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First DNA Barcoding Based Record of *Lysiosquillina Maculata* (Fabricius, 1793) (Crustacea: Stomatopoda) From Chennai Coast, Tamil Nadu, India

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Abstract

Taxonomic identification of mantis shrimp *Lysiosquillina maculata* through DNA barcoding analysis collected from Kasimedu fisheries harbour, Chennai coast, Tamil Nadu, India. The mitochondrial cytochrome oxidase sub unit I gene (mtcoI) with 650 bp region was sequenced for phylogenetic analysis. The present record, mitochondrial gene sequences were used to identify the mantis shrimp *Lysiosquillina maculata.* This is the first confirmed record of Indian waters and the mt COI sequence was deposited in GenBank. The neighbor joining method was used for phylogenetic analysis. The pair wise genetic distance calculated with 08 closely related species varied form 0.03-0.404%. Phylogenetic tree based on 13 protein coding genes shows that *Lysiosquillina maculata* has a closer phylogenetic relationship to *Harpiosquilla harpax.*

Introduction

A total of 500 species belonging to more than 120 genera, 18 families and 7 superfamilies were recorded in worldwide (Ahyong 1997; Ahyong and Harling 2000; Ahyong 2012). According to Ahyong (2001), under the family Lysiosquillidae Giesbrecht, 1910 there are three genera and four valid species, *Lysiosquillina glabriuscula* (Lamarck 1818), *L. lisa* (Ahyong and Randall 2001), *L. maculata* (Fabricius 1793), and *L. sulcata* (Manning 1978b). Although, some misidentification and unauthenticated information has been reviewed on this group, but the through the taxonomic investigation and generation of molecular data are precisely attempted throughout the world (Tang et al. 2010). Moreover, the morphological characters are not enough to the identification of species level. Recently, Hebert et al. (2003) suggested that DNA barcodoing technique is made to use of the cytochrome c oxidase subunit-I (COI) in the mitochondrial DNA (mtDNA) markers of an organism, it is rapid and effective species identification by studying the genetic variability among different taxa, and recently the technique has been used to identify the varied life-history stages of single species (Vogler and Monaghan 2007). DNA barcoding is very well developed in worldwide it has been successfully applied in a wide range of animals, including both terrestrial and aquatic taxa (Hebert et al. 2004a; Ward et al. 2005; Hajibabaei et al. 2006; Wakabayashi et al. 2006; Hubert et al.2008). This method has become popular in identifying and studying the genetic diversity of stomatopods by comparing the sequence of other stomatopods of the unknown species with the earlier collected sequences of stomatopod (Barber and Erdmann 2000; Barber et al. 2002; Barber et al. 2006; Barber and Boyce 2006; Tang 2010). So far, a very few study was conducted in the diversity of stomatopods in east coast of India through DNA barcode methods (Kundu et al. 2018).

The common banded/striped/zebra mantis shrimp *Lysiosquillina maculata* was first described as *Squilla maculata* by Fabricius (1793) from India orientali, which was reported as *Lysiosquilla 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) list the species occurring in Indian waters. However, Trivedi et al. (2020). Synonymised *L. maculata* with *Lysiosquilla tredecimdentata* Holthuis, 1941. The recent collection of *Lysiosquillina* specimens from

Chennai were critically analysed and confirmed that they belonged to *Lysiosquillina maculata* (Fabricius 1793) through DNA barcoding, the results of which are presented here.

Materials And Methods

Sampling and morphological identification

The mantis shrimp *Lysiosquillina maculata* were collected from trawl by-catches at Kasimedu, Fisheries Harbour, Chennai. The specimen was collected by hand picking and its identification was carried out using standard guidelines (Manning 1978; Ahyong et al. 2008). The specimen was preserved in 70% alcohol and deposited in the museum of Fishery Survey of India, Chennai.

Genomic DNA isolation, PCR and sequencing

The sample was used for the extraction of total genomic DNA using Phenol Chloroform method, standardized by CAGL. Quality of the genomic DNA was assessed using 0.7% agarose gel along with 1kb DNA ladder as size standard and the quantity of the genomic DNA was assessed in Biophotometer (Eppendorf). Amplification of *COI* gene was carried out for all the test samples using *COI* Forward and Reverse primers. PCR-generated amplicon for 06 samples were confirmed and purified using GeneJET PCR purification kit (Thermo Scientific, EU-Lithuania) to remove the primer dimer and other carryover contaminations. The quality of the products was assessed using 2% agarose gel along with 100bp DNA marker as size standard and the products were found to be good for sequencing.

Purified PCR products were prepared for Cycle sequencing using the Big Dye® Terminator 3.1 sequence kit (Applied Biosystems, Foster City, California, USA). After cycle sequencing, the products were purified using Ethanol-EDTA purification protocol to remove the un-incorporated dNTP's, ddNTP's and primer dimer. After purification the products were dissolved in 12µl Hi-Di formammide and the samples were subjected for denaturation at 95^{0}C for 5 mins. Denatured products were used for sequencing in forward and reverse directions using Genetic Analyzer 3500 (Life Technologies Corporation, Applied Biosystems®, California 94404, USA) as per manufacture's instruction. Sequences were aligned, edited, and analyzed using Clustal W and Mega software version 6 (Tamura et al. 2013).

Dataset preparation and sequence analysis

The sequence was converted to FASTA format and submitted to nucleotide BLAST through the NCBI website (http://www.ncbi.nlm.nih.gov/). The first five sequence matches with highest similarity to database references (mat ident) were analysed for species consistency. All matches were analysed using the percent similarity score.

Taxonomic designations were derived from phylogenetic analysis of mtDNA gene sequences. Sequence was analysed using Bioedit software (Version 7.2.5) (Hall 2004). Final alignment the sequence was compared with the sequences deposited in the National Center for Biotechnology Information (NCBI) in 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 1000 bootstrap replications were performed in MEGA 6.0 software (Kumar et al. 2016). The K2P genetic distances for defining the species, genus and family levels were based on Ward et al. (2005).

Results And Discussion

The genus *Lysiosquillina* contains 04 species that are difficult to identify the morphological characters. More over these characters are not enough to the accurate identification of the species level. In the present study, the mtDNA sequences of COI gene of the mantis shrimp *Lysiosquillina maculata* belongs to the family Lysiosquillina were initially compared with same genus of 03 different species like, *L. glabriuscula* (Lamarck 1818), *L. lisa* Ahyong and Randall (2001) and *L. sulcata* (Manning 1978b). It was the first conformational record in Indian waters. The GenBank accession number is MT490885. A 650bp segment of the 5' margin of mitochondrial cytochrome oxidase subunit I gene is currently used for classification of molecular taxonomy. The A,T,G and C contents of the sequence *L. maculata* were 154%, 140%, 86% and 51% respectively. The GC content was observed at 31.8% in the species. Other sequence variation was not observed among those specimens.

In the present study we have examined the morphological characters and identified the specimen as *Lysiosquillina maculata* (Fabricius 1793). We recorded two specimens with total length ranged from 161 to 178 mm and the specimens are having the characters of ocular scales triangular, erect, and inclined anteriorly. The carapace is convex and broad the rostrum is cordiform-shaped occasionally sub triangular, width usually greater than length. The blunt longitudinal carina present on anterior third plate. Dactylus of raptorial claw possessing 8-11 teeth; normally 10-11 but the larger females it may be varied in 10-11 teeth. Mandibular palp present and 3 segmented. TS8 sternal keel rounded. Uropodal protopod is triangular lobe and ventero laterally anterior to articulation of each uropod and uropodal exopod proximal segment outer margin with 7–9 movable spines; endopod with distal ¾ dark. Dorsum base colour pale yellow, with black transverse bands.

The genetic information of taxonomically identified species is essential for perform the genetic similarity search in the global database (Moritz and Cicero 2004). Hence, before submitting any novel sequences in GenBank and BOLD, it is essential to identify the studied specimens. Earlier, the genetic information on the two known species of genus *Harpiosquilla, H.harapax* and *H. raphidea* are accessible in the GenBank database. The generated sequence of *L. maculata* from Indian waters were first time annotated (650 bp) and submitted in to the GenBank datasets. The generated sequences are shown 91% similarity with *Lysiosquillina maculata* in both GenBank and BOLD database.

The genetic distance of *L. maculata* with eight closely related species were calculated based on Kimura's 2-parameter method (Table 1). The pair wise genetic distance was calculated it varied from (0.03-0.404) that showed the smaller genetic distance indicate a close genetic relationship whereas a large genetic distance indicate a more distant genetic relationship. Genetic variation within populations can be lost

through genetic drift or bottleneck in the population (You et al. 2001). Hebert et al. (2003) suggested that DNA bar coding has the powerful tool to provide valuable insight in to patterns of genetic divergence affected by species level or ecological variation. The average genetic distance among species does not exceed the average genetic distance between “sister” species.

The phylogenetic relationship of *Lysiosquillina maculata* with 01 closely related species and one out group were analysed in this study. Complete mitochondrial genes of these 04 species are available on GenBank. The maximum-liklihood evolutionary tree (ML tree) was constructed by MEGA 7 (Kumar et al. 2016) based on 1st and 2nd codon sequences of 19 protein coding genes.

In the ML phylogenetic tree, *Lysiosquillina maculata* and *Harpiosquilla harpax* formed one clade with strong support. But *Oratosquilla oratoria, Traisquilla profunda, Heterosquillatri carinata, Austrosquilla tsangi, Platysquilla eusebia, Madeirasquilla tuerkayi* and *Lysiosquilla maculata* in the second clade. These two clades mentioned above were all classified into family Lysiosquillidae or order Lysiosquilloidea (Fig. 1). All the above results shows that *L. maculata* has close phylogenetic relationship to *H. harapax.* As expected, species from the same genera were clustered in to a two different clades with well supported bootstrap proportion (Steinke et al. 2005).

Declarations

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Conflicts of interest/Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Ethics approval (including appropriate approval or waivers)

Not applicable

Consent to participate (include appropriate statements)

Not applicable

Consent for publication (include appropriate statements)

Not applicable

Availability of data and material (data transparency)

All the relevant data have been presented in the manuscript

Code availability (software application or custom code)

Not applicable

Author’s contributions

All the authors of this manuscript have contributed significantly towards the execution of this work.

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Tables

Table 1 is not available with this version.

Figures

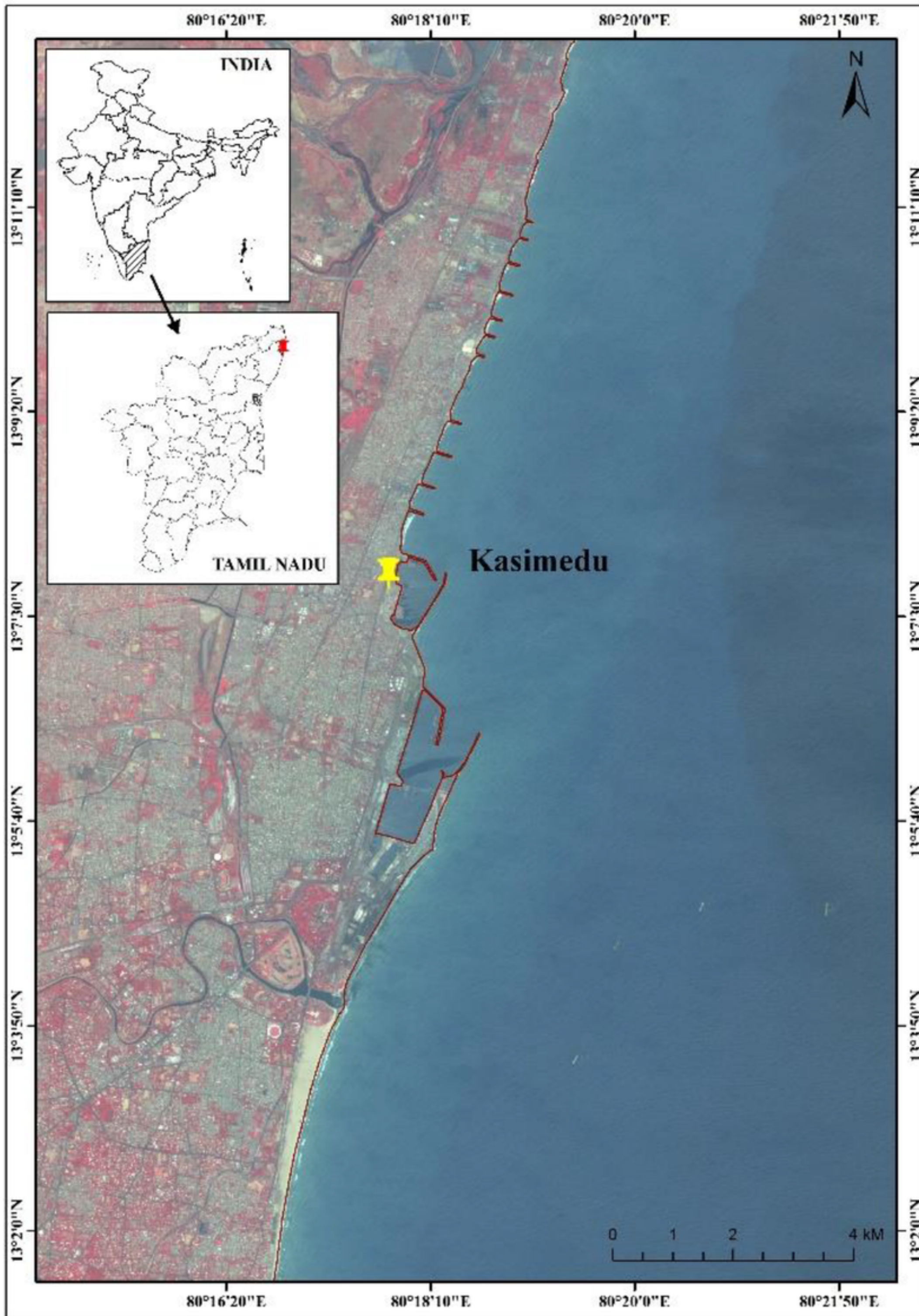

Figure 1

Collection site of the *Lysiosquillina maculata* from Kasimedu fisheries harbour

(a) (b)

Figure 2

Lysiosquillina maculata (TL-178 mm) (a) dorsal view (b) ventral view

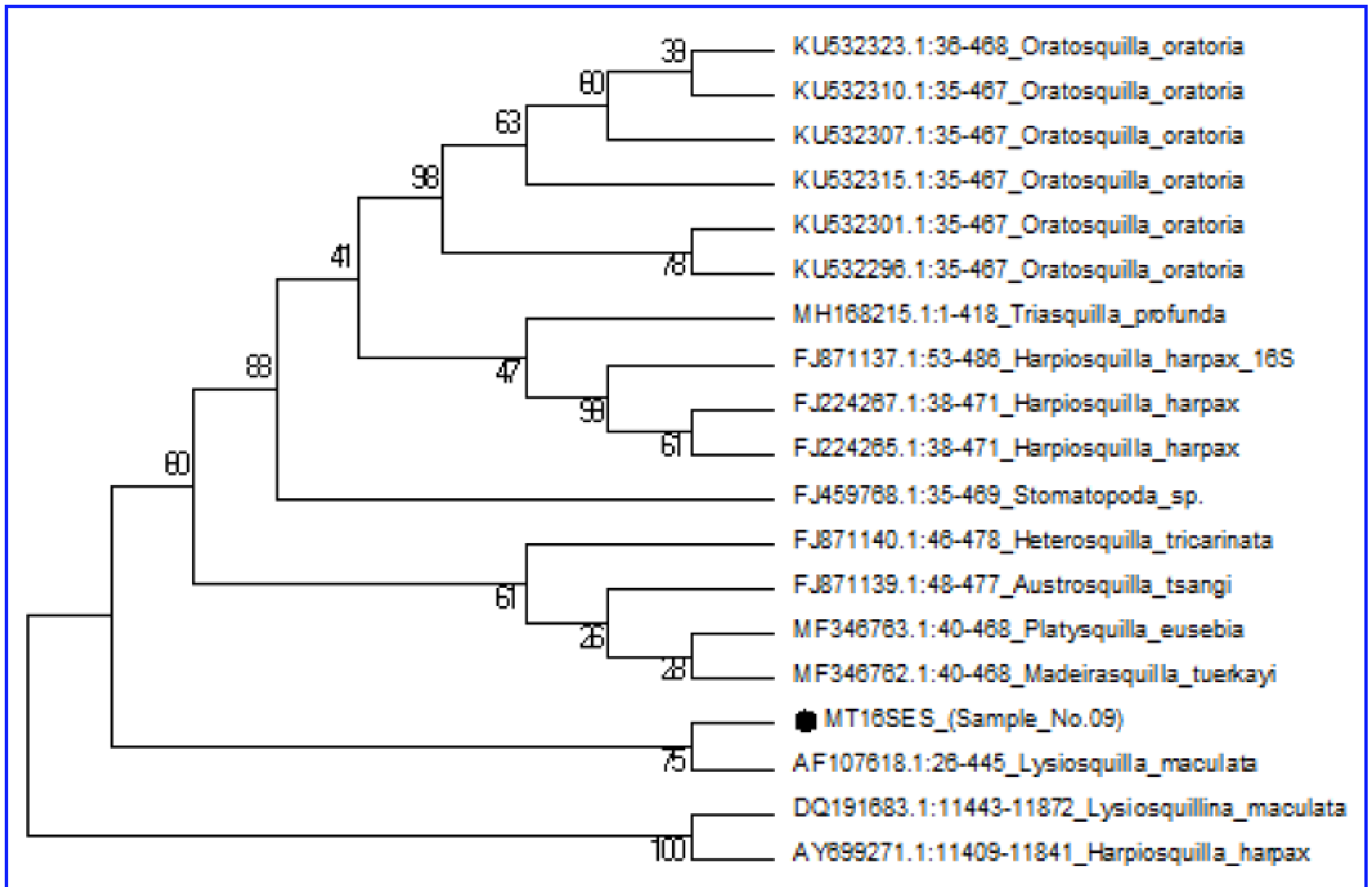

Figure 3

Phylogenetic analysis of *L. maculata* with closely related species by Neighbour Joining method. The black dots represent the generated sequences in this study.