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RESEARCH PAPER

First Genetic Record of *Cyprinion watsoni*, Indus Lotak (Day, 1872) Cypriniformes: Cyprinidae from Indus River District Swabi Khyber Pakhtunkhwa, Pakistan

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Abstract

Cyprinion watsoni is fresh water fish commonly known as Indus lotak or Watson cyprinid is native fish to the Indus River basin, Pakistan, but also found in Afghanistan, Iraq, Iran, Arabian Peninsula and Oman. This fish inhibits a verities of aquatic environments i.e. rivers, deep water pools, lakes, streams, and brackish water. A field survey of the present study was conducted in August, 2024 to investigate the morphological and genetic relationship of *C. watsoni* Population using Cytochrome oxidase gene sub unit I (*COI*) from Indus River, district Swabi, Khyber Pakhtunkhwa, Pakistan. A fish sample were collected from Tarbela region of the Indus River and morphologically identified as *Cyprinion* species using standard taxonomic key. We sequenced and submitted a Cytochrome oxidase sub unit I (*COI*) gene to GenBank with accession number PV444642 from first time of Pakistan. The Phylogenetic analysis of the reported *COI* gene from Pakistan showed high similarity of almost 91.34% with *C. watsoni* reported from Saudi Arabia. For further conformation, we establish a Median joining haplotype network and calculate the nucleotide and haplotype diversity of *COI* gene. The calculated Haplotypes diversity (hd) was 0.9848 and nucleotide diversity (π) was 0.0801865.

INTRODUCTION

Indus River is one of the longest Asian's river that is originated from Tibetan plateau, Ladakh region of Gilgit-Baltistan and pass from entire length of Pakistan before merging the Arabian Sea in Karachi, Sindh (Khan & Hassan, 2025). The Indus River is home to fish fauna that include 180 freshwater fish species, among which 22 species are endemic (Rafique & Khan, 2012). The upper region of Indus River support fewer fish species due to food scarcity and cold water. However, after merging of the Kabul River into the Indus River the fish diversity increased by the availability of food and rise of the water temperature (Khan, 2025).

Cyprinion watsoni is a freshwater fish commonly known as Indus lotak or Watson cyprinid or Indus cyprinid belonging to order Cypriniformes and family Cyprinidae. It is native to the Indus River basin, Pakistan, but also exist in Afghanistan, Iran, Arabian Peninsula and Oman (Harrison, 2015). This fish inhabits a variety of aquatic environments, including rivers, deep water pools, lakes, streams, and brackish waters (Talwar & Jhingran, 1991). The population of *C. watsoni* was first reported from Indus River, Sakkar District, Sindh, Pakistan (Day, 1872). This fish has been referred to by various synonyms over time, including: *Scaphiodon watsoni, Scaphiodon irregularis* (Day, 1872), *Scaphiodon muscatensis* (Boulenger, 1888), *Cirrhina afghan* (Boulanger, 1889), *Barbus bampurensis* (Nilkoiskii, 1900), *Scaphiodon macmahoni* (Regan, 1906), *Scaphiodon baluchiorum* (Jenkins, 1910), *Scaphiodon readingi* (Hora, 1923) and *Semiplotus dayi* (Fowler, 1958). This fish is primarily herbivore and shows vegetarian tendency to eat filamentous algae i.e., spirogyra and Cladophora and wide range of diatoms. Sometimes it acts as omnivore due to the

presence of insects in its gut (Nasari et al., 2018). *C. watsoni* spawn once per year. Although the breeding season varies geographically: In Iran, peak spawning is reported from July to August (Motlagh et al., 2019). In Islamabad, Pakistan, spawning occurs in late March to mid-April (Shaikh & Jalali, 1991). In Rawalpindi, Pakistan, it occurs from April to May (Shaikh & Hafeez, 1993). This fish spawns in slow running shallow water with submerged vegetation. The mature eggs are dark yellow k and have a diameter of about 1.2mm. Females lay very small number of eggs only around 150 eggs per breeding season. Males have small tubercles on the Snout and anal fin rays with creamy color testes (Regan, 1906). The maximum total length was ranged from 8.34 to18.7 cm and maximum weight is recorded 53 g (Esmaeili et al., 2006; Motlagh et al., 2019). These species are not economically important but in Pakistan they are used as a source of protein in human diet (Shah, 2002).

Fish are the most diverse group of vertebrates with over 32,500 species worldwide (Nalson, 2006). All types of fish are different sizes, shape and color (Lipinski & Tweed, 2003). Fish fauna are generally identified on the base of morphology such as physical appearance, size, color, type of scale, meristic and morphometric measurements. Sometimes also identified by number of gill and fin rays, various stages of life cycle and on the base of habitats (Ullah, 2014). However, some closely related species i.e., larval stage and dead decomposed part fish are impossible to identify through morphology. Nowadays, DNA-based identification is widely used. DNA based identification is most authentic method, for this purpose various marker genes such as 16Sr, 18Sr, 12Sr, Cytb, and *COI* of mitochondrial DNA were used. Among these, the *COI* gene is the most accurate global identifier due to its low intra- and interspecific variation (Avise et al., 2003; Hebert, 2004). In this study, *C. watsoni* was sequenced for the first time in Pakistan, shedding light on its systematic status and haplotype analysis. This study clarifies the taxonomic status of *Cyprinion* species in Pakistan through morphological and genetic analysis.

MATERIALS AND METHOD

Study Area

The study area for collecting fish samples was the Indus River in Swabi District, Khyber Pakhtunkhwa, Pakistan. The field survey was conducted in August 2024, and a total of 5 fish specimens were collected from Tarbela region, Indus River with geographic coordinates 34.21N and 72.81E using cast nets and trammel nets. The Tarbela region of Swabi District is surrounded by District Buner and Haripur to the north, Punjab Province to the South, Islamabad and Rawalpindi to the East and District Mardan to the west.

Specimens Identification

The collected fish samples were morphologically identified using standard taxonomic keys such as "Inland Fishes of India and Adjacent Countries" (Talwar & Jhingran, 1991), "Fishes of Punjab" (Mirza, 2003), "Freshwater Fishes of the Indian Region" (Jayaram, 1999). After identification, fish samples were preserved in 70% fresh ethanol for DNA extraction and molecular analysis. The voucher specimens were deposited in the Fisheries and Aquaculture Laboratory, Department of Zoology, University of Peshawar, Pakistan.

Molecular and Phylogenetic Analysis

For DNA extraction and phylogenetic analysis two fresh fish specimens were collected from Tarbela region of Indus River, Swabi District. The Ammonium Acetate based protocol was used to extract Mitochondrial DNA from muscle tissue of fish specimen (Bruford et al., 1998). PCR amplicons were sequenced by the next generation Microgen sequencer in Korea. The new *COI* sequence chromatogram was trimmed, edited and read using Bio-Edit (version 7.2.5) and Finch TV (1.4.0). The available sequence was blasted through NCBI to find similar related sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The new sequences were deposited in GenBank with accession number PV444642 (Tarbela) and the taxonomic status was assessed using MEGA11 (Hall, 1999) to construct their phylogenetic tree. Nucleotide and Haplotype diversity were calculated using DnaSPv. 5.10.1, and a median join network was constructed in PopART v.1.7. (Librado & Rozas, 2009; Leigh & Bryant, 2015).

RESULTS

Morphological identification

The body shape of the fish is elongated,

slender and laterally compressed with Total length (TL) ranged from 10.6 to 17.8 cm. The head is pointed and elongated with 3.2 to 4.6 times to standard length (SL). The snout is round but slightly pointed with 1.2 to 2.1 times of head length (HL). The mouth was terminal or slightly inferior, small and horseshoe-shaped, pharyngeal teeth was 2, 3, 5 or 5, 3, 2 with round crowns. The eyes were round and moderate in size with 0.25 to 0.36 of the head length. The gills rakers were 10-18. Skin was covered by small cycloid scales, lateral line scales were 30-45. The dorsal fin has a total of 14 rays: 4 unbranched and thick rays, including a serrated first ray, and 10 branched and soft rays. The pectoral fin has 16 branched and seven unbranched rays. The pelvic fin has 8-9 branched rays. The anal fin consists of 2 to 3 spinous unbranched rays and 7 branched rays. The caudal fin is forked in shape, with the upper lobe longer than the lower lobe, and contains 19-21 branched rays (Figure 1 & 2).

Figure 1. Lateral view of preserved sample of *C. watsoni*

Figure 2. a & b) Lateral view of live sample of *C. watsoni*

Body Color

The belly area and lower flank are light - pink or yellowish, while the upper plank is copper brown, light green olive, or brown-grey, occasionally orange or bluish tones. Adult fish has 6-9 orange strips above the lateral line. Eyes were golden in color with black iris, cheek area and operculum are bluish? And slightly orange in color. The pelvic and pectoral fins are orange to pink in color, while the caudal fin is hyaline with black spots.

Sexual Dimorphism

Males have creamy color testes and adult males exhibit a depression in front of the nostrils. There are large tubercles on Snout and anal fin rays. During breeding season, males are more vibrant in color as compared to female. Female individuals have a rounded and swollen belly due to the presence of eggs.

Molecular and Phylogenetic Analysis

Mitochondrial DNA was extracted from fish samples collected from Tarbela area of Swabi Khyber Pakhtunkhwa, Pakistan. The sequenced mitochondrial cytochrome oxidase subunit-I gene (*COI*/mDNA/650-710bp) was blasted and submitted to GenBank for accession. The newly generated *COI* nucleotide sequence with accession number PV444642 was aligned with other available nucleotide sequences of related species. It showed a high similarity (91.34%) with *Cyprinion* watsoni reported from Saudi Arabia (see Table 1). For Phylogenetic analysis, different nucleotide sequences were downloaded and aligned in Mega file to construct Phylogenetic tree (Figure 3). The Maximum-likelihood phylogenetic tree of C. watsoni using the *COI* gene revealed high similarity with other related in-group species of the tree. The *Cyprinion* sp., *Cyprinion watsoni, Cyprinion macrostomum, Spinibarbichthys*

Table 1. Species name, Location, Accession numbers, Accession length, Similarity index

Species name	Location	Accession number	Accession length	Similarity Index %
Cyprinion Sp.	Iran	KM590429	770	91.22
Cyprinion watsoni	Saudi Arabia	PQ119925	633	91.34
Cyprinion watsoni	Pakistan	PV444642	617	91.34
Cyprinion watsoni	Iran	KM590435	770	91.06
Cyprinion watsoni	Saudi Arabia	PQ119922	647	91.06
Cyprinion macrostomum	Iraq	MW250387	629	91.06
Cyprinion macrostomum	Iran	KM590433	770	91.06
Cyprinion watsoni	Iran	KM590434	770	90.89
Spinibarbichthys denticulatus	Vietnam	MW446151	884	90.76
Spinibarbichthys denticulatus	Vietnam	MW446152	884	90.60
Cyprinion mhalense	Iran	PP111342	558	91.11
Schizothorax macropogon	China	KT439284	650	89.79
Cyprinus carpio	Iraq	OM669703	657	89.76

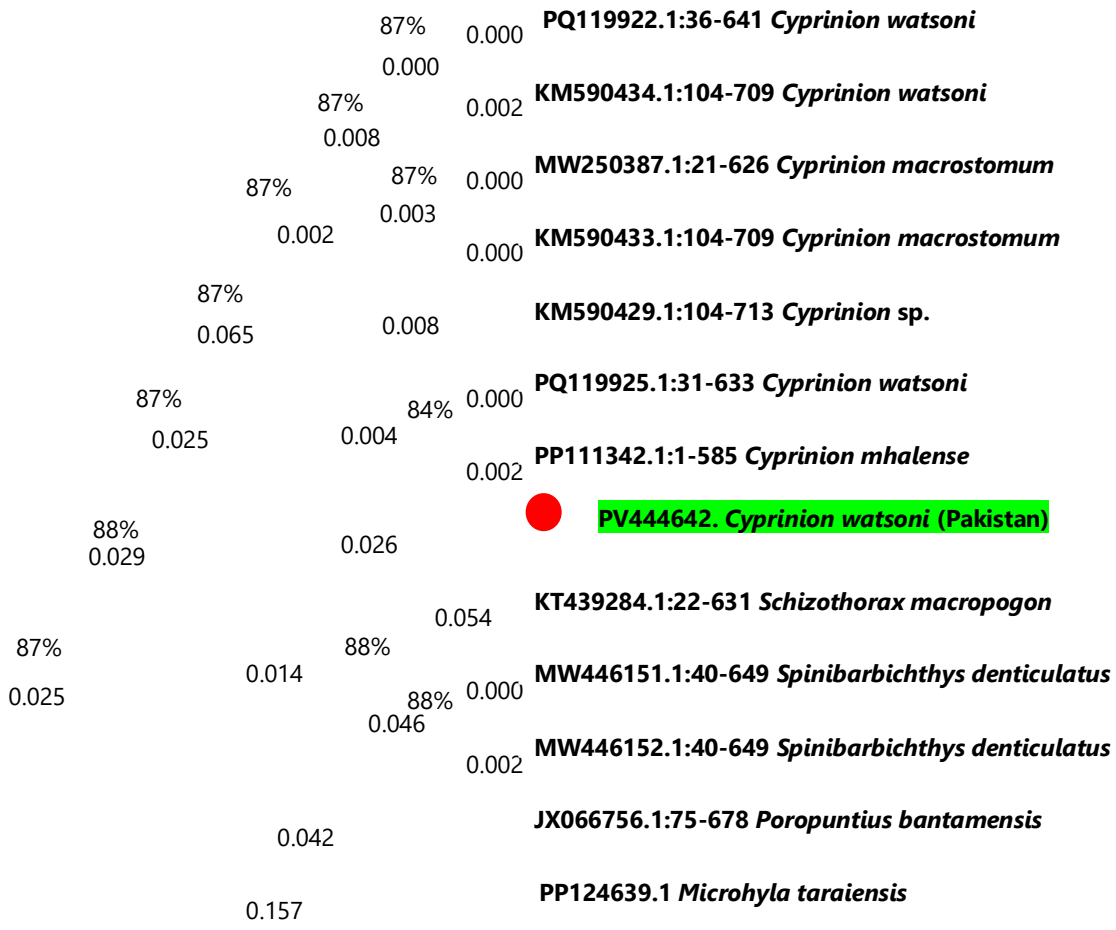

Figure 3. Maximum-likelihood phylogenetic tree of *C. watsoni* using the *COI* gene. *Microhyla taraiensis* was used as the out group, while *Cyprinion* sp., *Cyprinion watsoni, Cyprinion macrostomum, Cyprinion mhalense, Schizothorax macropogon, Poropuntius bantamensis* and *Spinibarbichthys denticulatus* was included in the group.

denticulatus, Cyprinion mhalense and *Schizothorax macropogon* were in-group species, while Minervarya agricola was out-group.

Median Joining Haplotype Network and Nucleotide Diversity

A new *COI* sequence was selected for construction of Median joining haplotype network and to find the haplotype and nucleotide diversity. A total of 12 *COI* sequences were downloaded from NCBI GenBank and aligned in MEGA11 to create a Nexus file. The Nexus file opened in DnaSP to make haplotype data file. In this file, 12 sequences were used. The selected region was 1,619 bp in length, with a total of 619 sites, and 585 sites excluding gaps. A total of 11 haplotypes were identified, with a haplotype diversity (hd) of 0.9848 and a nucleotide diversity (π) of 0.0801865. The haplotype data file and trait file used in Pop Art to create a Median-joining haplotype network. The median joining network shows intraspecific and geographic relationships of *C. watsoni* from Pakistan with the other species reported from Iran, Iraq, Vietnam, Saudi Arabia, China, and Louis America. We also calculated the single nucleotide polymorphism among the selected 11 haplotypes i.e. Hap_1; 1SNP, Hap_2; 3SNPs, Hap_3; 25SNPs, Hap_4; 4SNPs, Hap_5; 1SNP, Hap_6; 1SNP, Hap_7; 1SNP, Hap_8; 1SNP, Hap_9; 31SNPs, Hap_10; 44SNPs, Hap_11; 15SNPs, Hap_2 and Hap_8; 1SNP, Hap_4 and Hap_7; 1SNP (Figure 4 and 5).

DISCUSSION

C. watsoni is fresh water fish living in pools, streams, and rivers flowing in mountainous and sub mountainous regions of Pakistan, Iran, Türkiye, Saudi Arabia, Afghanistan and Iraq. The species were first collected by Watson from Sukkur Indus River basin Pakistan and first described by a pioneer Ichthyologist Day (1889) in "Fauna of British India". Several other Ichthyologist worked on morphology of

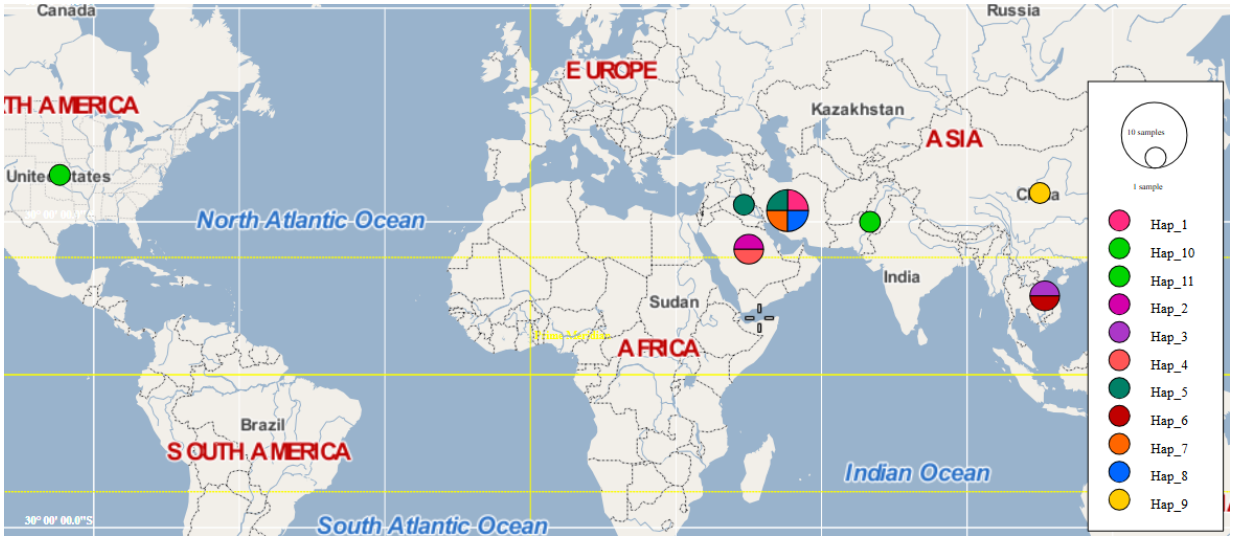

Figure 4. Haplotype network and geographic distribution of *C. watsoni* from Pakistan, Iran, Iraq, Vietnam, Saudi Arabia, China, and Louis America.

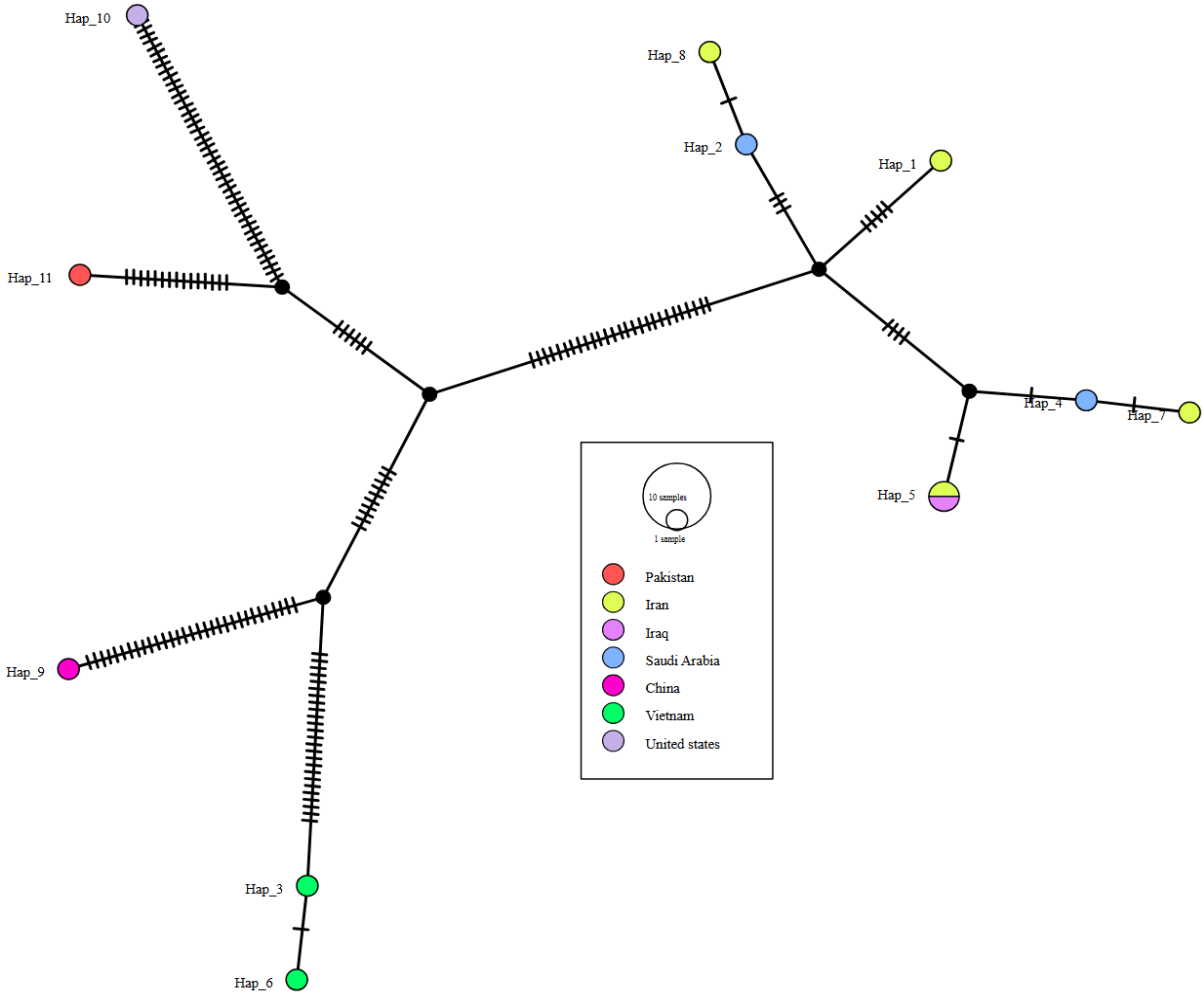

Figure 5. Haplotypes and single nucleotide polymorphisms (SNPs) among the *C. watsoni* species and other related species from Pakistan, Iran, Iraq, Vietnam, Saudi Arabia, China, and Louis America.

C. watsoni including Boulenger (1888), Günther (1889), Nikolskii (1900), Regan (1906), Jenkins (1910), Hora (1923) and Fowler (1958). In Pakistan, Naseeb et al. (2020) reported it from Zhob River, northern Balochistan, Ullah et al. (2014) from the River Panjkora, Ahmad et al. (2023) from the Khudo Khail stream District Buner. A fish sample was collected from Tarbela region of Swabi District, Khyber Pakhtunkhwa, Pakistan. The morphological findings, such as total length (TL) ranged from 10.6 to 17.8 cm, head was pointed and elongated with 3.2 to 4.6 times to Standard length (SL), snout was round but slightly pointed with 1.2 to 2.1 times of head length (HL), mouth was terminal or slightly inferior, small and horseshoe shaped, pharyngeal teeth's were 2, 3, 5 or 5, 3, 2 with round crowns, gills rakers were 10-18, skin was covered by small cycloid scales, and lateral line scales 30-45, were all similar to the findings of Motlagh et al. (2019) and Talwar and Jhingran (1991). A mitochondrial Cytochrome oxidase subunit I gene (*COI*) with accession number PV444642 from Tarbela region Swabi District, Khyber Pakhtunkhwa, Pakistan is sequenced for the first time. There is very little literature review about molecular and genetic characterization of *C. watsoni*. Hashemzadah segherloo et al. (2016) provided genetic record from Iran and Altowairqi et al. (2024) reported from Saudi Arabia. Esmaeili et al. (2024) separated *C. muscatese* and *C. watsoni* through Phylogenetic analysis of *COI* gene collected from Arabian Peninsula and Tiger River drainage of Persian Gulf basin. Additionally, we provide the Median joining haplotype network of *C. watsoni* for the first time and compared the geographic distribution and genetic variation of this species with other related species reported from Iran, Saudi Arabia, Iraq, Louis US, China and Vietnam.

CONCLUSION

This study was aimed to document the identification of C. watsoni based on morphological characters and on Cytochrome oxidase subunit I (*COI*) gene. The result of the study was based on maximum-likelihood inference assess the taxonomic status of the sample obtained from Pakistan. The first genetic record of C. watsoni from Tarbela Indus River, Swabi Districts was provided baseline data for *Cyprinion* genus and its distribution across Pakistan.

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