



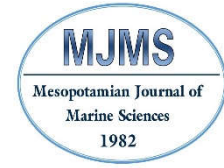
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**New record of *Macrobrachium equidens* shrimp (Dana, 1852) (Crustacea: Decapoda: Palaemonidae) as invasive species in Al- Mashaab River, East of Al- Hammar marsh, Southern Iraq**

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**Abstract** - A species of freshwater shrimp belonging to *Macrobrachium* Genus was recently recorded from Iraqi water. Morphological features accompanied by COI gene nucleotides sequencing identified that the shrimp species is *Macrobrachium equidens*, four forms of rostrum were observed. The total length of *M. equidens* recorded in this study was ranged from 40.38 - 55.33 mm for females and from 45.51 to 58.72 mm for males, while weight for females and males were 0.36 to 5.44g and 0.53 - 6.01 g respectively.

التسجيل الجديد للروبين *Macrobrachium equidens* كنوع غازي في نهر المسحب، شرق هور الحمار، جنوبي العراق

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**المستخلص** - سجل مؤخراً أنواع من روبين المياه العذبة العائدة للجنس *Macrobrachium* في المياه العراقية، وفي الدراسة الحالية شخص النوع *Macrobrachium equidens* مظهرياً وباستخدام تقنية التشخيص الجزيئي كنوع جديد وغازي في نهر المسحب، جنوبي العراق، وقد لوحظ تنوع شكل الخطم لهذا النوع. تراوح الطول الكلي لهذا النوع بين 40.38 – 55.33 ملم في الإناث، بينما تراوح في الذكور بين 45.51 – 58.72 ملم، وتراوح الوزن للإناث والذكور بين 0.36 – 5.44 غم وبين 0.53 – 6.01 غم على التوالي.

**الكلمات المفتاحية:** تسجيل جديد، أنواع غازي، *Macrobrachium equidens*، جنوب العراق.

**Introduction**

Caridean shrimp occur in all aquatic habitats, they exist in marine to freshwater habitats (Grave *et al.*, 2007). There are three families of Caridea freshwater species: Palaemonidae, Atyidae and Alpheidae. Genus *Macrobrachium* Bate, 1868 (Palaemonidae) have more than two-hundred and forty two species and subspecies live in different aquatic environments (De Grave and Fransen, 2011).

*Macrobrachium* is originated in many countries excluding in Europe (Holthuis and Ng, 2010). Based on previous studies, taxonomic records of *Macrobrachium* from Iraq back to 2006, the first record was by Salman *et al.* (2006) who reported *Macrobrachium nipponense*, and second species which *Macrobrachium lar* (Ghazi and Hassan, 2021). According to Choy (1984) *Macrobrachium equidens* has been founded in large area such as India, Southeast Asia and Islands. In this research, an comparative study for the morphological features of *Macrobrachium equidens* with the DNA barcode of the mitochondrial cytochrome oxidase I gene which identified that these samples refers to the shrimp *Macrobrachium equidens*. and widely distributed in estuarine; it prefers brackish water but sometimes arrives far away from the tidal areas, the mothers need salty aquatic environments for ovulation (Wowor, 2001). There are only a few studies on *Macrobrachium equidens* (Krishna-Murthy and Rajagopal, 1990; Ngoc-ho 1976; Short 2004).

## **Materials and Methods**

### **Study Area**

The prawns were collected from Al-Mashaab, near East of Al Hammer Marsh, Southern Iraq in October 2015. This is located within an area that lies between 30° 39' 34.27" N; 47° 39' 13.81" E., through Shatt Al-Arab River this location is connected with the Arabian Gulf (Figure. 1). This area is currently distinguished by being semi closed, receiving its water mainly from the Euphrates River and its branches, and it depends on the phenomenon of tide to changes its waters.

The study area is characterized by the spread of several aquatic plants such as *Ceratophyllum demersum*, *Myrophyllum* sp., *Nymphoides indica*, *Typha australis*, *Potamogeton* sp. and *Phragmites australis*.

### **Collecting Specimens**

Specimens were isolated by 3 meter long a trawl mesh with a 10 mm net size at depth between 3 - 4 meters. All specimens obtained were fixed in 70% ethanol. All collected samples were kept in cold box and transported to the laboratory. Dissecting microscope was used for specimen's examination and depending on some keys (Riek, 1951; Short and Meek, 2000; Wowor and Choy, 2001). Salinity, water temperature and potential hydrogen ion (pH) were measured by YSI 556 MPS models 2005 which usually calibrated before every field trip.

### **Molecular Identification**

The molecular study included DNA extraction purification, PCR amplification and Sequencing send and analysis.

### **DNA Extraction**

According to Wen and He (2003), 10 samples subjected to the whole genomic DNA extraction, 30µl STE buffer (10 mM/L sodium chloride, 1m M/L EDTA pH 8.0, 10 mM/L Tris-HCl, pH 8.0) and 2µl proteinase K at concentration 10 mg/ml were used to the homogenate

sample. In order to reduce the loss, the grinding tip was washed with 30µl STE buffer, finally, samples were incubated at 56 °C for two hours and then incubated at 95 °C for forty-five seconds centrifuged at 8000-10000 rpm for thirty seconds. For PCR reaction, the supernatant being used as amplification template and stored in the refrigerator.

### Agrose Gel Electrophoresis

The DNA gel electrophoresis required some of chemicals and stains will mentioned in below:

#### Protocol

There are three steps in the DNA gel electrophoresis.

1-Step one: Agarose gel preparation.

A- Up take of 1x TBE 25 ml.

B- Weighs of 0.2 gm agarose powder and mix with the 25 ml loading buffer.

C- Solve the mixed solution using (hot plate) at 80 °C.

D- Cooling the solution after heating down to 50-60 °C.

2- Step Two: Electrophoresis unit configuration.

A- The casting tray was filled up with mixture before solidification and the wells were made due to presence of the comb at the end of the cast.

B- Waiting up 30 minutes for complete solidification of the gel.

C- Removing the comb and the tray filled up with the loading buffer.

3- Step three: DNA immigration in agarose gel.

A- 9 µL DNA plus 3 µL bromophenol blue dye were mixed well depend on ratio of 3:1 than loaded in the wells of the 0.2 gm agarose gel.

B- The cathode and anode were connected to the specific side of each one in the power supply

C- The electrical parameters were fitted at 60 V and 2 mA for 30 min and stop the power supply when bromophenol blue reached to the near of the gel end.

D- The DNA bands were visualized using the gel documentation system after staining the gel with Ethidium bromide.

### Identification of *Macrobrachium equidens* using specific COI gene amplification

The amplification of COI gene 416 bp was subjected using set of primers according to (Liu, *et al.*, 2007) and the oligonucleotides were showed in table 1, 2 and 3, and designed on the sequence of COI gene which published in Genbank accession number JF737758.1).

Table 1. COI gene primers sequences

Primer	Sequence	Number
Forward primer	5'- AGTATAAGCGTCTGGGTAGTC -3'	21
Reverse primer	5- CCTGCAGGAGGAGGAGACCCA -3	21

Table 2. COI gene mixture contents

No	Reagent	Volume
A	DNA	10µl
B	Oligonucleotides 1	2 µl
C	Oligonucleotides 2	2 µl
D	Loading dye	11µl
E	Deionized water	25µl
	Total	50µl

Table3. COI gene PCR amplification protocol

No	Stage	No. of Cycle	Time in min
A	DNA Denaturation	1 Cycle	10 min
B	DNA Annealing	35 Cycle	1 min
C	DNA Extension	1 Cycle	10 min

### Sequencing preparation and sending

Ten samples of purified PCR products with 20 µl were send to Bioneer company in the South Korea for nucleotides data base sequencing analysis.

### Results and Discussion

The results of DNA extraction, purification, PCR amplification and sequencing analysis of *Macrobrachum equidens* were identical 100% with their reference strains in the Genbank data base. The results of current study were matched with the results of (Liu *et al.*, 2007). Sequences of this strains and showed in Table (4).

Table 4. The nucleotides sequence

	<p>ATTCTAATTTTACCTGCCTTCGGTATAATCTCTCATAT  TGTAAGACAAGAATCTGGTAAAAAAGAATCTTTTGG  CACTTTAGGAATAGTCTACGCTATGATAGCAATTGGT  GTTCTAGGATTTGTCGTGTGAGCACACCATATATTCA  CAGTAGGGATGGATGTAGACACACGAGCCTACTTCA  CATCTGCTACGATAATTATTGCTGTACCAACAGGAAT  TAAAATCTTCAGATGACTAGCCACCCTCCATGGGACT  CAATTCACCTACAGGCCCTCTCTAATTTGAGCGCTAG  GCTTTATTTTCTTTTACAATAGGAGGCTTAACAGG  AGTAGTTTTAGCTAAATCTTCCATCGATATCATCTTA  CACGACACTTACTTTGTAGAAGCCCACTTCCACTACG  TCTTATCAAT</p>
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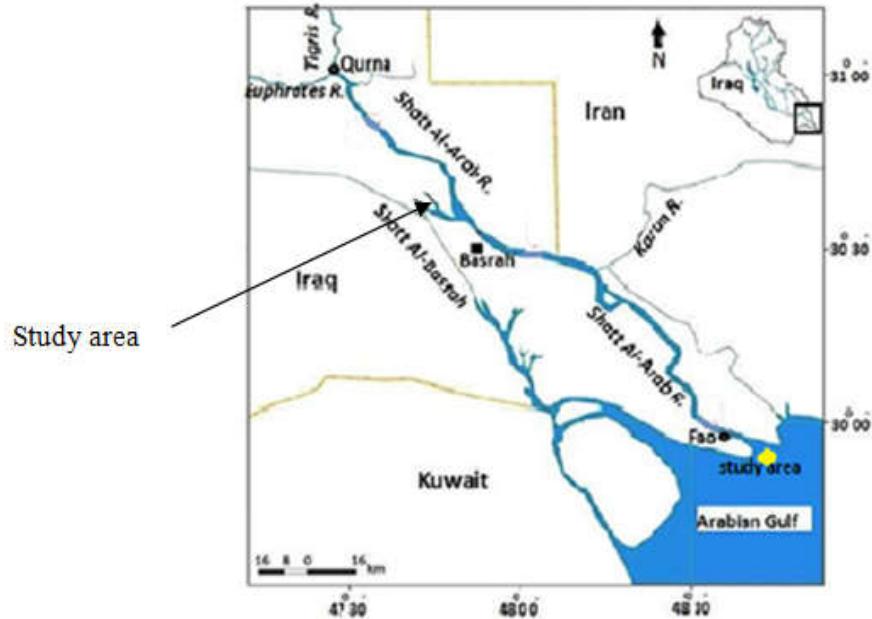


Figure 1. Geographical location in Al- Mashaab river, East Al- Hammar Marsh, Southern Iraq where specimens of *Macrobrachium equidens* were collected.

### Diagnosis

Size of males *M. equidens* are larger than females of the same ages, body length of females *M. equidens* was ranged from 40.38 to 55.33 mm and from 45.51 to 58.72 mm for males, while weight for females and males were 0.36 to 5.44g and 0.53 to 6.01 g respectively. Second pereiopod involving carpus longer than merus, carpus cylindrical shape. Rostrums contain from 1 to 3 teeth, the rostrum have lip overreaching end of third segment of antennular peduncle, no appressed scales that covered the second pereiopods, also fingers not have row of tuber post antennular carapace, margin not rounded, just in developed males, observed the seconds pereiopods with chela, carpus than surface of carapace, telson and uropod spiculate.

### Morphological remarks

In Southern Iraq marsh, there are four rostrum forms observed in *M. equidens*, the first is curved upwards distally, the second is almost straight, the third is straight forward, and the fourth is straight forward with a different front of the snout, and the results of the molecular analysis showed that all these shapes belong to the same type, the rostrum of the four forms observed has eleven dorsal and 3 to 4 ventral teeth (Figure 2. A and B).

### Description

Rostrum sinuous or straight, of normal depth, in developed male, second pair of pereiopod with pappose setal pubescence completely covering both fingers, segments marbled with dark irregular blotches in live specimens. First ventral rostral tooth located in half mid-length of ventral carina, superior margin with 9 to 12 teeth, of which teeth are distributed almost uniformly

between 2 to 4 behind the orbit, distal teeth, more widely spaced in comparison with the others: inferior margin with 4 to 7 teeth. Carapace very smooth. Second pair of periopod of male is developed without pappose setal or, if present, distal cutting edges non-crenulate; developed males with protective setation (spinule or tubercle-like) on anterolateral carapace.



Figure 2. A. *Macrobrachium equidens*: A, curved upward rostral form, B, almost straight rostral form, C, straight forward, and D, is straight forward with a different front of the rostrum.

In current study, record third species belonging to genus *Macrobrachium*, it was *Macrobrachium equidens* (Dana 1852), (Palaemonidae family), or commonly known as rough river shrimp. This investigated description the first record of *M. equidens*, in Al- Hammar marsh, Southern Iraq. Several of new species of freshwater *Macrobrachium* were recorded in Iraq water, and *M. equidens* the third invasive species of *Macrobrachium* to be recorded in the Iraq water, after the *Macrobrachium nipponense* (Salman *et al.*, 2006) and recently *Macrobrachium lar* (Ghazi and Hassan, 2021). In this study, presents a differential characterize of the morphological and confirmed with DNA barcoding, mitochondrial analysis COI gene was the most important key to confirm the species under this study. Rough river shrimp, *M. equidens* is preferred inhabits fresh and brackish water (Short, 2004). According to Powell (1986) that the first observed of *M. equidens* in Nigeria in 1982, while in the Philippines was recorded by Cai and Shokita (2006) and Krishna-Murthy *et al.* (1987) recorded it in India.

In recent years, the introduction of invasive species to new environments has increased. Although the reasons are not clear, but global climate change can be considered one of the most important causes. In previous periods, our local environment has witnessed major changes in the quality of water, especially regarding the difference in the level of salinity, because of the decrease in the quantities of fresh water coming through the Euphrates and Tigris the advancement of marine water (Al-Mahmood, 2015). It is known that global waters are linked with each other, and there is navigation and movement between different countries, which contributed to the transfer of these organisms from their main habitats to new environments, as happened with *M. equidens*. The native habitat of this shrimp is the Indo- Pacific, so it is



expected that this species arrived in the local environment via ships or accidentally together such as other species such as *M. nipponense*.

According to Short (2004) *M. equidens* can inhabit rivers and estuaries. Because of the local environmental conditions are appropriate to the basic requirements for this type of ideal temperatures for growth and reproduction, especially, this species have good able to adapt to new environments. Foreverymore, this species has a high fertility rate, ranged between 448 and 8,281 eggs per female, with a mean of 2,752 (Krishna-Murthy and Rajagopal 1990; Krishna-Murthy *et al.* 1987). Although the morphological studies give good results in the field of the diagnosis of living organisms, but the molecular diagnosis gives an unquestionable diagnosis in determining the organism. Therefore, the study relied on the genetic diagnosis in addition to the morphological diagnosis in determining this species. One of the reasons that prompted the reliance on genetic diagnosis is the variation in the shape of the rostrum for this species, which made the dependence on the morphological less reliable. The phenomenon of the difference in the shape of the rostrum of the same species is recorded in the shrimp under study and other types of shrimp and these forms are common among small and young specimens (Chace and Bruce, 1993).

## Conclusion

The water of Basra governorate, represented by Al-Mashab River, witness the registration of new invasive species of shrimp belonging to the Palaemonidae family. The species recorded in the current study, *M. equidens*, is one of these species, and it is considered the third invasive species after *M. nipponense* and *M. lar*.

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