

Research Paper

Molecular systematics and phylogeny of *Rutilus* s. str. (Teleostei: Leuciscidae) at the southern part of the Caspian Sea

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Introduction

Leuciscidae, with 14 genera and 41 endemic species, constitutes approximately 15% of the fauna of Iran's inland water fishes (Jouladeh-Roudbar et al., 2015; 2020). The genus *Rutilus* is one of the economically important genera of this family, which now consists of 10 valid species found in brackish, marine, and freshwaters throughout Eurasia (Eschmeyer et al., 2018; Coad, 2018; Levin et al., 2017). Based on prior research, three major lineages of the genus *Rutilus* have been recognized. 1- *R. frisii* lineage, 2- *Rutilus rutilus* s. str. 3. The group includes species found in the Aegean Sea, Caspian, Black-Azov, and Aral, as well as in the interior waters of Central Asia, the rivers of Siberia, and the Arctic Ocean. According to the sequencing results of the third-category Cytb gene, there is no significant genetic difference. *R. stoumboudae*, *R. r. aralensis*, and *R. schelkovnikovi* are therefore considered synonyms of *R. rutilus*. Previous studies demonstrated the presence of *R. caspius* (Yakovlev 1870), *R. lacustris* (Pallas 1814), *R. kutum* Kamenskii, 1901, *R. rutilus* (Linnaeus 1758), *R. r. caspius natio kurensis* Berg 1932, and *R. r. caspius natio knipowitschi* Pravdin 1927 in the southern Caspian Sea basin (Bogutskaya & Naseka, 2004; Kottelat & Freyhof, 2007; Coad 2018; Jouladeh-Roudbar et al., 2020). Most of these taxa have distinct morphological features, making them difficult to distinguish and identify (Berg 1949; Abdolhay et al., 2010; Kashеfi et al., 2012; Kohestan-Eskandari et al., 2014). Even though morphological studies have been conducted on populations of this group, their taxonomic position in the southern basin of the Caspian Sea has not been explored, and it is unknown which taxon or taxa are valid. This study used the COI gene sequence to evaluate the taxonomic status of populations of this group in the southern Caspian Sea to clarify the status of the valid species.

Methods and Materials

To conduct this study, adult specimens of the *Rutilus* with various morphological traits, including the number of scales on the lateral line, dorsal fin radius, pectoral fin radius, gill spine, and body color, were collected from beach seine between 2018 and 2019. The fin clips were stored in 96% ethanol and transferred to the laboratory

6/ Molecular systematics and phylogeny of *Rutilus s. str.* (Teleostei: Leuciscidae) at the ...

for molecular studies. DNA was extracted using a kit made by BIO-RAD. forward and reverse primers amplified the COI barcode region. After electrophoresis confirmed adequate amplification, PCR products were purified using Exo-SAP-IT® and sequenced by an external company (Macrogen, Seoul, South Korea) using the same forward primer. The results of the sequences and other sequences found in the NCBI gene bank were used to plot phylogenetic trees, which were done so using the Bayesian inference method, the Maximum likelihood estimation method, and the haplotype network.

Results & Discussion

Following the receipt and correction of the sequencing data from Macrogen Company, 630 bp of 20 samples were obtained for analysis. Furthermore, 112 sequences from members of this genus were taken from the NCBI World Bank and aligned with the above sequences, up to 630 bp, 123 polymorphic positions, and 507 fixed positions without polymorphism. In addition, 108 locations were identified as Parsim-informative out of 630 bases analyzed. Phylogenetic trees revealed that 10 species, including *R. rutilus*, *R. lacustiris*, *R. ylikiensis*, *R. panosi*, *R. virgo*, *R. albus*, *R. basaki*, *R. aulai*, *R. pigu*, and *R. frisii*, showed high levels of BI and ML. Also, the results of bPTP species approach were the same for all of the clusters, except for *R. lacustiris*.

Conclusion

Due to the insignificant difference in COI gene and phenotypic diversity, it seems the differences are due to ecological plasticity, tolerance to salted waters coupled degrees of salinity, and having resident and migratory populations among members of this genus. Generally, this study found no significant differences between the populations tested in the south of the Caspian Sea. As a result, it is recommended that *R. frisii* be regarded as a valid species and the other taxa listed as synonyms, based on the priority in naming. But because the above proposal was based on the results of sequencing the COI mitochondrial gene, it is suggested that nuclear genes be used better to measure the cognitive range of members of this species and come to a complete conclusion.

Keywords: *Carp*s, COI genetic distance, phenotype, Iran

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Declaration of conflict of interest: The authors declare that they have no conflicts of interest.