Molecular and morphological study of a new record Crab (*potamon persicum*) in the Greater Zab river Kurdistan region-Iraq

Youns K. Saber*
Biology Department, College of Education, Salahaddin University-Erbil, Kurdistan Region of Iraq.
Corresponding email: youns.saber@student.su.edu.krd

Luay A. Ali
Biology Department, College of Education, Salahaddin University-Erbil, Kurdistan Region of Iraq.

Abstract

The Brachyura (decapod;crustacean) of Kurdistan region–Iraq, *Potamon persicum* Pretzmann,1962.discovered in central Asia from The Euphrates-Tigris rivers in the southern Turkey. New record species in the Greater Zab river Kurdistan region –Iraq, as primary freshwater crab*(P.presicum)*reported in this study. Morphologically the recorded species has a transversely oval-shaped Carapace. Front margin is smooth and folded down. The third maxilliped's exopod has a flagellum on its exopod. Concerning to Molecular study, and the identity results showed evidence derived from the mitochondrial 16S rDNA supports the establishment of this species, identically species that they are 100% identical *P.persicum*.

Keywords: Brachyura, freshwater crab, new record, *Potamon persicum*, Iraq.

Introduction

Malacostraca is one of the largest class of Crustacea, includes nearly 20.000 taxa distributed in marine, freshwater, and groundwater environments. There are three subclasses which are Eumalacostraca, Haplocarida, and Phyllocarida (Özbek,and Ustaoğlu, 2006). Primary freshwater crabs include more than 1,476 species worldwide & nearly inhabit 20 percent of completely the brachyurans, Freshwater crabs live in both tropics and subtropics in most areas of the world. They can be discovered in utmost freshwater ecosystems (Yeo *et al.*, 2008,2012). Primary freshwater crabs, sometimes known as "wholly freshwater crabs," grow entirely on land (Yeo *et al.*, 2014).

The family potamoidae are freshwater crabs represented by a large number of genera and species found in different habitats in Africa, Asia, and Europe (Micheli,and Vannini 1990;Brandis *et al.*,2000). Family potamidae has15 Genera included more than 60 species (Guinot *et al.*, 1997).

In Turkey has nine primary freshwater crab described belonged to specimens have its place to the genus *Potamon: P. persicum Pretzmann, 1962; P. ibericum (Bieberstein, 1809), P. hueceste Pretzmann, 1962; P. magnum Pretzmann, 1962 P. bileki Pretzmann, 1971, P. potamios (Olivier, 1804), P. setiger Rathbun, 1904, P. rhodium Parisi, 1913, and P.mesopotamicum* Brandis, Storch & Türkay,1998. Nevertheless, engrossed and detailed information was not available on specimens distribution in Turkey (Harlioglu, *et al*,.2018).

In Iraq and the Kurdistan Region, there has been little research on crabs, especially their molecular identification, in Kurdistan region

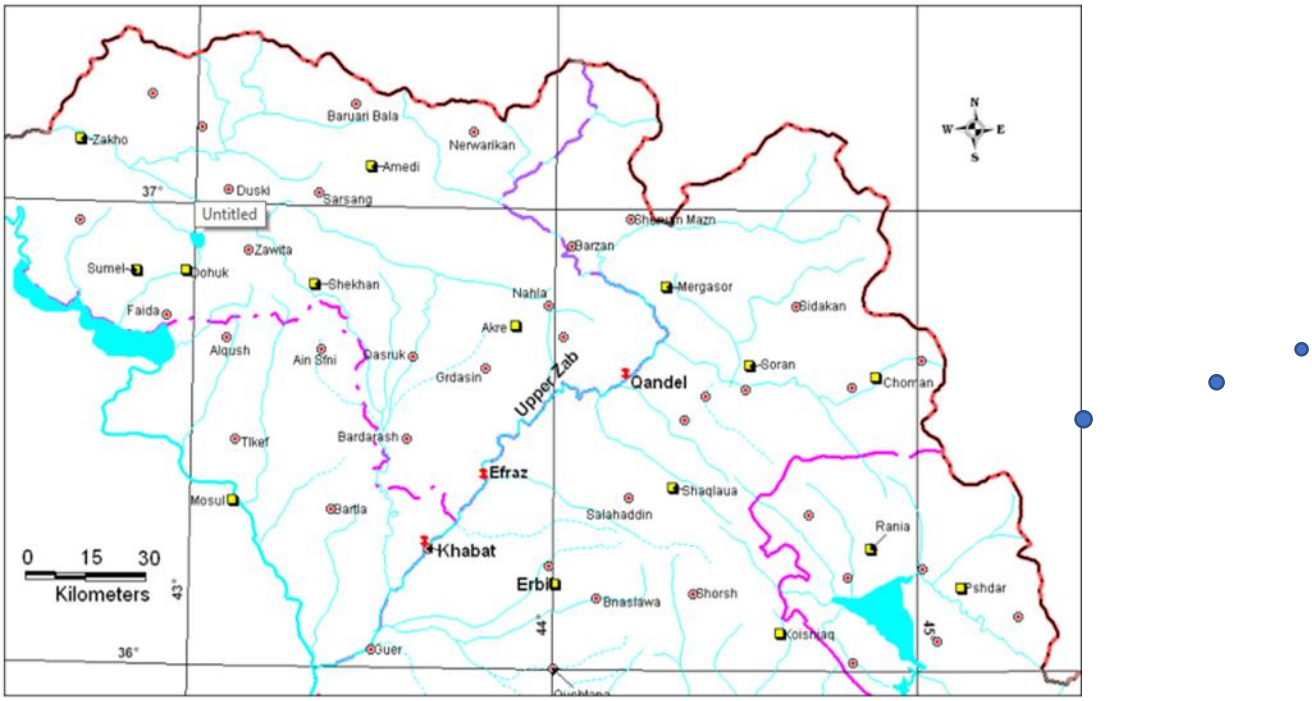
just one study; First discovered species *P.mangum*, in the Greater Zab river recording by (Ali and Jawair,2013) and the present study regards a new recorded species of *P. persicum* in the Greater Zab River of this group.

Material method

In the current study 117 specimens of *P.persicum* were gathered from the Greater Zab river from October 2021 until May 2022 samples freshwater crabs were conserved in (70%,96%,99%)ethanol for morphology and molecular study.

Study Area; The Greater Zab river 392km, located east of Tigris river in the northern Iraq (Kurdistan region). The sampling study is situated near the Khabat subdistrict (36.1884° N, 44.0331° E); the district lies 37km West of the city of Erbil.

Fig. 1. Map shows Greater Zab River and tributaries sites, Showing and sampling station.



DNA Extraction PCR Amplification DNA Analysis

Genomic DNA from Brachyura species was arranged by using rDNA Extraction kit (Add prep Bio Korea). Genomic DNA was isolated from the muscle tissue of the Ambulatory legs and juvenile crab body after two days to a week. DNA Extraction PCR Amplification DNA Analysis cut up to 20mg of tissue and transfer it into a 1.5mL Microcentrifuge tube (not provided); add 200µl of lysis Buffer and remain in the incubator for 48 hours. Qualification and quantification of DNA Concentration were carried out using Nanodrop (ND-1000 USA) Spectrophotometer 1µL buffer solution mixed samples used for measured DNA concentration for DNA purity, PCR was used to detect the 16S rDNA in the genomes of the expected to be specific crabs. The COI primers used were forward: 5'-ACCCGCTGAATTTAAGCAT-3' at position 25 and reverse primer: 5'-CTCTTCAGAGTACTTTTCAAC-3' (Mollaret et al., 2000) designed the position 390. PCR Analysis occurs by using One PCRPTMP master mix, Add prep (Bioneer, Korea) whole volume of 40µl included 10µl

master mix, 1.5µl of each of the 2 primers, 2µl DNA template, and 5µl double-demonized water (ddH2O). PCR process is start at (95ºC) for 5 min, then the second step is start at (95ºC) for 45 seconds, then annealing is start at 72ºC for 45 seconds, and extension at 72°C for 45 sec, repeated 35 times. The amplified products were visualized by Safe dye staining after gel electrophoresis of 7 µl of the final reaction mixture in 2% agarose. The typical size of the PCR produce was 710 bps.

DNA Sequencing

DNA sequencing was done using an onward primer of DNA amplicons utilizing an ABI 3730XLs nucleotide sequence analyzer (Macrogen-Korea). The evolutionary historical was inferred by performing the Maximum Likelihood method on the basis of Jukes-Cantor model (Jukes and Cantor, 1969).

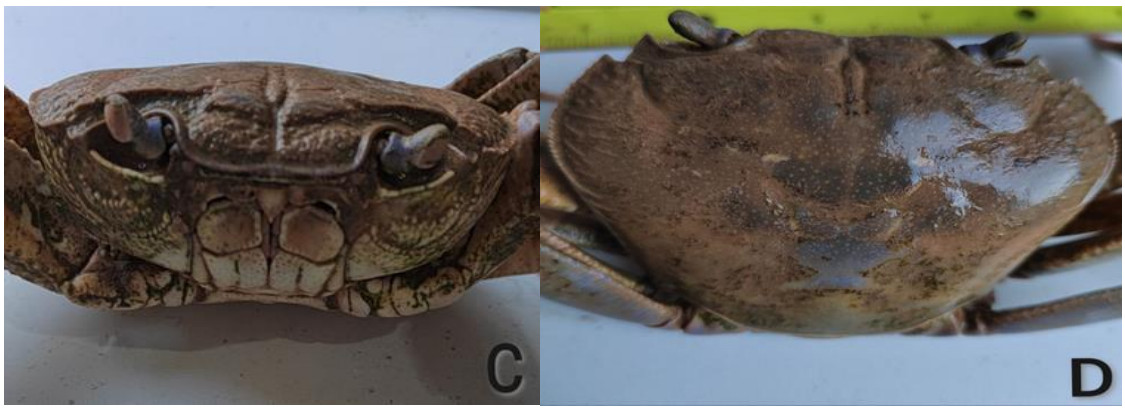
RESULT

Established *Potamon pesrsicum* based on the essential morphological and molecular study.

Morphology *potamon persicum*

Crosswise oval in shape is the carapace. The anterior frontal edge is clearly folded down, whereas the frontal margin is smooth. The front's external angles are smooth, lacking grains or spines. A short triangular process does not exist under the angles falling into the orbital hiatus. The inner suborbital margin does not have a spine or tooth. Mandibular palps comprise of three segment parts and the flagellum on the exopod of the third maxilliped is present. Superior margin of merus granular, inferior with single, less pointed teeth. The first cheliped carpal tooth is a massive, wide tooth with a pointed tip, whereas the second tooth is small. The gonopod 1's terminal article is just approximately between one-third and a fourth of the length of the gonopod's subterminal segment. The epigastric region of *P. persicum* is form in the posterior half of the sixth abdominal segment and includes the rectum and anus. usually this segment contain a gland, the shape is elliptical, a complement of the midgut lumen such as described by (Ceccaldi,1989).

Figure. 2. *Potamon persicum* A. Entire animal, male ventral aspect. B. Entire animal, female ventral aspect. C. Cephalothorax, frontal aspect. D. Entire animal, dorsal aspect.

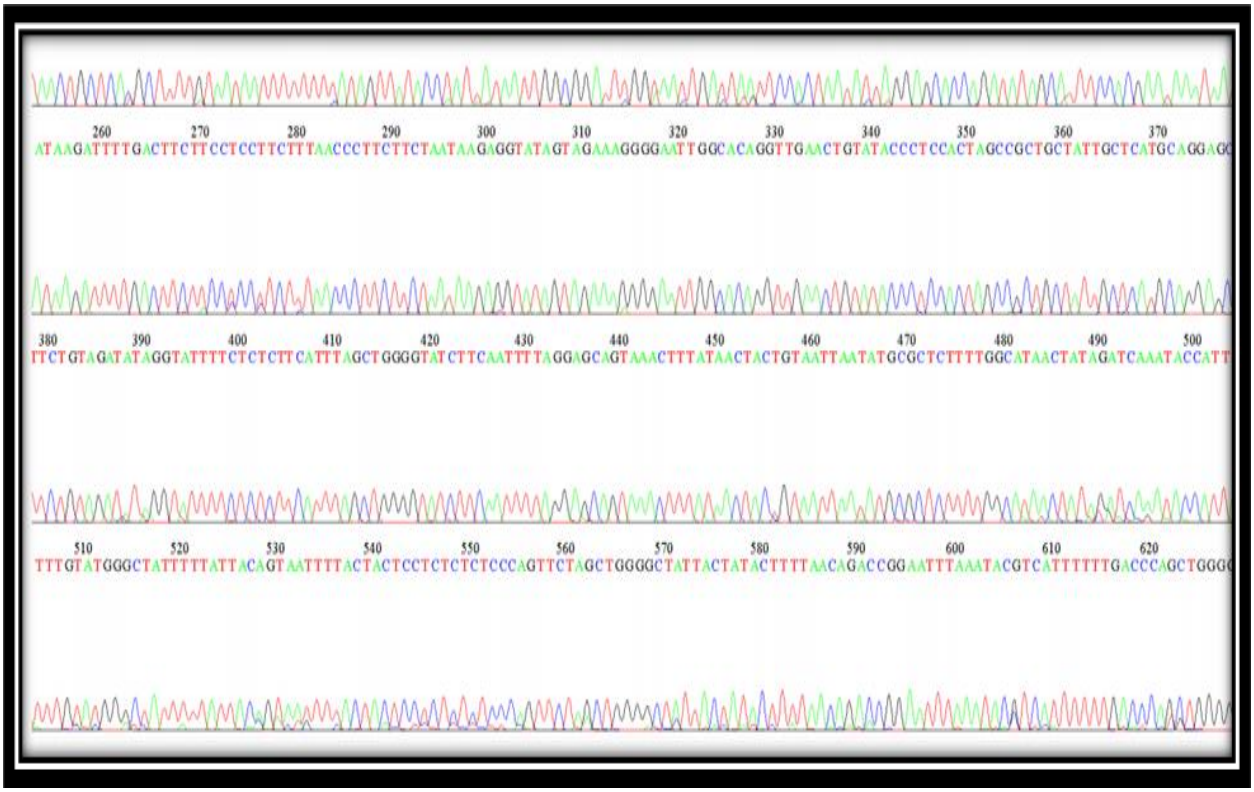


DNA analysis and phylogeny

Sequence alignment

The partial gene of COI sequences was applied to the Basic Local Alignment Search Tool (BLAST) (Stephen *et al.* 1997) is a searching tool that applies the sequence alignment method (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and is present at the NCBI (National Center for Biotechnology Information) website to comparing and alignment laboratory or query sequence with other biological sequences to find out more similarity with *P. persicum* species.

Figure.3. The result of chromatography sequence of the 16S rDNA sequence of crab species showed to be 100% identical to *Potamon persicm.*



Molecular identification of crabs

The partial gene of COI sequences of samples with size 692 bp is alimented by the BLAST database program from Gen bank (http://blast.ncbi.nlm.nih.gov/) and was acclimated differentiate our amplified sequences with other saved specimen of *P.persicum* sequences. The conclusion from the BLAST showed that the maximum identity number enquiry sequence was 100% identity are five samples, and another sample is more than 99.8 %. These ailments indicate submitting our query sequences inside of NCBI Gen bank and given accession numbers included below table (1):

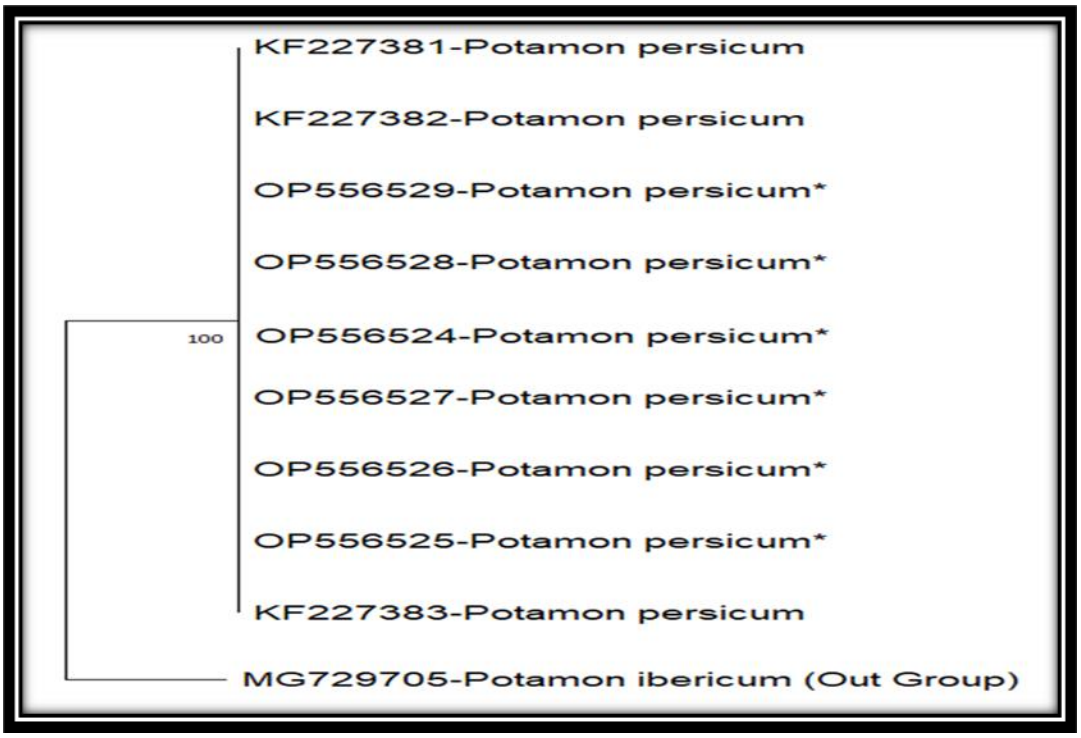
Table (1) partial large sub-unit of COI gene sequences in NCBI and alimented with same sequences after submission.

samples	Crab Identified	Accession Numbers	Query Cover %	Identic Numbe r %	Accession Number of BLAST Identification
1	*Potamon persicum*	OP556524	100	100	KF227381
2	*Potamon persicum*	OP556525	100	100	KF227382
3	*Potamon persicum*	OP556526			
4	*Potamon persicum*	OP556527	100	99.85	KF227383
5	*Potamon persicum*	OP556528			
6	*Potamon persicum*	OP556529			
7	*Potamon ibericum*	MG729705	**99**	**87.02**	(Out Group)

Phylogenetic inferences

Phylogenetic analysis according to COI nucleotide sequences showed a grouping of 6 investigated *P.persicum* on anticipated lines. From sequence deviation similarity data and phylogeny constructed, it was showed that specimen belonging to individual genera were adjacent to each other.

Fig.4. Phylogenic tree of *Potamon persicum* samples collected from Kurdistan region- Iraq. The phylogenic tree was created by performing the highest Likelihood technique according to the Tamura-Nei model in MEGA11 software and bootstrap investigation with six re-samplings. Partial DNA sequences of designed COI genes were used as input data.



Discussion

Potamon persicum was first discovered by Pretzmann,in1962. in the study, this species was observed for the first time in Iraq, in the Greater Zab river the samples were collected during period October 2021 until May 2022 in Khabat subdistrict. The investigation was included the molecular study first recorded species which obtained sequences and it was submitted in the NCBI double site the onward primer 5'- ACCCGCTGAATTTAAGCAT-3' at position 25 and inverse primer: 5'-CTCTTCAGAGTACTTTTCAAC-3' (Mollaret et al., 2000)designed the position 390. and the accession number were acquired. with accession number between (KF-227381-KF227383) as shown in (Figure 5).

Morphologically, *P.Persicum* the carapace of transversely oval in shape. The anterior frontal margin is visibly folded down, whereas the frontal margin is smooth. The front's exterior angles. Lack of short triangular process underneath angles falling into the orbital hiatus; they are smooth and devoid of spines or granules. The inner end of the suborbital border is smooth and devoid of a spine or tooth. The third maxilliped's exopod has a flagellum, and the mandibular palps contain three segments apiece. and those results close to that reported by (Keikhosravi,2014).

Merus inferior margin is granulated, merus superior margin has a row of granules, and merus distal margin has a single, less pointed teeth. The first cheliped carpal tooth is a massive , wide tooth with a pointed tip, while the second tooth is small.

The freshwater crab especially genus *Potamon* need to more research in the north Iraq, because only just one study has been recorded which is *Potamon magnum* in Khabat subdistrict, the

Greater Zab river, and the species record in this study is the second recorded species from the northern Iraq, and is expected to discover more new species of genus *Potamon*. in different water bodies in Iraq, because the Greater Zab river originates from the southeast Turkey, and many other species observed in this country may be discovered add, it to Iraqi fauna.

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