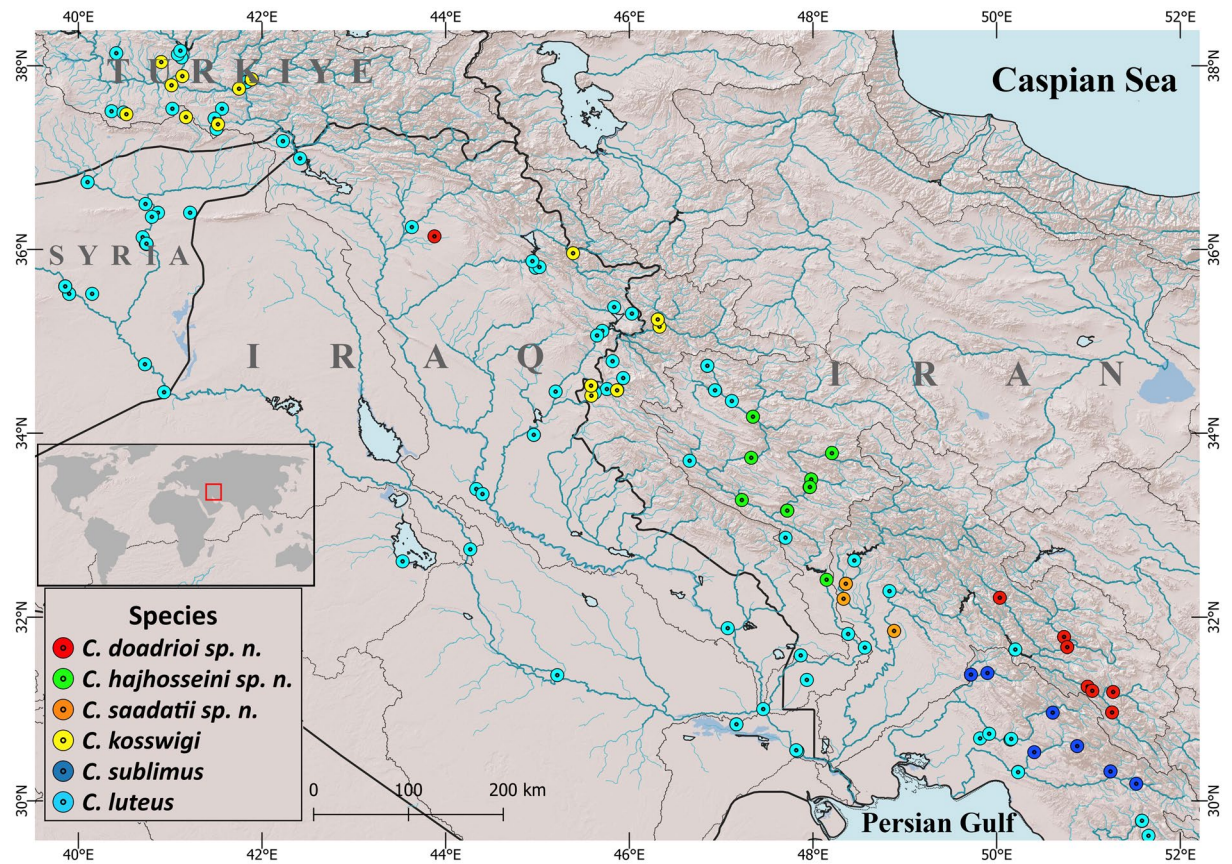


Fig. 1. Distribution map of *Carasobarbus* species in Persian Gulf basin.

| GenBank | Species | GenBank | Species |
|----------|---------------------|----------|----------------------------------|
| KJ552897 | <i>C. canis</i> | KJ552960 | <i>C. fritschii</i> |
| KJ552760 | <i>C. canis</i> | KJ553111 | <i>C. fritschii</i> |
| KJ552827 | <i>C. canis</i> | KJ553144 | <i>C. fritschii</i> |
| KJ553264 | <i>C. chantrei</i> | KJ553161 | <i>C. fritschii</i> |
| KJ553201 | <i>C. chantrei</i> | KJ553212 | <i>C. fritschii</i> |
| KJ553098 | <i>C. chantrei</i> | KJ553240 | <i>C. fritschii</i> |
| KJ553228 | <i>C. chantrei</i> | KJ552291 | <i>C. fritschii</i> |
| KJ552821 | <i>C. chantrei</i> | KM590427 | <i>C. hajhosseini</i> |
| KJ552958 | <i>C. chantrei</i> | KM590426 | <i>C. luteus</i> |
| KM590423 | <i>C. doadrioi</i> | KM590424 | <i>C. luteus</i> |
| MW250390 | <i>C. doadrioi</i> | KM590425 | <i>C. luteus</i> |
| KM590428 | <i>C. kosswigi</i> | MW250388 | <i>C. luteus</i> |
| KJ552798 | <i>C. harterti</i> | OR038182 | <i>C. luteus</i> |
| KJ552803 | <i>C. harterti</i> | OR038183 | <i>C. luteus</i> |
| KJ552814 | <i>C. harterti</i> | OR038192 | <i>C. luteus</i> |
| KJ552851 | <i>C. harterti</i> | OR038193 | <i>C. luteus</i> |
| KJ552906 | <i>C. harterti</i> | OP456596 | <i>Mesopotamichthys sharpeyi</i> |
| KJ552966 | <i>C. harterti</i> | OP456597 | <i>M. sharpeyi</i> |
| KJ552780 | <i>C. fritschii</i> | OP456598 | <i>M. sharpeyi</i> |
| KJ552819 | <i>C. fritschii</i> | KM590450 | <i>Arabibarbus grypus</i> |
| KJ552951 | <i>C. fritschii</i> | KM590451 | <i>A. grypus</i> |
| KJ552959 | <i>C. fritschii</i> | | |

Table 1. GenBank accession numbers of the COI sequences downloaded for this study.

| GenBank | Species | GenBank | Species |
|----------|---------------------|----------|----------------------------------|
| KU525007 | <i>C. apoensis</i> | KU524970 | <i>C. fritschii</i> |
| KU525008 | <i>C. apoensis</i> | KU524973 | <i>C. fritschii</i> |
| KU525009 | <i>C. apoensis</i> | KU524974 | <i>C. fritschii</i> |
| KU525006 | <i>C. apoensis</i> | KU524969 | <i>C. fritschii</i> |
| AF145947 | <i>C. canis</i> | KU524971 | <i>C. fritschii</i> |
| KU524924 | <i>C. canis</i> | MN961175 | <i>C. fritschii</i> |
| KU524925 | <i>C. canis</i> | KU525005 | <i>C. fritschii</i> |
| KU524926 | <i>C. canis</i> | AF287430 | <i>C. fritschii</i> |
| AF180852 | <i>C. chantrei</i> | KU524978 | <i>C. fritschii</i> |
| HQ167605 | <i>C. chantrei</i> | KU524979 | <i>C. fritschii</i> |
| KU524913 | <i>C. chantrei</i> | KU524980 | <i>C. fritschii</i> |
| KU524921 | <i>C. chantrei</i> | KU524981 | <i>C. fritschii</i> |
| KU524922 | <i>C. chantrei</i> | KU524982 | <i>C. fritschii</i> |
| KU524923 | <i>C. chantrei</i> | KU524983 | <i>C. fritschii</i> |
| KU524958 | <i>C. chantrei</i> | KU524984 | <i>C. fritschii</i> |
| KU524959 | <i>C. chantrei</i> | KU524985 | <i>C. fritschii</i> |
| KU524914 | <i>C. chantrei</i> | KU524986 | <i>C. fritschii</i> |
| KU524934 | <i>C. doadrioi</i> | KU524935 | <i>C. hajhosseini</i> |
| KU524901 | <i>C. exulatus</i> | AF180855 | <i>C. harterti</i> |
| KU524902 | <i>C. exulatus</i> | KU524975 | <i>C. harterti</i> |
| KU524904 | <i>C. exulatus</i> | KU524976 | <i>C. harterti</i> |
| KU524907 | <i>C. exulatus</i> | KU524977 | <i>C. harterti</i> |
| KU524908 | <i>C. exulatus</i> | KP712261 | <i>C. kosswigi</i> |
| KU524903 | <i>C. exulatus</i> | AF180853 | <i>C. kosswigi</i> |
| KU524905 | <i>C. exulatus</i> | KU524915 | <i>C. luteus</i> |
| AF180856 | <i>C. fritschii</i> | KU524964 | <i>C. luteus</i> |
| MN961176 | <i>C. fritschii</i> | KU524965 | <i>C. luteus</i> |
| KU524990 | <i>C. fritschii</i> | KU524912 | <i>C. luteus</i> |
| KU524993 | <i>C. fritschii</i> | KU524920 | <i>C. luteus</i> |
| KU524987 | <i>C. fritschii</i> | KU524928 | <i>C. luteus</i> |
| KU524988 | <i>C. fritschii</i> | KU524963 | <i>C. luteus</i> |
| KU524989 | <i>C. fritschii</i> | KP712262 | <i>C. luteus</i> |
| KU524991 | <i>C. fritschii</i> | KU524933 | <i>C. luteus</i> |
| KU524992 | <i>C. fritschii</i> | KU524927 | <i>C. luteus</i> |
| KU524995 | <i>C. fritschii</i> | KU524929 | <i>C. luteus</i> |
| KU524999 | <i>C. fritschii</i> | KU524909 | <i>C. sublimus</i> |
| MN961177 | <i>C. fritschii</i> | KU524931 | <i>C. sublimus</i> |
| KU524994 | <i>C. fritschii</i> | KU524930 | <i>C. sublimus</i> |
| AF287429 | <i>C. fritschii</i> | KU524910 | <i>C. sublimus</i> |
| KU524968 | <i>C. fritschii</i> | KU524911 | <i>C. sublimus</i> |
| KU524996 | <i>C. fritschii</i> | KU524932 | <i>C. sublimus</i> |
| KU524997 | <i>C. fritschii</i> | KF876032 | <i>Mesopotamichthys sharpeyi</i> |
| KU525000 | <i>C. fritschii</i> | KF876033 | <i>M. sharpeyi</i> |
| KU525001 | <i>C. fritschii</i> | KF876031 | <i>M. sharpeyi</i> |
| KU525002 | <i>C. fritschii</i> | KF876029 | <i>Arabibarbus grypus</i> |
| KU525004 | <i>C. fritschii</i> | KF876028 | <i>A. grypus</i> |
| KU524966 | <i>C. fritschii</i> | KF876021 | <i>A. arabicus</i> |
| KU524967 | <i>C. fritschii</i> | KF876022 | <i>A. arabicus</i> |
| KU525003 | <i>C. fritschii</i> | KF876023 | <i>A. adharami</i> |
| KU524998 | <i>C. fritschii</i> | KF876024 | <i>A. adharami</i> |
| KU524972 | <i>C. fritschii</i> | | |

Table 2. GenBank accession numbers of the Cyt *b* sequences downloaded for this study.

| N | Species | I | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|----|--------------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1 | <i>C. doadrioi</i> sp. n | 0.06 | | | | | | | | | |
| 2 | <i>C. hajhosseini</i> sp. n | 0.00 | 4.1 | | | | | | | | |
| 3 | <i>C. saadatii</i> sp. n | 0.09 | 2.5 | 4.7 | | | | | | | |
| 4 | <i>C. canis</i> | 0.00 | 5.0 | 5.3 | 4.7 | | | | | | |
| 5 | <i>C. chantrei</i> | 0.21 | 4.9 | 5.3 | 5.0 | 2.0 | | | | | |
| 6 | <i>C. kosswigi</i> | 0.00 | 1.6 | 3.8 | 1.6 | 4.1 | 4.2 | | | | |
| 7 | <i>C. luteus</i> | 0.19 | 5.3 | 5.3 | 5.4 | 2.8 | 1.6 | 4.7 | | | |
| 8 | <i>C. sublimus</i> | 0.16 | 3.9 | 3.3 | 5.1 | 5.3 | 5.5 | 4.2 | 5.4 | | |
| 9 | <i>C. harterti/fritschii</i> 1 | 0.72 | 4.2 | 4.2 | 4.2 | 4.7 | 4.0 | 3.8 | 4.9 | 5.3 | |
| 10 | <i>C. harterti/fritschii</i> 2 | 0.55 | 5.2 | 5.1 | 4.8 | 4.4 | 4.1 | 4.5 | 4.8 | 5.4 | 2.6 |

Table 3. Uncorrected-p genetic distances (%) in COI gene between different species of *Carasobarbus* (I.: intraspecific distance).

| N | Species | I | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|----|-----------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1 | <i>C. doadrioi</i> sp. n | 0.32 | | | | | | | | | | |
| 2 | <i>C. hajhosseini</i> sp. n | 0.20 | 8.5 | | | | | | | | | |
| 3 | <i>C. saadatii</i> sp. n | 0.32 | 4.6 | 7.6 | | | | | | | | |
| 4 | <i>C. canis</i> | 0.04 | 7.6 | 8.2 | 6.5 | | | | | | | |
| 5 | <i>C. chantrei</i> | 0.10 | 7.1 | 8.0 | 6.4 | 3.2 | | | | | | |
| 6 | <i>C. exulatus</i> | 0.10 | 7.2 | 7.9 | 6.5 | 3.0 | 2.6 | | | | | |
| 7 | <i>C. fritschii</i> | 0.66 | 8.3 | 9.9 | 8.8 | 6.6 | 7.1 | 6.9 | | | | |
| 8 | <i>C. harterti</i> | 0.04 | 8.7 | 9.9 | 8.3 | 5.7 | 6.2 | 6.0 | 3.1 | | | |
| 9 | <i>C. luteus/apoensis</i> | 0.59 | 7.6 | 8.1 | 6.9 | 3.4 | 2.6 | 3.2 | 7.0 | 6.2 | | |
| 10 | <i>C. kosswigi</i> | 0.56 | 4.2 | 7.1 | 3.8 | 6.3 | 5.6 | 5.6 | 8.3 | 8.5 | 6.0 | |
| 11 | <i>C. sublimus</i> | 0.34 | 9.0 | 5.0 | 8.8 | 9.2 | 9.2 | 8.6 | 9.4 | 9.7 | 8.8 | 7.9 |

Table 4. Uncorrected-p genetic distances (%) in Cyt *b* gene between different species of *Carasobarbus* (I.: intraspecific distance).

gap width of 1.5 over ten steps. The ASAP analysis was also made, using Simple Distance (p-distances), via its web interface (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>). The bPTP analysis was run only on the in-group on the online implementation of it (<https://species.h-its.org/>) using default settings.

Results

We were able to generate 38 new sequences (22 COI + 16 Cyt *b*) for six species of *Carasobarbus* from Iran, Iraq and Türkiye, in addition to 173 sequences from NCBI GenBank (Tables 1 and 2). The final alignment for COI consisted of 770 base pairs, with 676 positions being constant, 88 being parsimony informative and 6 being singletons (calculated just between in-group species), and for Cyt *b* the alignment was 1143 base pairs, with 872 positions being constant, 240 being parsimony informative and 29 being singletons (calculated just between in-group species for both genes).

The COI gene of *Carasobarbus* displayed an interspecific uncorrected-p genetic distance of 1.6% between *C. luteus* and *C. chantrei* as well as *C. doadrioi* sp. n., *C. saadatii* sp. n. and *C. chantrei* to 5.5% between *C. sublimus*. Average intraspecific distance for *Carasobarbus* species was 0.20%, ranging from 0.0 in *C. canis*, *C. hajhosseini*, and *C. kosswigi* to 0.72% in clade 1 of *C. fritschii/harterti* species group (Table 3).

For the Cyt *b* gene, the genetic distances between species ranged from 2.6% between *C. luteus/apoensis*, *C. chantrei* and *C. exulatus* to 9.9% between *C. harterti* and *C. chantrei* as well as between *C. hajhosseini*, *C. fritschii* and *C. harterti*. Also, the average intraspecific distance was 0.30%, ranging from 0.04% in *C. canis* and *C. harterti* to 0.66% in *C. fritschii*. Table 4 shows the genetic distances between and within the *Carasobarbus* species for Cyt *b* gene.

The general topology of Cyt *b*, COI and concatenated dataset trees (Figs. 2, 3 and 4) were in agreement with previously published phylogenies that focused on the genus *Carasobarbus*^{8,25}. The COI and Cyt *b* dataset both resulted in acceptable trees with some nodes which were harder to resolve (not well supported). The increased sampling size, in the case of individual gene datasets, appears to improve the result compared to the prior phylogenetic works. The concatenation of the two genetic markers resulted in the best resolved tree even though the number of represented species was reduced. In general, all species analysed in any of the datasets was recovered as monophyletic apart from *C. harterti* and *C. fritschii* in the COI dataset. In this case, the resolution of the COI dataset for this part seems to not be adequate, and some samples identified as *C. harterti* are placed with *C. fritschii* and vice versa.

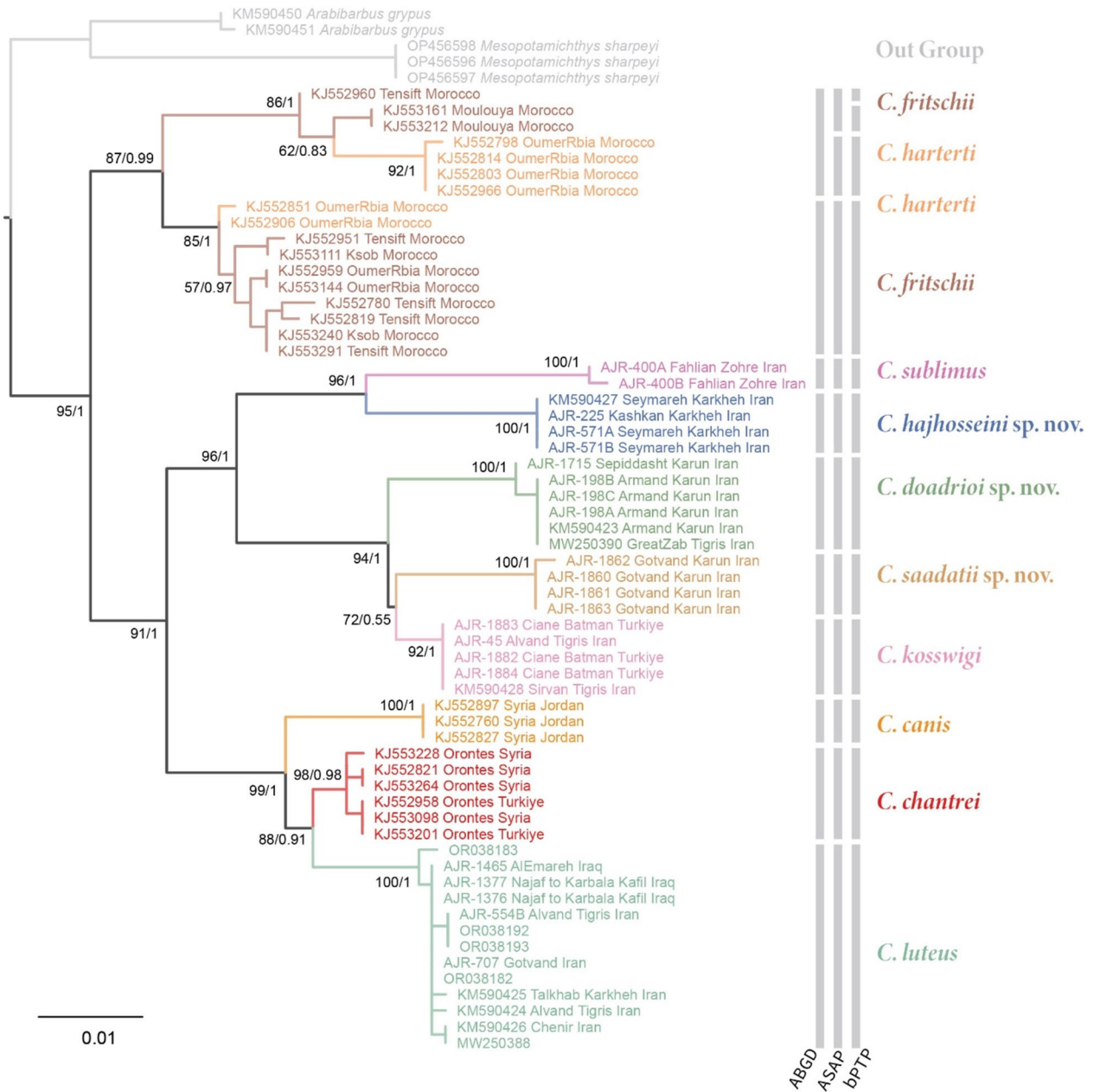


Fig. 2. Phylogenetic tree of *Carasobarbus* based on the maximum likelihood and Bayesian analyses of the mitochondrial COI barcode region. Numbers present at each node are bootstrap/posterior probability support values. The result of the three different species delimitation methods is shown using the vertical bars.

Key to species of *Carasobarbus* in Persian Gulf basin

- 1a - Lower lip without median lobe; one pair of barbels (two pair in the Makran population).
.....*C. luteus*
- 1b - Lower lip with median lobe; two pair barbels.
.....2
- 2a - 24 – 29 total lateral-line scales; lower lip lobe well-developed (Coad and Najafpour, (1) data included).
.....*C. sublimus*
- 2b - 32–44 total lateral-line scales; lower lip lobe slightly to relatively developed.
.....3
- 3a - 32–37 total lateral-line scales.
.....4
- 3b - 38–44 total lateral-line scales.
.....5
- 4a - Lower lip lobe well-developed; 32–77 [mode 36] total lateral-line scales; head length 25–27% SL; posterior barbel 13–20% HL; snout length 36–44% HL.

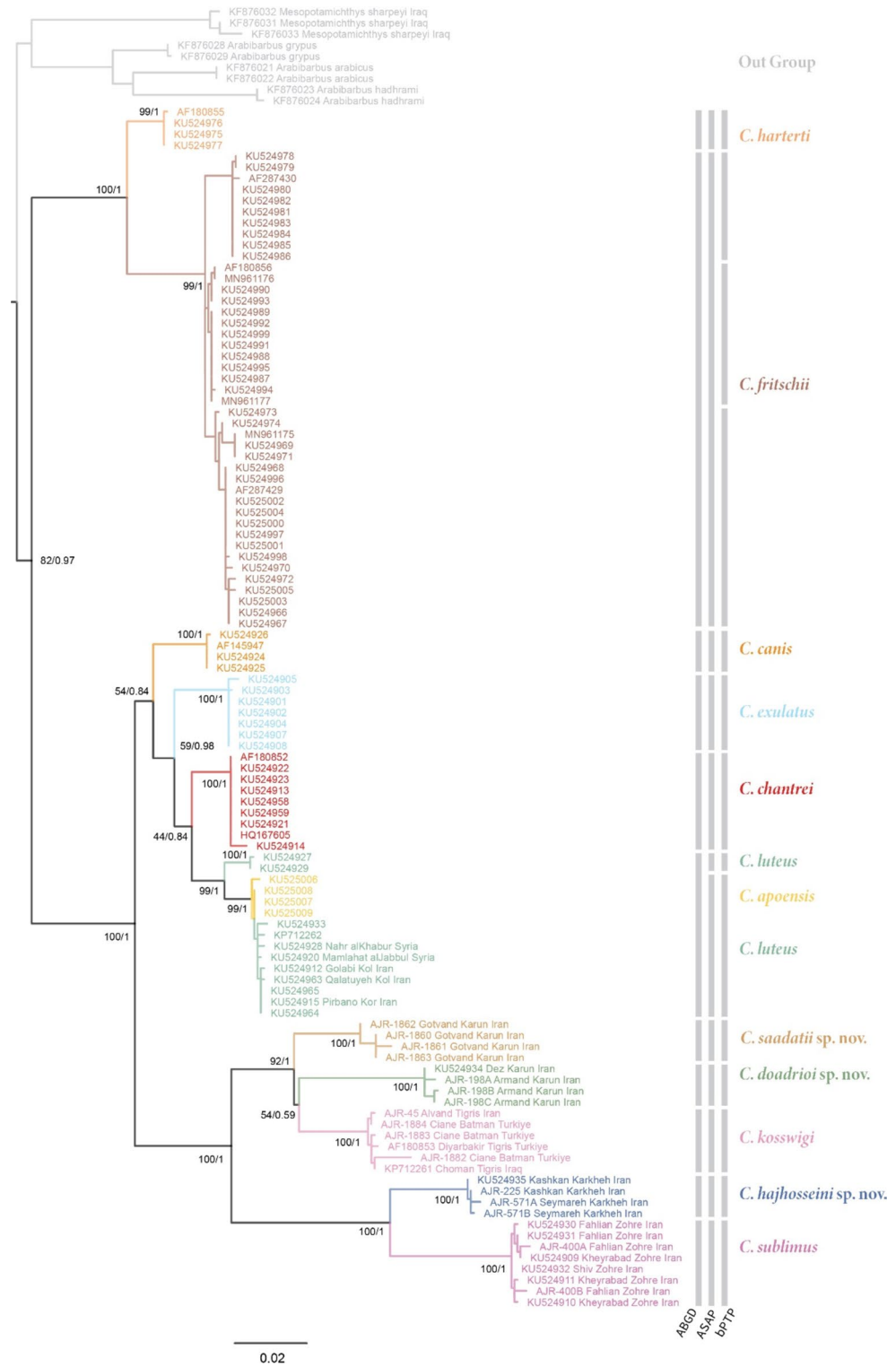


Fig. 3. Phylogenetic tree of *Carasobarbus* based on the maximum likelihood and Bayesian analyses of the *Cyt b* gene. Numbers present at each node are bootstrap/posterior probability support values. The result of the three different species delimitation methods is shown using the vertical bars.

.....*C. kosswigi*
 4b - Lower lip lobe slightly developed; 32–34 [mode 33–34] total lateral-line scales; head length 20–24% SL; posterior barbel 21–38% HL; snout length 25–31% HL.

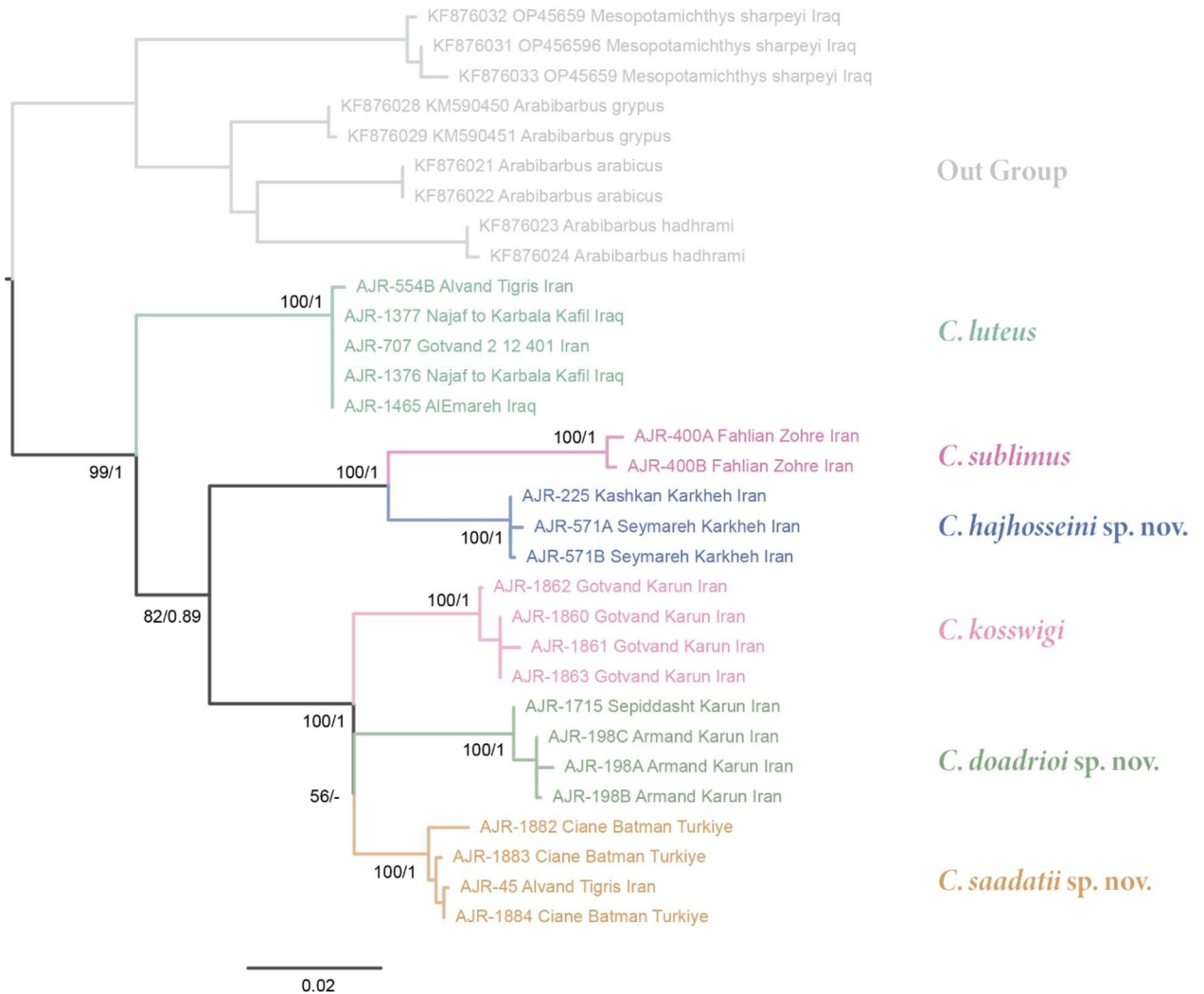


Fig. 4. Phylogenetic tree of *Carasobarbus* based on the maximum likelihood and Bayesian analyses of the mitochondrial COI barcode region and the *Cyt b* markers concatenated. Numbers present at each node are bootstrap/posterior probability support values.

.....*C. hajhosseini* sp. n.

5a – A prominent black blotch on end of caudal peduncle in specimens < 85 mm SL; head length 22–25% SL; dorsal fin height 19–26% SL; distance between base of pelvic and anal fins 24–25% SL.

.....*C. doadrioi* sp. n.

5b – No black blotch on end of caudal peduncle in specimens < 85 mm SL; Head length 19–20% SL; dorsal fin height 26–30% SL; distance between base of pelvic and anal fins 26–28% SL.

.....*C. saadatii* sp. n.

***Carasobarbus doadrioi*, new species**

(Figs. 5, 6, 7 and 8).

Holotype. BIAUBM 6-H, 75.3 mm SL; Iran: Chaharmahal and Bakhtiari prov., Khersan River at Atishgah, Karun River drainage, Persian Gulf Basin, 31.24358, 50.99075.

Paratypes. AJRPC 17-P, 7, 69.3–45.2 mm SL; data same as holotype.

New material used in molecular genetic analysis. AJRPC-DNA 198A (COI: PP515175, *Cyt b*: PP548209), 198B (COI: PP515176, *Cyt b*: PP548210), 198C (COI: PP515177, *Cyt b*: PP548211), same data as holotype; AJRPC-DNA 1715 (COI: PP515188, *Cyt b*: not sequenced), Iran: Lorestan prov., Sezar River at Absardeh, Karun River drainage, Persian Gulf Basin, 33.20562, 48.88326.

Diagnosis

Carasobarbus doadrioi is distinguished from *C. sublimus*, *C. hajhosseini* sp. n. and *C. kosswigi* by having more scales on lateral line (40–44 vs. 27–37). *Carasobarbus doadrioi* sp. n. is similar to *C. saadatii* sp. n. and is distinguished by having a prominent black blotch on end of caudal peduncle in specimens < 85 mm SL (vs. no black blotch), longer head length (22–25 vs. 19–20% SL), shorter dorsal fin height (19–26 vs. 26–30% SL) and shorter distance between base of pelvic and anal fins (24–25 v. 26–28% SL). It is distinguished from *C. luteus* by having



Fig. 5. *Carasobarbus doadrioi* sp. n.; BIAUBM 6-H, holotype, 75 mm SL; Iran: Khersan River, Karun drainage.



Fig. 6. *Carasobarbus doadrioi* sp. n., AJRPC 17-P, paratypes, from top: 69 mm SL, 63 mm SL; Khersan River, Persian Gulf basin.

two pair of barbels (vs. one pair), well-developed median lobe on the lower lip (vs. without median lobe) and more scales on the lateral line (40–44 vs. 25–30) (Table 5).

Description

See Figs. 5, 6, 7 and 8 for general appearance, Table 6 for morphometric data. Body moderately high, laterally compressed, without nuchal hump. The greatest body depth in front or at dorsal-fin origin. Ventral head profile straight, dorsal head profile with a slight to pronounced hump near nostrils. Head short and narrow. Maximum body depth larger than head length. Triangular axillary scale at pelvic-fin base present. Pelvic-fin origin below vertical of last unbranched or first branched dorsal-fin ray. Caudal fin forked. Pectoral fin reaching approximately 70–90% of distance between pectoral- to pelvic-fin origin. Pelvic fin not reaching anus. Eye large, markedly



Fig. 7. *Carasobarbus doadrioi* sp. n.; uncatalogued, about 150 mm SL; Iran: Khersan River, Karun drainage.



Fig. 8. *Carasobarbus doadrioi* sp. n.; uncatalogued, about 150 mm SL; Iran: Khersan River, Karun drainage.

| Species | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 |
|-----------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| <i>C. luteus</i> | 1 | 2 | 3 | | 1 | 1 | | | | | | | | | | | | | | |
| <i>C. hajhosseini</i> sp. n | | | | | | | | 3 | 4 | 4 | | | | | | | | | | |
| <i>C. kosswigi</i> | | | | | | | | 1 | 2 | 3 | 3 | 6 | 1 | | | | | | | |
| <i>C. saadatii</i> sp. n | | | | | | | | | | | | | | 1 | 2 | 2 | | | | |
| <i>C. doadrioi</i> sp. n | | | | | | | | | | | | | | | | 3 | 1 | 1 | | 1 |
| <i>C. sublimus</i> | | | 1 | 1 | 2 | | | | | | | | | | | | | | | |

Table 5. Lateral line scale count. Significant values are bold.

smaller than snout. Mouth inferior, lips thick and fleshy with a well-developed median lob. Two pairs of barbels, rostral barbel reaches to anterior part of eye and maxillary barbel reaching to posterior part of eye.

Dorsal fin with 4 (n = 8) unbranched rays and 11½ (n = 8) branched rays, outer margin deeply concave. Anal fin with 3 (n = 8) unbranched and 6½ (n = 8) branched rays, outer margin straight. Pectoral fin with 14 (n = 5), 15 (n = 3) rays. Pelvic fin with 7 (n = 1)–8 (n = 7) rays. Lateral line with 40 (n = 3), 41 (n = 2), 42 (n = 1), 43 (n = 1), 44 (n = 1) scales. Scale rows between dorsal-fin origin and lateral line 7 (n = 8). Scale rows between anal-fin origin and lateral line 6 (n = 11).

| Characters | <i>C. hajhosseini</i> | | | | | <i>C. doadrioi</i> | | | | |
|-------------------------------------|------------------------|------|------|------|-----|------------------------|------|------|------|-----|
| | Holotype and paratypes | | | | | Holotype and paratypes | | | | |
| | H | Min | Max | Mean | SD | H | Min | Max | Mean | SD |
| Standard length (SL) | 191 | 86 | 184 | | | 75 | 45 | 69 | | |
| In percent of standard length | | | | | | | | | | |
| Head length | 22.5 | 19.8 | 24.1 | 22.3 | 1.4 | 22.1 | 22.1 | 24.7 | 23.5 | 1.2 |
| Body depth at dorsal fin origin | 30.8 | 26.6 | 32.9 | 30.2 | 1.6 | 29.9 | 26.2 | 30.3 | 28.3 | 1.6 |
| Body depth at anal fin origin | 21.0 | 19.0 | 22.4 | 20.9 | 1.1 | 20.8 | 16.7 | 20.8 | 19.2 | 1.5 |
| Pre-dorsal length | 53.5 | 50.4 | 57.1 | 53.4 | 2.1 | 50.0 | 50.0 | 52.3 | 51.1 | 0.9 |
| Pre-pelvic length | 53.2 | 49.4 | 53.6 | 51.2 | 1.3 | 51.3 | 49.1 | 53.4 | 51.4 | 1.7 |
| Pre-anal length | 78.5 | 74.6 | 78.7 | 76.6 | 1.6 | 76.0 | 73.7 | 76.0 | 75.2 | 0.9 |
| Dis. betw. pectoral and anal fins | 56.3 | 50.8 | 58.9 | 54.2 | 2.3 | 55.8 | 49.2 | 55.8 | 52.0 | 2.2 |
| Dis. betw. pectoral and pelvic fins | 30.2 | 25.5 | 31.0 | 28.6 | 1.7 | 31.4 | 25.6 | 31.4 | 28.0 | 2.0 |
| Dis. betw. pelvic and anal fins | 27.2 | 23.9 | 28.4 | 25.9 | 1.3 | 24.2 | 24.1 | 25.2 | 24.6 | 0.5 |
| Dorsal fin height | 23.3 | 22.1 | 28.2 | 24.8 | 1.9 | 18.8 | 18.8 | 25.6 | 23.1 | 2.3 |
| Anal fin height | 23.9 | 19.1 | 27.2 | 23.0 | 2.6 | 20.6 | 19.7 | 25.4 | 21.8 | 2.2 |
| Pectoral fin length | 20.7 | 19.9 | 25.6 | 22.7 | 1.9 | 24.0 | 20.8 | 24.0 | 22.1 | 1.3 |
| Pelvic fin length | 18.1 | 18.1 | 22.5 | 20.1 | 1.6 | 18.0 | 17.0 | 21.1 | 18.9 | 1.4 |
| Upper caudal fin lobe | 28.8 | 28.6 | 36.2 | 31.4 | 2.4 | 28.7 | 28.7 | 32.8 | 30.9 | 1.6 |
| Length of middle caudal fin | 12.0 | 11.3 | 14.4 | 12.9 | 1.0 | 13.8 | 12.0 | 16.1 | 14.0 | 1.6 |
| Caudal peduncle length | 15.7 | 14.7 | 18.0 | 15.9 | 1.2 | 15.5 | 14.8 | 16.4 | 15.4 | 0.6 |
| Caudal peduncle depth | 11.7 | 11.1 | 13.5 | 12.4 | 0.8 | 12.3 | 10.8 | 12.3 | 11.4 | 0.6 |
| In percent of head length | | | | | | | | | | |
| Snout length | 31 | 25 | 31 | 27.4 | 2.2 | 26 | 22 | 29 | 25.5 | 2.2 |
| Eye diameter | 21 | 21 | 26 | 23.1 | 1.3 | 24 | 20 | 28 | 24.2 | 3.0 |
| Head depth at pupil | 78 | 56 | 79 | 65.5 | 8.7 | 59 | 54 | 59 | 56.2 | 2.1 |
| Head depth at nape | 88 | 81 | 96 | 87.0 | 4.2 | 85 | 72 | 85 | 78.9 | 4.8 |
| Posterior barbel | 25 | 21 | 38 | 27.7 | 5.4 | 17 | 17 | 24 | 21.3 | 2.2 |
| Anterior barbel | 13 | 13 | 19 | 16.9 | 2.3 | 20 | 13 | 20 | 15.2 | 2.9 |

Table 6. Morphometric data of *C. hajhosseini* sp. n. (holotype BIAUBM 7-H and paratypes AJRPC 18-P to 23-P; n = 11) and *C. doadrioi* sp. n. (holotype BIAUBM 6-H and paratypes AJRPC 17-P; n = 8).

Coloration

In life: Body silverish or cream-white. Back darker than belly. Series of scales over the lateral line outlined by dark pigmentation, evident in anterior and fade in posterior. Fins with scattered dark melanophores on rays and membranes. In formalin: Cream-brown, back darker than belly. Series of scales over the lateral line with dark anterior pigmentation, fading posteriorly. Fins with scattered dark melanophores on rays and membranes.

Distribution

Known from the lower Dez and Karun drainages.

Etymology

This species name derives from the name of the Spanish ichthyologist Ignacio Doadrio Villarejo, in honour of his invaluable contribution to the study of the fishes of the world.

Habitat

Carasobarbus doadrioi sp. n. is found in the deep, slow current of large rivers (Fig. 9). It typically favours areas with abundant vegetation with rocky substrates during the summer. Generally, the species is most abundant in the middle and lower Karun drainage. *Luciobarbus esocinus* Heckel, 1843, *Garra rufa* (Heckel, 1843), *Acanthobrama marmid* Heckel, 1843, *Alburnus sellal* Heckel, 1843, *Chondrostoma regium* (Heckel, 1843), *Squalius berak* Heckel, 1843, *Oxynoemacheilus euphraticus*, *Glyptothorax cous* (Linnaeus 1766) and *G. alidaei* Mousavi-Sabet, Eagderi, Vatandoust & Freyhof, 2021 were found coexisting with the new species.

Carasobarbus hajhosseini, new species

(Figs. 10, 11, 12 and 13).

Holotype. BIAUBM 7-H, 190.6 mm SL; Iran: Ilam prov. Seymareh River at Talkhab, Karkheh drainage, Persian Gulf basin, 33.27771, 47.21252.

Paratypes. AJRPC 18-P, 4, 85.8–184.3 mm SL; same data as holotype. AJRPC 19-P, 2, 95.0–108.9 mm SL; Iran: Lorestan prov. Kahman River at Doab, Karkheh drainage, Persian Gulf basin, 33.78557, 48.20640. AJRPC 20-P, 1, 117.5 mm SL; Iran: Lorestan prov. Karkheh River at Pa Alam, Karkheh drainage, Persian Gulf basin, 32.83141, 48.03337. AJRPC 21-P, 1, 136.9 mm SL; Iran: Lorestan prov. Karkheh River at Mamulan, Karkheh drainage,



Fig. 9. Khersan River at Atishgah, Karun drainage, type locality of *Carasobarbus doadrioi* sp. n.



Fig. 10. *Carasobarbus hajhosseini* sp. n.; BIAUBM 7-H, holotype, 191 mm SL; Iran: Seymareh River, Karkheh drainage.

Persian Gulf basin, 33.37823, 47.95654. AJRPC 22-P, 1, 113.2 mm SL; Iran: Lorestan prov. Karkheh River at Kal Sefid, Karkheh drainage, Persian Gulf basin, 33.08346, 47.53871. AJRPC 23-P, 1, 93.7 mm SL; Iran: Ilam prov. Karkheh River at Pol Zaal, Karkheh drainage, Persian Gulf basin, 32.98729, 47.76504.

New material used in molecular genetic analysis. AJRPC-DNA 225 (COI: PP515178, Cyt *b*: PP548212), Iran: Lorestan prov. Kahman River at Doab, Karkheh drainage, Persian Gulf basin, 33.78557, 48.20640; AJRPC-DNA 571A (COI: PP515182, Cyt *b*: PP548215), 571B (COI: PP515183, Cyt *b*: PP548216) same data as holotype.

Diagnosis

Carasobarbus hajhosseini sp. n. is distinguished from *C. sublimus*, *C. saadatii* sp. n. and *C. doadrioi* sp. n. by having more scales on lateral line (32–34 vs. 24–29 in *C. sublimus*; 40–44 in *C. doadrioi* sp. n.; 38–40 in *C. saadatii* sp. n.).

Carasobarbus hajhosseini sp. n. is similar to *C. kosswigi* but can be distinguished by slightly developed lower lip lobe (vs. well-developed), shorter head (20–24 vs. 24–27% SL), shorter posterior barbel (13–20 vs. 21–38% HL) and shorter snout (25–31 vs. 36–44% HL).

Also, the new species can be distinguished from *C. luteus* by having two pair of barbels (vs. one pair), well-developed median lobe on the lower lip (vs. without median lobe) and more scales on the lateral line (32–34 vs. 25–30).



Fig. 11. *Carasobarbus hajhosseini* sp. n., paratypes; from top: AJRPC 19-P, 109 mm SL; AJRPC 22-P, 113 mm SL; AJRPC 23-P, 94 mm SL; Iran: Karkkeh drainage.

Description

See Figs. 10, 11, 12 and 13 for general appearance, Table 6 for morphometric data. Body moderately high, laterally compressed, without nuchal hump. The greatest body depth at a level in front of or point of dorsal fin origin. Ventral head profile straight, dorsal profile has a slight to pronounced hump near nostrils. Head short and narrow. Maximum body depth larger than head length. Triangular axillary scale at pelvic-fin base. Pelvic-fin origin below vertical of last unbranched dorsal fin ray. Caudal fin forked. Tip of anal fin, when pressed to body, reaching to hypural complex. Pectoral fin reaching approximately 70–90% distance from pectoral-fin origin to pelvic-fin origin. Pelvic fin not reaching anus. Eye large, but smaller than snout. Mouth inferior, lips thick and fleshy with a small median lobe. Two pairs of barbels, rostral not/or reaches to anterior part of eye and maxillary reaching to the posterior part of eye.

Dorsal fin with 4 unbranched rays and $10\frac{1}{2}$ (n = 6)– $11\frac{1}{2}$ (n = 5) branched rays, outer margin deeply concave. Anal fin with 3 (n = 11) unbranched and $6\frac{1}{2}$ (n = 11) branched rays, outer margin straight. Pectoral fin with 13 (n = 4), 14 (n = 6), 15 (n = 1) rays. Pelvic fin with 8 (n = 7)–9 (n = 4) rays. Lateral line with 32 (n = 3), 33 (n = 4), 34 (n = 4) scales. Scale rows between dorsal-fin origin and lateral line 6 (n = 11). Scale rows between anal-fin origin and lateral line 5 (n = 11).



Fig. 12. *Carasobarbus hajhosseini* sp. n.; BIAUBM 7-H, holotype, 191 mm SL; Iran: Seymareh River, Karkheh drainage.



Fig. 13. *Carasobarbus hajhosseini* sp. n.; AJRPC 19-P, paratype, 109 mm SL; Iran: Kahman River, Karkheh drainage.

Coloration

In fresh: Body silverish or cream-white. The back darker than the belly. Upper lateral line scales outlined by dark pigmentation, evident in anterior and fade in posterior. Fins with scattered dark melanophores on rays and membranes. In formalin: Body cream-brown, back darker than belly. Upper lateral line scales outlined by dark pigmentation, prominent in anterior section, fades towards posterior.

Distribution

The new species is known from the Gamasiab, Kahman, Kashkan and Seymareh in Karkheh drainage.

Etymology

The species is named in honour of Haj Hossein Javadi Pour (HHJP), who is the father of the first author of this study (AJR).

Habitat

Carasobarbus hajhosseini is commonly found in the deep, swiftly flowing sections of rivers and dam reservoirs (Fig. 14). It typically favours areas with abundant vegetation, and during the summer, it can also be observed in shallower waters. Generally, the species is most abundant in the middle and lower Karkheh drainage. *Luciobarbus esocinus*, *Capoeta shajariani* Jouladeh-Roudbar, Eagderi, Murillo-Ramos, Ghanavi & Doadrio, 2017, *Garra gymnothorax* Berg, 1949, *Chondrostoma regium*, *Alburnus sellal*, *Squalius lepidus* Heckel, 1843, *Squalius berak*, *Turcinoemacheilus saadiei* Esmaili, Sayyadzadeh, Özuluğ, Geiger & Freyhof, 2014, *Glyptothorax cous* and *G. alidaei* were found coexisting with the new species.

***Carasobarbus saadatii*, new species**
(Figs. 15, 16 and 17).

Holotype. BIAUBM 8-H, 187.6 mm SL; Iran: Khuzestan prov., Karun River at Gotvand, Persian Gulf Basin, 32.27319, 48.83521.

Paratypes. AJRPC 24-P, 4, 122.9–179.4 mm SL; data same as holotype.

New material used in molecular genetic analysis. AJRPC-DNA 1860 (COI: PP515189, Cyt *b*: PP548217), 1861 (COI: PP515190, Cyt *b*: PP548218), 1862 (COI: PP515191, Cyt *b*: PP548219), 1863 (COI: PP515192, Cyt *b*: PP548220) same data as holotype.

Diagnosis. *Carasobarbus saadatii* sp. n. is distinguished from *C. sublimus* (Fig. 18), *C. hajhosseini* sp. n. and *C. kosswigi* (Figs. 19, 20 and 21) by having more scales on lateral line (38–40 vs. 27–37).

The new species can be distinguished from *C. luteus* (Fig. 22) by having two pair of barbels (vs. one pair), well-developed median lobe on the lower lip (vs. without median lobe) (Fig. 23) and more scales on the lateral line (38–40 vs. 25–30).



Fig. 14. Seymareh River at Talkhab, Karkheh drainage, type locality of *Carasobarbus hajhosseini* sp. n.



Fig. 15. *Carasobarbus saadatii* sp. n.; BIAUBM 8-H, holotype, 188 mm SL; Iran: Karun River, Persian Gulf basin.



Fig. 16. *Carasobarbus saadatii* sp. n., AJRPC 24-P, paratypes, from top: 174 mm SL; 177 mm SL; 188 mm SL; 123 mm SL; Karun River, Persian Gulf basin.



Fig. 17. *Carasobarbus saadatii* sp. n.; uncatalogued, 175 mm SL; Iran: Karun River, Persian Gulf basin.

Description

See Figs. 15, 16 and 17 for general appearance, Table 7 for morphometric data. Body moderately high, laterally compressed, without nuchal hump. The greatest body depth at point of origin of dorsal fin. Ventral head profile straight, dorsal profile has a slight to pronounced hump near the nostrils. A rounded keel on back in front of dorsal fin. Head short and narrow. Maximum body depth larger than head length. Triangular axillary scale at pelvic-fin base. Pelvic-fin origin below vertical of last unbranched dorsal fin ray. Caudal fin forked. Tip of anal fin, when pressed to body, reaching to hypural complex. Pectoral fin reaching approximately 70–80% distance from pectoral-fin origin to pelvic-fin origin. Pelvic fin not reaching anus. Eye large, but smaller than snout. Mouth inferior, lips thick and fleshy with a well-developed median lob. Two pairs of barbels, rostral reaches to eye and maxillary reaching to the posterior part of eye.

Dorsal fin with 4 (n = 5) unbranched rays and 10½ (n = 5) branched rays, outer margin deeply concave. Anal fin with 3 (n = 5) unbranched and 6½ (n = 5) branched rays, outer margin straight. Pectoral fin with 14 (n = 2)–15 (n = 3) rays. Pelvic fin with 8 (5) rays. Lateral line with 38 (n = 1), 39 (n = 2), 40 (n = 2) scales. Scale rows between dorsal-fin origin and lateral line 6 (n = 4)–7 (n = 1). Scale rows between anal-fin origin and lateral line 5 (n = 6).

Coloration

In fresh: Body silverish or cream-white. The back darker than the belly. Upper lateral line scales outlined by dark pigmentation, evident in anterior and fade in posterior. Fins with scattered dark melanophores on rays



Fig. 18. *Carasobarbus sublimus*; VPFC Fahlian 1400.10., 132 mm SL; Iran: Fahlian River.



Fig. 19. *Carasobarbus kosswigi*; VPFC NeypahN Seyfolah 1400.7., 85 mm SL; Iran: Alvand River, Tigris drainage.



Fig. 20. *Carasobarbus kosswigi*; uncatalogued, about 175 mm SL; Türkiye: Tigris River.



Fig. 21. *Carasobarbus kosswigi*; VPFC Hajij 1394.4., 114 mm SL; Iran: Sivan River.



Fig. 22. *Carasobarbus luteus*; VPFC SiyahGav 1400.9., 81 mm SL; Iran: Siyah Gav Lake.



Fig. 23. The ventral view of the head. From left to right: *Carasobarbus kosswigi*, VPFC NeypahnSeyfolah 1400.7., 85 mm SL; *C. sublimus*, VPFC Fahlian 1400.10., 132 mm SL; *C. doadrioi* sp. n., uncatalogued, about 150 mm SL; *C. hajhosseini* sp. n., AJRPC 21-P, 137 mm SL.

| Characters | <i>C. saadatii</i> | | | | | <i>C. sublimus</i> | | | | <i>C. kosswigi</i> | | | |
|---|------------------------|------|------|------|-----|--------------------|------|------|-----|--------------------|------|------|-----|
| | Holotype and paratypes | | | | | Min | Max | Mean | SD | Min | Max | Mean | SD |
| | H | Min | Max | Mean | SD | | | | | | | | |
| Standard length (SL) | 188 | 123 | 179 | | | 72 | | | | 85 | 176 | | |
| In percent of standard length | | | | | | | | | | | | | |
| Head length | 20.1 | 18.7 | 20.1 | 19.1 | 0.6 | 23.1 | 26.8 | 25.5 | 1.7 | 24.5 | 27.4 | 25.7 | 0.8 |
| Maximum body depth at dorsal fin origin | 27.2 | 27.0 | 29.9 | 28.1 | 1.2 | 27.1 | 32.2 | 29.0 | 2.5 | 25.4 | 29.9 | 27.8 | 1.3 |
| Body depth at anal fin origin | 18.2 | 18.0 | 20.3 | 19.3 | 1.0 | 19.1 | 22.4 | 20.8 | 1.5 | 17.0 | 19.9 | 18.4 | 1.0 |
| Pre-dorsal length | 54.1 | 48.9 | 54.1 | 51.9 | 2.2 | 51.7 | 56.6 | 54.3 | 2.1 | 47.0 | 53.1 | 50.5 | 1.7 |
| Pre-pelvic length | 53.1 | 47.6 | 53.1 | 49.6 | 2.2 | 51.5 | 55.5 | 53.6 | 1.9 | 49.2 | 52.6 | 51.1 | 0.9 |
| Pre-anal length | 75.1 | 74.2 | 76.3 | 75.1 | 0.8 | 74.5 | 77.8 | 75.8 | 1.6 | 73.9 | 78.9 | 75.9 | 1.4 |
| Dis. betw. pectoral and anal fins | 55.1 | 55.0 | 57.6 | 56.4 | 1.2 | 46.3 | 51.4 | 49.0 | 2.1 | 50.2 | 56.4 | 53.4 | 1.4 |
| Dis. betw. pectoral and pelvic fins | 29.8 | 29.5 | 30.6 | 29.9 | 0.4 | 25.0 | 28.5 | 27.2 | 1.5 | 25.0 | 29.7 | 27.6 | 1.1 |
| Dis. betw. pelvic and anal fins | 25.6 | 25.6 | 28.1 | 26.9 | 1.1 | 21.1 | 23.8 | 22.6 | 1.2 | 18.6 | 30.1 | 26.0 | 2.4 |
| Dorsal fin height | 28.2 | 26.2 | 29.7 | 27.9 | 1.4 | 21.1 | 26.1 | 23.2 | 2.2 | 25.3 | 30.5 | 27.5 | 1.2 |
| Anal fin height | 22.0 | 20.4 | 22.9 | 21.3 | 1.1 | 20.4 | 27.2 | 23.5 | 3.0 | 17.5 | 27.4 | 22.2 | 3.3 |
| Pectoral fin length | 22.1 | 21.7 | 24.0 | 22.6 | 1.0 | 20.6 | 22.3 | 21.4 | 0.7 | 20.2 | 22.4 | 21.0 | 0.5 |
| Pelvic fin length | 19.1 | 18.8 | 21.3 | 19.8 | 1.0 | 19.1 | 21.0 | 20.1 | 0.8 | 18.6 | 20.1 | 19.3 | 0.5 |
| Upper caudal fin lobe | 29.7 | 29.7 | 33.7 | 31.6 | 1.7 | 29.5 | 34.9 | 32.8 | 2.5 | 27.8 | 32.0 | 29.9 | 1.2 |
| Length of middle caudal fin | 9.7 | 9.7 | 13.3 | 11.2 | 1.4 | 13.0 | 16.6 | 15.2 | 1.6 | 11.6 | 14.1 | 12.9 | 0.8 |
| Caudal peduncle length | 16.3 | 15.3 | 17.1 | 16.3 | 0.7 | 13.3 | 14.9 | 14.0 | 0.7 | 15.2 | 17.6 | 16.5 | 0.7 |
| Caudal peduncle depth | 10.2 | 10.2 | 12.1 | 11.0 | 0.7 | 10.9 | 13.1 | 11.8 | 1.1 | 9.3 | 10.9 | 10.2 | 0.4 |
| In percent of head length | | | | | | | | | | | | | |
| Snout length | 28 | 26 | 28 | 26.6 | 0.8 | 29 | 34 | 30.6 | 2.2 | 36 | 44 | 39.8 | 2.2 |
| Eye diameter | 23 | 20 | 24 | 22.2 | 1.7 | 22 | 30 | 25.3 | 3.1 | 16 | 26 | 19.2 | 2.5 |
| Head depth at pupil | 60 | 56 | 62 | 59.9 | 2.2 | 55 | 61 | 57.6 | 2.5 | 53 | 61 | 57.0 | 2.3 |
| Head depth at nape | 88 | 88 | 95 | 90.8 | 3.0 | 78 | 81 | 80.0 | 1.6 | 65 | 76 | 70.7 | 2.9 |
| Posterior barbel | 16 | 16 | 21 | 19.4 | 2.4 | 24 | 35 | 29.1 | 4.9 | 13 | 20 | 16.5 | 1.5 |
| Anterior barbel | 19 | 17 | 21 | 19.1 | 1.9 | 12 | 20 | 17.0 | 3.4 | 18 | 25 | 20.8 | 2.0 |

Table 7. Morphometric data of *C. saadatii* sp. n. (holotype BIAUBM 8-H and paratypes AJRPC 24-P; n = 5) and *C. sublimus* (VPFC Zard 1400.9., VPFC Fahlian 1400.10; n = 8) and *C. kosswigi* (FFR 416, FFR 417, FFR 421; n = 17).

and membranes. In formalin: Body cream-brown, back darker than belly. No dark pigmentation on anterior and posterior section of scales.

Distribution

The new species distributed in the lower Karun drainage as well as the Great Zab in the Tigris drainage.

Etymology

The species is named in honour of Mohamadali Saadati (Mashhad), acknowledging his significant contributions to the taxonomy of freshwater fishes in Iran. He holds the distinction of being the first Iranian Ichthyologist, conducting a systematic study on the taxonomy and distribution of freshwater fishes in Iran in 1977. To this day, his findings continue to be utilized by several Ichthyologists in Iran.

Habitat

The new species is usually found in the deeper parts of rivers and dam reservoirs, where water flows are slower and there is ample vegetation and cover (Fig. 24). During the summer months, it disperses into faster-flowing waters as well, likely due to warming water temperatures in their typical habitat. It prefers areas along the banks and around islands where tree roots and aquatic plants are accessible. This allows it to forage while remaining hidden among the vegetation to avoid predators. The species appears to be most abundant in the middle and lower Karun. *Luciobarbus barbulus* (Heckel, 1847), *Capoeta aculeate* (Valenciennes, 1844), *Garra rufa*, *Chondrostoma regium*, *Alburnus sellal*, *Squalius lepidus*, *Squalius berak* and *Glyptothorax cous*, were found coexisting with the new species.

Discussion

In general, fishes of the genus *Carasobarbus* are bottom feeders, with morphological characters specialised for such behaviour. This is especially visible in the differences in the development of their mouth structure and lips. Similar developments have been observed in other species of barbs^{26,27}. The lips development in *Carasobarbus* fishes, seems to be a suitable character to separate species²⁸. In the newly described species, the *C. hajhosseini* species present the smaller lips (less developed). On the other hand, *C. doadrioi* species, appear to show the most developed lips among them. Check the ventral head view figure (Fig. 23) to compare these differences and observe that both latter mentioned species show both ends of the spectrum. *Carasobarbus saadatii* species also present intermediate lips development similar to *C. sublimus* for example, but we do not have an acceptable picture to show in this work.

Borkenhagen and Krupp² questioned the locality data of the *C. sublimus* specimen (CMNFI 1979-0277), as the morphometric and meristic characters (scales in the lateral line, above the lateral line, and around the least circumference of the caudal peduncle; length of the dorsal, pectoral, ventral, and anal fins) of this specimen are within the range of *C. sublimus* and outside the range of *C. kosswigi*. This discrepancy is unsurprising because the Karkheh population belongs to *C. hajhosseini*, and the range of these characters matches the locality mentioned for this voucher specimen. However, they considered *C. hajhosseini* populations as *C. sublimus*, and *C. doadrioi* and *C. saadatii* as *C. kosswigi*, which caused the range of morphometric characters to expand and positioned *C. kosswigi* and *C. sublimus* as paraphyletic in the phylogenetic trees.

In general, nearly all the internal nodes are well resolved in all three datasets (COI, *Cyt b* and concatenated datasets) used in molecular phylogenetic analyses. But as expected, the concatenated dataset resulted in the best resolved tree. Both genetic markers used in the concatenated dataset are mitochondrial markers, i.e. they share the same evolutionary history. This point out that the improvement in the phylogenetic resolution is most probably



Fig. 24. Karun River, between Gotvand and Shushtar, Persian Gulf basin, type locality of *Carasobarbus saadatii* sp. n.

due to the increment in the phylogenetic signal coded in a longer sequence fragment. This point underlines the importance of including multiple markers to be able to resolve remaining obscure relationships within the genus. On the other hand, being hexaploid, complicates the inclusion of any nuclear marker in any genetic study in near future²⁹. This point is important as some species of the genus (for example *C. luteus*) is widespread in a variety of habitats and therefore will not be surprising to find that different populations does not share the same evolutionary history. This will not be visible without analysing both mitochondrial and nuclear genomic markers.

In the obtained mitochondrial phylogenetic results in the actual study, the only unresolved relationship, is the one between *C. doadrioi*, *C. hajhosseini* and *C. saadatii*. The very short internal branch at this level, when present, shows a potential rapid speciation event, resulting in small number of conserved changes to resolve this relationship. In our results, based on the partial COI gene, two clearly separate clades are formed with both containing sequences identified as *C. harterti* and *C. fritschii*. This is most probably the result of misidentification, or also it can be due to introgression events. As we do not have access ourselves to the material used in this case (genetic material was retrieved from GenBank), we cannot further develop on this and corroborate the identity of each of the clades. On the other hand, using other individuals identified as these two species, they do separate well in the results of the *cyt b* gene dataset, with no further issues. Another possible issue which will need further investigation is the inclusion of samples identified as *C. apoensis* within the *C. luteus* clade, with practically no genetic difference with them. This point was also mentioned in Borkenhagen²⁸. Based on this observation we recommend a systematic revision of both *C. apoensis* and *C. luteus* in further studies.

Comparative materials examined

Carasobarbus kosswigi. Iran: – VPFC Neypahn Seyfolah 1400.7., 1, 85 mm SL; Iran: Kermanshah prov.: Alvand River at Neypahn Seyfolah, Karkheh, 34.408611, 45.586944. – VPFC Hajji 1394.4., 1, 114 mm SL; Iran: Kermanshah prov.: Sirvan River at Hajji, Tigris drainage, 35.15678, 46.32132 (now under dam).

Türkiye: – FFR 416, 17, 124–176 mm SL; FFR 417, 1, 170 mm SL; FFR 421, 4, 129–168 mm SL; Siirt prov.: Botan River at 8 km southwest of Siirt, Tigris drainage, 37.85268, 41.88749.

Carasobarbus sublimus. Iran: – VPFC Zard 1400.9., 2, 72–132 mm SL; Iran: Fars prov., Zard River at Zard Mashin, Marun drainage, 31.37633, 49.72072. – VPFC Fahlian 1400.10., 2, 111–92 mm SL; Iran: Fars prov., Fahlian River at Fahlian bridge, Zohre drainage, 30.18520, 51.52443.

Carasobarbus luteus. Iran: – VPFC Siyahgav 1400.9., 7, 65–95 mm SL; Iran: Ilam prov., Siyah Gav Lake, near Abdanan, Tigris drainage, 32.86564, 47.70155. – VPFC Golabi 1400.10., 1, 130 mm SL; Iran: Fars prov., Golabi spring, near Darab, Kol drainage, 28.78766, 54.37183.

New material used in molecular genetic analysis

Carasobarbus kosswigi. Türkiye: AJRPC-DNA 1882 (COI: PP515193, *Cyt b*: PP548221), 1883 (COI: PP515194, *Cyt b*: PP548222), Şırnak prov.: Tigris River at 4 km north of Cizre, 37.375610 42.147106; 1884 (COI: PP515195, *Cyt b*: PP548223), Şırnak prov.: Tigris River at Damlarca, 37.404131 42.070865. Iran: AJRPC-DNA 45 (COI: PP515174, *Cyt b*: PP548208), Kermanshah prov.: Alvand River at Neypahn Seyfolah, Karkheh, 34.408611, 45.586944.

Carasobarbus sublimus. Iran: AJRPC-DNA 400A (COI: PP515179, *Cyt b*: PP548213), 400B (COI: PP515180, *Cyt b*: PP548214), Iran: Fars prov., Zard River at Zard Mashin, Marun drainage, 31.37633, 49.72072.

Carasobarbus luteus. Iran: AJRPC-DNA 554B (COI: PP515181) Kermanshah prov.: Alvand River at Neypahn Seyfolah, Karkheh, 34.408611, 45.586944; 707 (COI: PP515184) Khuzestan prov., Karun River at Gotvand, Persian Gulf Basin, 32.27319, 48.83521; Iraq: AJRPC-DNA 1465 (COI: PP515187), Al Najaf prov.: Euphrates River at Kafal, Persian Gulf Basin, 32.22339, 44.36113; 1376 (COI: PP515185), 1377 (COI: PP515186), Maysan prov.: Tigris River at Amareh, Persian Gulf Basin, 31.85783, 47.13605.

Data availability

All the specimens obtained in this study are deposited in local publicly accessible (upon request) zoological collections. The genetic data obtained in this study is deposited in NCBI's GenBank, the accession numbers for each gene marker is mentioned after each specimen's voucher code.

Received: 24 April 2024; Accepted: 28 August 2024

Published online: 18 September 2024

References

- Coad, B. W. & Najafpour, N. *Barbus sublimus*, a new species of cyprinid fish from Khuzestan province, Iran. *Ichthyol. Explor. Freshw.* **7**, 273–278 (1997).
- Borkenhagen, K. & Krupp, F. Taxonomic revision of the genus *Carasobarbus* Karaman, 1971 (Actinopterygii, Cyprinidae). *ZooKeys* **339**, 1–53. <https://doi.org/10.3897/zookeys.339.4903> (2013).
- Coad, B. W. *Carp and Minnows of Iran (Families Cyprinidae and Leuciscidae) Volume I: General Introduction and Carps (Family Cyprinidae)* (Canadian Museum of Nature, 2021).
- Kaya, C., Turan, D. & Unlu, E. The latest status and distribution of fishes in upper Tigris River and two new records for Turkish freshwaters. *Turk. J. Fish. Aquat. Sci.* **16**(3), 545–562. https://doi.org/10.4194/1303-2712-v16_3_07 (2016).
- Jouladeh-Roudbar, A., Ghanavi, H. R. & Doadrio, I. Ichthyofauna from Iranian freshwater: Annotated checklist, diagnosis, taxonomy, distribution and conservation assessment. *Zool. Stud.* **59**, e21. <https://doi.org/10.6620/ZS.2020.59-21> (2020).
- Bianco, P. G. & Bănărescu, P. M. A contribution to the knowledge of the Cyprinidae of Iran (Pisces, Cypriniformes). *Bull. Soc. Fr. Ichthyol.* **6**(2), 75–96 (1982).
- Karaman, M. S. Süßwasserfische der Türkei. 8. Teil: Revision der Barben Europas, Vorderasiens und Nordafrikas. *Mitt. Hambg. Zool. Mus. Inst.* **67**, 175–254 (1971).

8. Borkenhagen, K., Esmaeili, H. R., Mohsenzadeh, S., Shahryari, F. & Gholamifard, A. The molecular systematics of the *Carasobarbus* species from Iran and adjacent areas, with comments on *Carasobarbus albus* (Heckel, 1843). *Environ. Biol. Fish.* **91**, 327–335. <https://doi.org/10.1007/s10641-011-9787-1> (2011).
9. Kottelat, M. & Freyhof, J. *Handbook of European Freshwater Fishes* (Kottelat, Cornol & Freyhof, 2007).
10. Ward, R. D. *et al.* DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **360**(1462), 1847–1857. <https://doi.org/10.1098/rstb.2005.1716> (2005).
11. Machordom, A. & Doadrio, I. Evidence of a Cenozoic Betic-Kabilian connection based on freshwater fish phylogeography (Luciobarbus, Cyprinidae). *Mol. Phylogenet. Evol.* **18**(2), 252–263. <https://doi.org/10.1006/mpev.2000.0876> (2001).
12. Katoh, K., Misawa, K., Kuma, K. I. & Miyata, T. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **30**(14), 3059–3066. <https://doi.org/10.1093/nar/gkf436> (2002).
13. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **30**(4), 772–780. <https://doi.org/10.1093/molbev/mst010> (2013).
14. Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. MEGA 6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**, 2725–2729. <https://doi.org/10.1093/molbev/mst197> (2013).
15. Nguyen, L. T., Schmidt, H. A., Von Haeseler, A. & Minh, B. Q. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **32**(1), 268–274. <https://doi.org/10.1093/molbev/msu300> (2015).
16. Trifinopoulos, J., Nguyen, L. T., von Haeseler, A. & Minh, B. Q. W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Res.* **44**(W1), W232–W235. <https://doi.org/10.1093/nar/gkw256> (2016).
17. Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K., Von Haeseler, A. & Jermini, L. S. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* **14**(6), 587–589. <https://doi.org/10.1038/nmeth.4285> (2017).
18. Guindon, S. *et al.* New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst. Biol.* **59**(3), 307–321. <https://doi.org/10.1093/sysbio/syq010> (2010).
19. Ronquist, F. *et al.* MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**(3), 539–542. <https://doi.org/10.1093/sysbio/sys029> (2012).
20. Huelsenbeck, J. P., Larget, B. & Alfaro, M. E. Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. *Mol. Biol. Evol.* **21**(6), 1123–1133. <https://doi.org/10.1093/molbev/msh123> (2004).
21. Rambaut, A., Drummond, A. J., Xie, D., Baele, G. & Suchard, M. A. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* **67**(5), 901–904. <https://doi.org/10.1093/sysbio/syy032> (2018).
22. Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. ABGD, automatic barcode gap discovery for primary species delimitation. *Mol. Ecol.* **21**(8), 1864–1877. <https://doi.org/10.1111/j.1365-294X.2011.05239.x> (2012).
23. Puillandre, N., Brouillet, S. & Achaz, G. ASAP: Assemble species by automatic partitioning. *Mol. Ecol. Resour.* **21**(2), 609–620. <https://doi.org/10.1111/1755-0998.13324> (2021).
24. Zhang, J., Kapli, P., Pavlidis, P. & Stamatakis, A. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* **29**(22), 2869–2876. <https://doi.org/10.1093/bioinformatics/btt499> (2013).
25. Borkenhagen, K. A new genus and species of cyprinid fish (Actinopterygii, Cyprinidae) from the Arabian Peninsula, and its phylogenetic and zoogeographic affinities. *Environ. Biol. Fish.* **97**, 1179–1195. <https://doi.org/10.1007/s10641-014-0315-y> (2014).
26. Nagelkerke, L.A.J. The barbs of Lake Tana, Ethiopia. Morphological diversity and its implications for taxonomy, trophic resource partitioning, and fisheries. Dissertation, *Univ. Diss.*, 296 (1997).
27. Levin, B. A. *et al.* Adaptive radiation of barbs of the genus *Labeobarbus* (Cyprinidae) in an East African river. *Freshw. Biol.* **64**, 1721–1736 (2019).
28. Borkenhagen, K. Taxonomy, phylogeny and zoogeography of the hexaploid *Torini* of the Middle East and North Africa. Dissertation, *Frankfurt am Main, XIII*, 148 (2017).
29. Yang, L. *et al.* Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Mol. Phylogenet. Evol.* **85**, 97–116 (2015).

Author contributions

A.J.R. and H.R.G. designed the experiment. A.J.R., S.V. and C.K. sampled the individuals. A.J.R. performed the wet laboratory experiments and morphological analyses. A.J.R. and H.R.G. performed the molecular analyses, and wrote the manuscript with inputs from all authors. S.V. and C.K. helped obtain permits for sampling. H.R.G. secured the funding and supervised the work.

Funding

Open access funding provided by Lund University.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to H.R.G.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2024