

NOTE

First DNA barcoding-based record of *Lysiosquilla maculata* (Crustacea: Stomatopoda) from Chennai coast, Tamil Nadu, India

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ABSTRACT. The taxonomic identification of mantis shrimp *Lysiosquilla maculata* was performed through DNA barcoding analysis of specimens collected from the Kasimedu fishing port, Chennai coast, Tamil Nadu, India. The mitochondrial cytochrome oxidase subunit I (mtCOI) gene with a region of 650 bp was sequenced for phylogenetic analysis. In the present record, mitochondrial gene sequences were used to identify the mantis shrimp. This is the first confirmed DNA barcoding record from Indian waters, whose mtCOI sequence was deposited in GenBank. The Neighbor-joining method was used for phylogenetic analysis. The calculated pairwise genetic distance with five closely related species ranged from 0.01 to 0.094%. The morphological and molecular analysis confirm that the specimens collected correspond to *L. maculata*.

Key words: Biodiversity, mantis shrimp, mitochondrial DNA, phylogeny, genetic distance.

Primer registro basado en código de barras de ADN de *Lysiosquilla maculata* (Crustacea: Stomatopoda) de la costa de Chennai, Tamil Nadu, India

RESUMEN. La identificación taxonómica del camarón mantis *Lysiosquilla maculata* se realizó mediante análisis de códigos de barras de ADN de especímenes recolectados en el puerto pesquero de Kasimedu, costa de Chennai, Tamil Nadu, India. Se secuenció el gen de la subunidad I de la citocromo oxidasa mitocondrial (mtCOI) con una región de 650 pb para análisis filogenético. En el presente registro, se utilizaron secuencias de genes mitocondriales para identificar el camarón mantis. Este es el primer registro confirmado de códigos de barras de ADN en aguas indias, cuya secuencia de mtCOI se depositó en el GenBank. Se utilizó el método *Neighbor-joining* para el análisis filogenético. La distancia genética por pares calculada con cinco especies estrechamente relacionadas osciló entre 0,01 y 0,094%. Los análisis morfológicos y moleculares confirman que los ejemplares colectados corresponden a *L. maculata*.

Palabras clave: Biodiversidad, camarón mantis, DNA mitocondrial, filogenia, distancia genética.



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A total of 500 species of mantis shrimps belonging to 120 genera, 18 families and 7 superfamilies have been recorded to date worldwide (Ahyong 1997; Ahyong and Harling 2000; Ahyong 2012). *Lysiosquillidae* is the largest known stomatopod, with a total length exceeding 380 mm, mostly showing the presence of dark and light stripes on the dorsal region of the body (Ahyong 2001). According to Ahyong and Lin (2022), the presence of transitional species between *Lysiosquilla* and *Lysiosquillina* makes the validity of the genus difficult,

so molecular tools as DNA barcodes are important to enhance the taxonomy of the group and achieve a correct species identification. Consequently, *Lysiosquilla* was synonymized with *Lysiosquilla*, leaving two genera in the family. Although, some misidentification and unauthenticated information on this group have been reviewed, the taxonomic ambiguity was resolved based on the molecular studies and phylogenetic analysis (Tang et al. 2010). Moreover, in some cases morphological characters are not enough for identification to species level. DNA barcoding techniques have been successfully applied to resolve the correct identification of a wide range of animals, including both terrestrial and aquatic taxa (Hebert et al. 2004; Ward et al. 2005; Hajibabaei et al. 2006; Wakabayashi et al. 2006; Hubert et al. 2008). The DNA barcode has become more popular for identifying and studying the genetic diversity of stomatopods (Barber and Erdmann 2000; Barber et al. 2002; Barber et al. 2006; Barber and Boyce 2006; Tang et al. 2010). So far, few studies on the diversity of stomatopods have been conducted on the eastern coast of India using DNA barcode (Kundu et al. 2018).

The common banded/striped/zebra mantis shrimp *Lysiosquilla maculata* was first described as *Squilla maculata* by Fabricius (1793) from eastern India, which was reported as *L. maculata* by Kemp (1913) from Kakinada, Chennai, Andaman and Nicobar Islands in Indian waters. Lyla et al. (1997) reported *L. maculata* from Parangipettai, while Kathirvel (2008) and Dev Roy and Gokul (2012) listed the species from Indian waters. However, Trivedi et al. (2020) synonymized *L. maculata* with *L. tredecimdentata* Holthuis, 1941. The present study intended to make a morphological description and molecular identification of *L. maculata* from Chennai coastal waters.

Mantis shrimp *L. maculata* were collected by hand from trawl bycatch landings in Kasimedu, Fisheries Harbour, Chennai (Figure 1). Specimens were identified using standard guidelines (Manning 1978; Ahyong et al. 2008) and preserved in 70% alcohol. Some specimens were deposit-

ed in the museum of Fishery Survey of India, Chennai (FSI CRUS-2021/17). The extraction of total genomic DNA from samples was performed by the Phenol Chloroform method, standardized by CAGL. Genomic DNA quality was assessed using 0.7% agarose gel together with a 1 kb DNA ladder as a size standard in a bio-photometer (Eppendorf). Amplification of *COI* gene was carried out for all tested samples using the published primer pair LCO1490:5'-GGTCAA-CAAATCATAAAGATTGG-3' and HCO2198:5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994), and used for amplification of partial mitochondrial cytochrome c oxidase subunit I (mtCOI) gene segment in a Veriti®. The PCR-generated amplicon for 6 samples was confirmed and purified with a GeneJET PCR purification kit (Thermo Scientific, EU-Lithuania) to remove the primer dimer and other carryover contaminations. The quality of products was assessed using 2% agarose gel together with 100 bp DNA marker as size standard and products were found to be good for sequencing.

Purified PCR products were prepared for cycle sequencing using the Big Dye® Terminator 3.1 sequencing kit (Applied Biosystems, Foster City, California, USA). After cycle sequencing, products were purified using the Ethanol-EDTA purification protocol to remove the unincorporated dNTP's, ddNTP's and primer dimer. After purification, products were dissolved in 12 µl Hi-Di formamide, and samples were denatured at 95 °C for 5 min. Denatured products were used for forward and reverse sequencing using the Genetic Analyzer 3500 (Life Technologies Corporation, Applied Biosystems®, California 94404, USA) according to the manufactures' instructions. Sequences were aligned, edited, and analyzed using Clustal-W and Mega software version 7 (Kumar et al. 2016). The sequence was converted to FASTA format and submitted to nucleotide BLAST via the NCBI website (<http://www.ncbi.nlm.nih.gov/>). The first five sequence matches shared the highest similarity to database references (mat ident) and were analyzed for species consis-

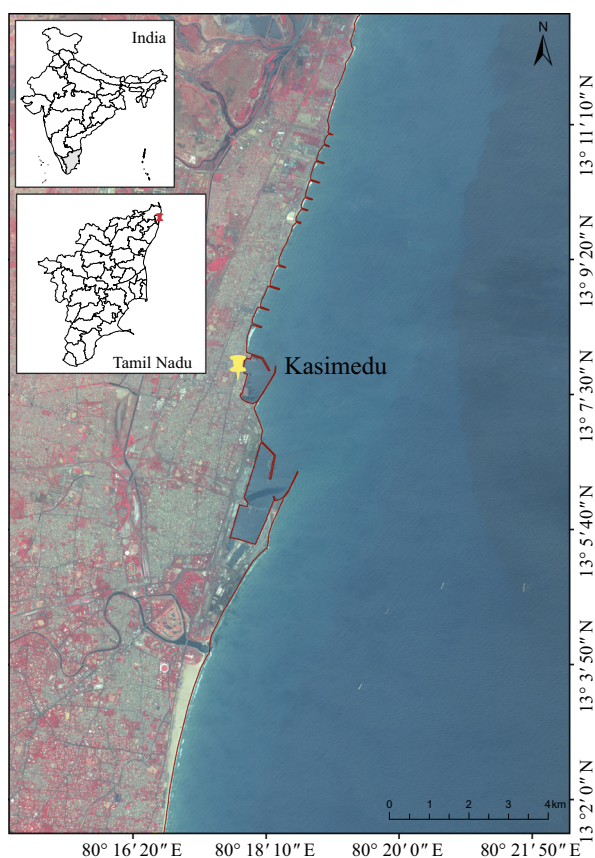


Figure 1. *Lysiosquilla maculata* collection site in the Kasimedu fishing harbor.

tency. All matches were analyzed using the percent similarity score.

Taxonomic designations were derived from phylogenetic analysis of mtDNA gene sequences. Sequence was analyzed using the Bioedit software (Version 7.2.5) (Hall 2004). Final sequence alignment was compared with sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST). Neighbor-joining (NJ) trees of Kimura two-parameter (K2P) distance were created to provide a graphic representation of the pattern of divergence between species (Saitou and Nei 1987). The 1,000 bootstrap replications were performed in MEGA 7 software (Kumar et al. 2016). The K2P genetic distances to

define species, genus and family levels were based on Ward et al. (2005).

The Genus *Lysiosquilla* contains 16 species that are difficult to identify by morphological characters. In the present study, mtDNA sequences of the COI gene from the mantis shrimp *L. maculata* (Family Lysiosquillidae) were initially compared with five different species. It was the first genetically confirmatory record in Indian waters. The COI sequence obtained was deposited in GenBank (accession number: MT490885). A 650 bp segment of the 5' margin of mitochondrial cytochrome oxidase subunit I gene is currently used for molecular taxonomy classification. The A, T, G, and C contents of *L. maculata* sequence were 154%, 140%, 86%, and 51%, respectively. The GC content was observed at 31.8% in the species. No other sequence variation was observed between those specimens.

Two stomatopod specimens with a total length ranging between 161 to 178 mm were recorded. They had a dorsoventrally flattened and weakly calcified body, triangular, erect, and inclined anteriorly ocular scales. The carapace was convex and wide, the rostrum was cordiform-shaped occasionally sub-triangular. Width was usually greater than length. The blunt longitudinal carina was presents in anterior third plate. Raptorial dactylus claw possesses 8-11 teeth, usually 10-11 but, larger females can exhibit some variation with 10-11 teeth. A three-segmented mandibular palp and five epipods were present. Sternal keel TS8 rounded. The uropodal protopod was a ventral, triangular lobe laterally anterior to the articulation of each uropod and the outer margin of the proximal segment of the uropodal exopod with 7-9 movable spines; endopod with distal 3/4 dark. Color of the dorsum base was pale yellow, with black transverse bands (Figure 2). Morphological data and the use of taxonomic keys (Ahyong and Lin 2022) allowed us to confirm that collected specimens corresponded to *Lysiosquilla maculata* (Fabricius 1793).

Genetic information on taxonomically identified species is essential to perform genetic similarity searches in the global database (Moritz and Cicero



Figure 2. *Lysiosquilla maculata* 178 mm total length. A) Dorsal view. B) Ventral view.

2004). Hence, before submitting novel sequences in GenBank and BOLD, it is essential to identify the studied specimens. Previously, the genetic information of *Harpiosquilla harpax*, *Oratosquilla oratoria*, *Miyakea nepa*, *Clorida decorata*, and *Anchisquilla fasciata* was obtained from the GenBank database. The generated sequence of *L. maculata* from Indian waters was annotated (650 bp) for first time and submitted to GenBank datasets. Generated sequences showed 91% similarity with *L. maculata* in the GenBank and BOLD databases. The genetic distance of *L. maculata* from five closely related species was calculated based on Kimura's 2-parameter method. The pairwise estimated genetic distance varied from 0.01 to 0.094, showing that smaller genetic distance indicated close genetic relationship, whereas large genetic distance indicated more distant genetic relationship (supplementary material, Table S1). Hebert et al. (2003) suggested

that DNA barcoding is a powerful tool to provide valuable insight on patterns of genetic divergence affected by species level or ecological variation. The average genetic distance between species does not exceed the average genetic distance between 'sister' species.

In this study, the phylogenetic relationship of *L. maculata* with five closely related species was analyzed. Complete mitochondrial genes of these five species are available on GenBank. The Neighbor-joining (NJ tree) was constructed by MEGA 7 (Kumar et al. 2016) based on 1st and 2nd codon sequences of 19 protein-coding genes. In the NJ phylogenetic tree, *Oratosquilla oratoria* and *Miyakea nepa* formed one clade, but *Harpiosquilla harpax* was the second clade of the group. The third clade consisted of *Clorida decorata* and *Anchisquilla fasciata*. The fourth clade supported *Lysiosquilla maculata* and *Lysiosquillina maculata* (Figure 3).

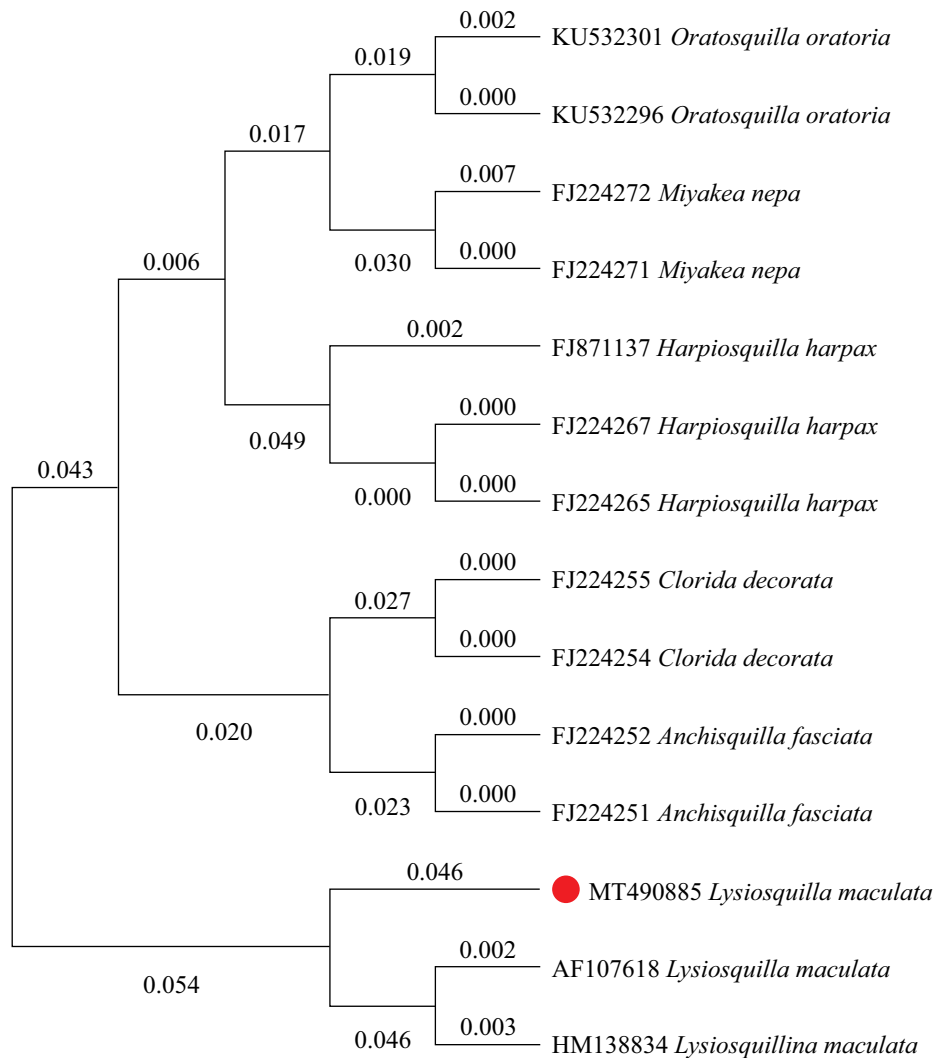


Figure 3. Phylogenetic analysis of *Lysiosquilla maculata* with closely related species by the Neighbor-joining method. Red dot represents the generated sequence in this study.

All the above results show that *Lysiosquilla maculata* has a close phylogenetic relationship with *A. fasciata*, *C. decorata*, *H. harpax*, *M. nepa* and *O. oratoria*. As expected, species of the same genera were clustered into a single clade with a well-supported bootstrap ratio (Steinke et al. 2005). The DNA barcoding results resolved and supported the ambiguity of the species identity; moreover, it was the first confirmatory record of this species based on DNA barcoding analysis.

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Author contributions

Chelliah Babu: conceptualization, methodology, resources and formal analysis. Krishnan Silambarasan: conceptualization, methodology, investigation, formal analysis and writing original draft of the manuscript. Antony P. Anrose: methodology, formal analysis and revised it critically the manuscript. Antony P. Tiburtius: conceptualization, investigation and supervision. All authors listed have made a substantial direct, and intellectual contribution to the work and approves this publication.

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