



# New Record of *Squilla mantis* from Coastal Waters of Iraq

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**Abstract:** A new record of the stomatopod, *Squilla mantis*, of the family Squillidae from the coastal waters of Iraq at the North-West of the Arabian Gulf made. The morphological features of this species have been diagnosed. The total length of specimens were 110 – 170 mm. DNA barcoding is used for the identification of new species involving DNA extraction subjected to PCR and sequencing of COI gene, indicated that the specimens are belonging to *S. mantis*, which was not recorded in the region before.

**Keywords:** Stomatopod, *Squilla mantis*, DNA sequences, Arabian gulf

Stomatopod is a small group of marine Malacostraca, occupy a wide range of habitats, which live in burrows in sand or near coral reefs. They are benthic animals that live in marine water at depths of less than 200 meters, and some live in brackish habitats, although there are species that live in depths of up to 1500 meters in the soft bottoms (Ahyong et al 2017). The group was recorded from the Mediterranean Sea and the Atlantic Ocean (Maynou et al 2004, Colmenero et al 2009). The Indo-West Pacific area, contains the highest percentage of them, reaching 67 % of all stomatopod of the world (Ahyong et al 2017). *Squilla mantis* is a benthic species mostly present on the coastal soft bottoms of the continental shelf down to 100 m in depth (Manning 1977). There are many studies on *S. mantis*, including biology (Maynou et al 2005, Rossetti et al 2005), growth and behavior (Heitler et al 2000), reproductive parameters and seasonal variations in the fatty acids composition (Mili et al 2011). In the Iraqi coastal waters, Ahmed (1971) described *Oratosquilla arabica*, as a new species in the region, but was synonymous with *S. mantis* (Linnaeus 1758). The genetic code technique is an important tool for diagnosing new species in different environments. Cytochrome c oxidase I (COI) is very important gene, called the mitochondrial encoded Cytochrome C Oxidase I (MT-CO1) which is a protein that in humans is encoded by the MT - CO1 gene. Cytochrome c oxidase subunit I (CO1 or MT-CO1) is one of three mitochondrial DNA (mt DNA) encoded subunits of the electron transfer chain of mitochondrial oxidative phosphorylation. It is a gene widely used DNA barcode to diagnose organisms species, furthermore it, MT-CO1 gene sequence is so perfect for this role in order to its mutation rate is often fast enough to identification exactly related species (Hebert et al 2003). The present article represents first

records of the stomatopod *S. mantis*, which was not reported before in the region.

## MATERIAL AND METHODS

About 120 specimens of *S. mantis* were collected by trawling net during 2017. The approximate GPS coordinates is 29° 44 200 N; 48° 40 00 E. The sampling sites were located at a depth between 7 to 12 m under water. The collected specimens were measured and preserved in 75% alcohol. The taxonomic identification depended on some related references (Manning 1971, 1978, 1995). Water parameters like temperature, pH, salinity and dissolved oxygen were measured in the field by multi meter.

**Identification by COI gene amplification:** DNA Extraction according to QIAGEN Kit (Serial NO: 56404) for alcohol embedded sample was carried out. The mitochondrial Cytochrome Oxidase subunit I is based on published COI sequences deposited in GenBank, amplification of a 688 bp fragment. The process of sequencing of PCR amplification for COI gene reagents and PCR program given in Table 1 and 2).

## RESULTS AND DISCUSSION

**Environments conditions:** The environmental conditions recorded in this study were, water temperature between 29-33°C; pH 6.8 - 7.5, salinity 40 - 41 ppt. and dissolved oxygen 6.6 - 7.3 mg l<sup>-1</sup>.

**Identification and morphological diagnosis of *S. mantis*:** The eyes of *S. mantis* were T-shaped, cornea bilobed, carapace, thorax, and abdomen with longitudinal ridges or carina. The total length of the specimens recorded in the current study was 110 – 170 mm. The individuals have paired dark spots on the dorsal part of the telson. The telson is with

distinct median carina. Dectylus of raptorial claw has six teeth on inner edge, slender, no inflated or buttressed basally, propodus raptorial claw lined with blunt pectinations. Carapace rounded poster laterally, with no excavation, there are clear interrupted in the carapace carina at base of bifurcation.

**Molecular identification using COI gene:** The extracted DNA from the sample was subjected to PCR for the amplification of the COI gene (Fig. 1). The specimens' band of the gene was marked by 688 bp in comparison with the standard molecular DNA Ladder (100-1500 bp). Agarose gel (2 % g and 60V, 2MA) form of electrophoresis show PCR amplified products of gene COI. Lane M: 1.5 kb DNA ladder, lanes 1-9: COI gene bands of *S. mantis*.

**Sequencing for COI gene of *S. mantis*:** COI gene and nucleotides sequencing for the extracted DNA of *S. mantis* from coastal waters of Iraq are listed below:

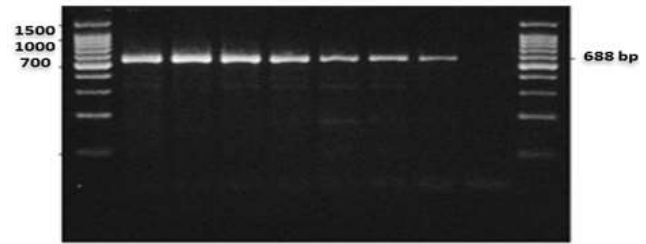
CATAAAGATATTGGAACCTTTATATTTTCATTCTAGGGGCTT  
 GATCAGGAATAGTAGGGACAGCCCTTAGTTTGATTATTC  
 GAGCTGAGCTAGGTCAACCAGGTAGGTTAATTGGAGAT  
 GACCAAATCTACAATGTTATCGTTACAGCACACGCTTTT  
 GTTATAATTTTTTTATAGTTATACCTATTATAATTGGGGG  
 GTTTGGAAACTGATTAGTGCCTTTAATATTAGGGGCC  
 CTGATATAGCATTCCCCCGTATAAATAACATAAGATTTTG  
 ATTACTACCTCCCGCACTCACCTTATTACTATCTAGGGG  
 CTTAGTTGAAAGAGGGGTTGGTACTGGATGAACAGTTT

**Table 1.** PCR amplification reagents for COI gene

No	Reagent	Volume
I	DNA template	5 µl
II	Forward primer	1 µl
III	Reverse primer	1 µl
IV	Master Mix	5 µl
V	Nuclease-free water	8 µl
	Total	20 µl

**Table 2.** Touchdown PCR Condition for COI gene

Steps	Temperature	Time
One	95 °C	2 min
Two	95 °C	30 sec
Three	59.8 °C decrease 0.5 percycle	30 sec
Four	72 °C	90.0 sec
Five	Repeat steps 2-4	14 more times
Six	95 °C	30 sec
Seven	52.8 °C	30 sec
Eight	72 °C	90.0 sec
Nine	Repeat steps 6-8	19 more time
Ten	72 °C	5 min



**Fig. 1.** Agarose gel (2%gm and 60V, 2 MA) electrophoresis patterns show PCR amplified products of gene COI. Lane M: 2kb DNA ladder, lanes 1- 9: Gene COI bands of *Squilla mantis*

ATCCCCCTTTATCAGCAGGAATTGCGCATGCCGGGGCT  
 TCTGTAGATATGGGTATTTTCTCTTTACATTTAGCAGGA  
 GCTTCTTCAATTTTAGGAGCTGTAAATTTACTACTACG  
 GTAATTAATATACGATCAAACGGAATGACTATAGACCGTA  
 TACCTTTATTTGTATGGTCTGTTTTTACTACAGCAATTTT  
 ACTACTTTTATCACTACCTGTTTTAGCGGGTGCCATTAC  
 CATACTACTAACAGACCGTAATTTAAACACATCGTTTTTT  
 GATCCTGCTGGAGGGGAGACCCTGTACTATATCAACA  
 TTTATTTTGATTTTTTGGTCACC.

Generally, *S. mantis* reached a maximum size of 200 mm as recorded in the Mediterranean (Ragonese et al 2012). But however in study, collected some lesser sizes specimens that ranged between 110-170 mm. The stomatopods are widely spread in tropical and temperate regions, and hide between coral rocks or bury themselves in the soft sand bottoms (Ahyong et al 2017). The *S. mantis* was recorded for the first time from the Iraqi marine waters, but (Ahmed 1981) described a new species of stomatopods commonly collected with the fishing catches by trawling nets. However, recent reports from a quite close spot, from the Kuwait coastal region a species designated as *Gonodactylus demani*, were recorded (Jones 1986). Apparently, the *Oratosquilla arabica* is possibly a synonymous to the present species *S. mantis* or *G. demani*, but DNA sequencing technique revealed that it is *S. mantis*. Many factors such as, climate changes, competition for food and habitat, pollution and existence of species that act as carrier like large animals, so that many species are leaving their native habitat and spreading into a new habitats (Silva et al 2003). This species is distributed in all the Mediterranean Sea, Atlantic Ocean, Indian Ocean and Indo-Pacific Ocean (Atkinson et al 1997, Al-Zaidan et al 2006). Local environmental conditions are suitable for the prevalence of *S. mantis*, is confirmed by (Raicevich et al (2014). The stomatopod is common on coral reefs habitats (Connolly et al 2003, Reaka et al 2008). Coral reef were recorded in the coasts of various Arab countries such Saudi Arabia, United Arab Emirates, Bahrain and Qatar. (Pohl et al

2014) have recorded coral reefs in the coastal water of Iraq. The presence of coral reefs, favorable environmental conditions, additionally, mixture of sand and mud bottom are expected to encourage the migration and survival of this species.

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