Classical taxonomy and 16S rRNA gene marker confirmed first record of the Menippid crab *Menippe rumphii* (Fabricius, 1798) from the West coast of India

Vivek Rohidas Vartak¹, Rajendran N² & Wazir S. Lakra³

¹Khar Land Research Station, Panvel-410206, Maharashtra, India
²Vellore Institute of Technology, Vellore- 632 014, Tamil Nadu, India
³Central Institute of Fisheries Education, Andheri (E), Mumbai-400061, India

* [E-mail: vivekvartak_arombrs@yahoo.com]

Received 22 February 2014; revised 7 July 2014

The *Menippe rumphii* (Fabricius, 1798), marine crab, occurring along the rocky shores of coastal areas of east coast of India was located along the rocky shores of the Konkan coastal region of Maharashtra. Classical taxonomy is applied for identification of this crab. Taxonomic descriptions and morphometric analysis confirmed the species as *Menippe rumphii*. The 16S rRNA gene from *Menippe rumphii* adults was sequenced to corroborate species identification. Phylogenetic analysis showed ostensible clade between sequences of *Menippe rumphii* from NCBI and study crab. Classical taxonomy and 16S rRNA gene marker substantiated the record of the specimens from the Konkan coast.

[Key words: First record, *Menippe rumphii*, 16S rRNA gene, Classical taxonomy]

Introduction

Menippid crab, *Menippe rumphii* (Fabricius, 1798) known as maroon stone crab is a coastal crab species distributed in the Pacific Ocean from Taiwan to Indonesia¹. This crab is found in Brunei Darsm, Cambodia, China, Indonesia, Malaysia, Singapore, Taiwan, Thailand, Viet Nam, Brazil, India (east coast), Sri Lanka, Djibouti, Somalia, and Oman²-³. Indian Museum possesses 100 specimens of this crab from Penang, Tavoy, Mergui, Chennai coast, Ceylon, Lakshadweep, Karachi and Persian Gulf ⁴. This crab is found along the rocky shores beneath stones, corals and other hiding spots. They have large maroon colored oval body with red eyes. This crab is edible and is captured with the help of gill net from rocky sea shores of Sindhudurg district of coastal region of Maharashtra state, India. This crab is a delicacy of the local people of the Konkan coastal region of Maharashtra. This species falsely resembles to bashful crab *Atergatis integerrimus* and stone crab *Myomenippe hardwickii*. Preliminary identification on the basis of pictures of related species suggested that the species might be stone crab *Myomenippe hardwickii*. Perusals of the pictures of Chhapgar⁵ on the collections from this region also shored up this observation. After detailed taxonomic examination as per Alcock⁴ this species was identified as *Menippe rumphii* (Fabricius, 1798) and has a valid record from the Chennai, east coast of India. To authenticate the taxonomic identity of this crab we decided to develop molecular marker for this species. Proper species identification is important for effective fisheries resource management and commercialization⁶, ⁷, ⁸, ⁹, ¹⁰. Taxonomic identification by DNA based methods is more sophisticated way which identifies specimens in all stages. Molecular methods are more exact, saves time and an advanced step of species identification if utilized along with classical taxonomy. Earlier studies on molecular methods showed effectiveness in identifying crustaceans⁹, ¹¹, ¹², ¹³, ¹⁴, ¹⁵, ¹⁶, ¹⁷. The use of 16S rRNA gene in crustaceans is reviewed by Schubart and Neigel¹⁸ suggesting molecular systematics as a highly effective tool for documenting and classifying the biodiversity of this world. The 16SrRNA gene is used for phylogenetic studies¹⁹ due to high conservancy between different species²⁰. Hence, we decided to make use of classical
taxonomy and 16S rRNA gene marker for identification and validation of this species.

Material and Methods

Sample collection and morphometrics

The first recorded Menippid crab *Menippe rumphii* caught in February of 2013 on the rocky shores of Kelus (15° 54' 12.0528'' N; 73° 35' 35.7864'' E), located along the Konkan coastal region of Maharashtra State of West coast of India (Figure 1).

Figure 1 Location of *Menippe rumphii* crab record in Sindhudurg district (dark shade) of Maharashtra state of West coast of India

First specimen found was female with 90 mm carapace width (Figure 2). Total ten crabs were found in catch and all were female. All crabs have been carefully stored in absolute alcohol in plastic bottles and taken to the laboratory for identification. Crabs were identified based on phenotypic criteria such as morphology, colour and appendages. Voucher specimens were stored at -80°C in DNA barcoding laboratory. Hundred mg muscle tissue from chelipeds was removed from both the crabs with sterile scissors and stored in absolute alcohol at -20°C for molecular study. Nine morphometric measurements with five ratios were used in analysis (Table 1a).

Table 1a Measurements used for morphometric analysis

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Measurements taken</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carapace length (CL)</td>
</tr>
<tr>
<td>2</td>
<td>Carapace width (CW)</td>
</tr>
<tr>
<td>3</td>
<td>Major cheliped merus length (MEL)</td>
</tr>
<tr>
<td>4</td>
<td>Major cheliped merus width (MEW)</td>
</tr>
<tr>
<td>5</td>
<td>Major cheliped manus length (MAL)</td>
</tr>
<tr>
<td>6</td>
<td>Major cheliped dactylus length (DAL)</td>
</tr>
<tr>
<td>7</td>
<td>Penultimate segment length (PL)</td>
</tr>
<tr>
<td>8</td>
<td>Penultimate segment width (PW)</td>
</tr>
<tr>
<td>9</td>
<td>Telson width (TW)</td>
</tr>
</tbody>
</table>

DNA extraction, polymerase chain reaction and data analysis

The DNA extraction was carried out using the standard SDS-phenol/chloroform method with slight modifications. Concentration of isolated DNA was estimated using a UV spectrophotometer and diluted to a final concentration of 100 ng/µl. Amplification of 16S rRNA gene was carried out with universal primers:16Sar(5'-CCTGTTTATCAAAAAACAT-3') and 16Sbr(5'CCGGTCTGAACTCAGATCAGT-3') 22. PCR reaction includes 10X Taq polymerase buffer, 10 mM of dNTP mix; 0.6 unit of Taq polymerase; 0.01 mM of each primer and 100 ng of DNA template. General thermocycler conditions were an initial step of 2 min at 95°C and 34 cycles of 30 s at 95°C, 40 s at 50°C and 1 min at 72°C, followed by a final extension at 72°C for 10 min. Amplified products were sequenced at Eurofins Genomics India Pvt. Ltd, Bangalore India. Sequences were aligned using CLUSTALW in BioEdit v. 7.2.3. For comparison and validation, the 16S rRNA gene sequences for other sympatric species were obtained from NCBI Gene Bank (Table 2). These sequences includes one sequence of *Menippe rumphii* from Singapore and 9 sequences of *Menippe adina*, *Menippe mercenaria*, *Menippe nodifrons*, *Myomenippe hardwickii*, *Chlorodiella Cytherea*, *Xantho poressa*, *Paraxanthus barbiger*, *Xantho pilipes* and *Atergatis integerrimus* belonging to two sympatric families, *Menippidae* and *Xanthidae* of Brachyuran subsection Heterotremata. Evolutionary history was inferred using the Neighbor-Joining (NJ) method with bootstrap support of 10000 replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown above the branches. All positions containing gaps and missing data were eliminated. Maximum Composite Likelihood method was used for computing evolutionary distances, which are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA5.

Results

Systematics

Family: *Menippidae* Ortmann, 1893
Genus: *Menippe* De Haan, 1833

Synonymized taxa:

*Alpheus rumphii* Weber, 1795 (nomen nudum)
*Cancer rumphii* Fabricius, 1798
*Pseudocarcinus bellangerii* H. Milne Edwards, 1834
Classification
Kingdom: Animalia, Phylum: Arthropoda
Class: Malacostraca, Order: Decapoda
Super family: Eriphioidea,
Family: Menippidae
Species: *Menippe rumphii*

Morphological description
Gastric region is distinct and fairly subdivided into three lobes. There are four blunt tubercles positioned in a square towards front between lobes and eyes. Brachial region has two low indistinct transverse granular elevations which run nearly parallel with the curve of the antero-lateral border. First elevation is the more distinct and can generally be traced across the gastric region also. Cardiac region has transverse interrupted ridge. Surface of the carapace is smooth except antero-lateral fine pitting. Front of the carapace has two prominent round pointed lobes which ends with a prominent rounded tooth separated from the supra orbital margin by groove (for eyes). Antero-lateral border is fairly sharp and distinguished by the four-tooth which divides it into four broad lobes. Last two lobes are distinctly acuminate than first two lobes. Chelipeds are big and unequal as left is smaller than right. They are smooth with fine and distant pitting on outer surface. Inner angle of wrist is bluntly prominent. Fingers are stout and black with short thumb. Legs are stout and smooth, except the upper border which is granular. Upper border of carpopodites is sparsely without any hairs. Borders of propodites and dactylus are thicker with hairs. Color of chelipeds is brownish yellow with a fine pattern of dark markings. This evaluation of description was first step to identify *Menippe rumphii* with classical taxonomy. The ratios for morphometric measurements for female of *Menippe rumphii* is given in Table 1b.

<table>
<thead>
<tr>
<th>Ratios</th>
<th><em>Menippe rumphii</em> (♀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CW/CL</td>
<td>1.64±0.01</td>
</tr>
<tr>
<td>MEL/MEW</td>
<td>1.66±0.08</td>
</tr>
<tr>
<td>MAL/DAL</td>
<td>2.18±0.07</td>
</tr>
<tr>
<td>PL/PW</td>
<td>0.45±0.05</td>
</tr>
<tr>
<td>PL/TW</td>
<td>0.48±0.09</td>
</tr>
</tbody>
</table>

Molecular analysis
Neighbour Joining (NJ) tree involving 18 nucleotide sequences is given in Figure 3. Sequences of *Menippe rumphii* obtained under this study (Haplotypes 1-5) showed distinctive clade with sequence of its haplotype (6) from Singapore. Other species under genus *Menippe* clustered separately from *Menippe rumphii* haplotypes without ambiguity, which indicated robustness of 16S rRNA gene in phylogenetic studies. Apart from this, NJ tree resulted in comprehensible clade between two families supported with higher bootstrap values indicating proper molecular differences between these species. Number of base differences per site between sequences is shown in Table 3. Base differences per site between sequences of *Menippe rumphii* from NCBI and present study was zero. Divergence was 0.08 and 0.18 between *Menippe rumphii* with other falsely resembling species viz. *Myomenippe hardwickii* and *Atergatis integerrimus* respectively. Thus, results of molecular data analysis supports that the species identified and recorded was *Menippe rumphii*.

Discussion
Identifying and recognizing a species without an adequate morphological description is unfeasible\(^29\). The wrong species identification shores up threats for biodiversity and food safety. Sea food labeling is modern thought emerging worldwide to avoid fraud associated with the mislabeled sea food. In such cases, the proper identification of species becomes vital for
Table 2 List of crabs used for phylogenetic analysis with Gene Bank accession numbers

<table>
<thead>
<tr>
<th>Name of species</th>
<th>Family</th>
<th>Common Name</th>
<th>Country</th>
<th>Gene Bank acc. numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Menippe rumphi</em></td>
<td>Menippidae</td>
<td>Maroon Stone crab</td>
<td>Maharashtra, India</td>
<td>KF220514 to KF220518*</td>
</tr>
<tr>
<td><em>Menippe rumphi</em></td>
<td>Menippidae</td>
<td>Maroon Stone crab</td>
<td>Singapore</td>
<td>HM637976</td>
</tr>
<tr>
<td><em>Menippe adina</em></td>
<td>Menippidae</td>
<td>Gulf stone crab</td>
<td>Singapore</td>
<td>HM637973</td>
</tr>
<tr>
<td><em>Menippe mercenaria</em></td>
<td>Menippidae</td>
<td>Florida stone crab</td>
<td>Singapore</td>
<td>HM637974</td>
</tr>
<tr>
<td><em>Menippe nodifrons</em></td>
<td>Menippidae</td>
<td>Cuban stone crab</td>
<td>Singapore</td>
<td>HM637975</td>
</tr>
<tr>
<td><em>Menippe nodifrons</em></td>
<td>Menippidae</td>
<td>Cuban stone crab</td>
<td>Mexico</td>
<td>AJ130817</td>
</tr>
<tr>
<td><em>Myomenippe hardwickii</em></td>
<td>Menippidae</td>
<td>Musca stone crab</td>
<td>Singapore</td>
<td>HM637977</td>
</tr>
<tr>
<td><em>Chlorodelia cytherea</em></td>
<td>Xanthidae</td>
<td>Venus green crab</td>
<td>USA</td>
<td>JQ277186</td>
</tr>
<tr>
<td><em>Xantho poressa</em></td>
<td>Xanthidae</td>
<td>Purple crab</td>
<td>USA</td>
<td>JQ277185</td>
</tr>
<tr>
<td><em>Paraxanthus barbiger</em></td>
<td>Xanthidae</td>
<td>Not available</td>
<td>Chile</td>
<td>FJ031225</td>
</tr>
<tr>
<td><em>Xantho pilipes</em></td>
<td>Xanthidae</td>
<td>Risso's Crab</td>
<td>Singapore</td>
<td>HM637956</td>
</tr>
<tr>
<td><em>Atergatis integerrimus</em></td>
<td>Xanthidae</td>
<td>Bashful crab</td>
<td>Singapore</td>
<td>HM798423</td>
</tr>
<tr>
<td><em>Atergatis integerrimus</em></td>
<td>Xanthidae</td>
<td>Bashful crab</td>
<td>Maharashtra, India</td>
<td>KF220494</td>
</tr>
</tbody>
</table>

* - Submitted to NCBI under this study

Figure 3 The Neighbour joining tree of sequences of *Menippe rumphi* with other nine species belonging to Xanthidae and Menippidae family. Bootstrap values are presented above the branch. Bootstrap values below 50% are not shown on the tree. ‘A’ indicates haplotypes for *Menippe rumphi* from present study and ‘B’ indicates haplotypes for *Menippe rumphi* obtained from NCBI Gene Bank. The separate clades for families are represented by right brace.
Table 3 Evolutionary divergence estimates between sequences of *Menippe rumphii* and other nine species

<table>
<thead>
<tr>
<th></th>
<th>MR1</th>
<th>MR2</th>
<th>MR3</th>
<th>MR4</th>
<th>MR5</th>
<th>MR6</th>
<th>MM</th>
<th>MA</th>
<th>MN1</th>
<th>MN2</th>
<th>MH</th>
<th>CC</th>
<th>XP</th>
<th>PB</th>
<th>XP1</th>
<th>XP2</th>
<th>AT1</th>
<th>AT2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Menippe rumphii</em> (Haplotype-1)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td><em>Menippe rumphii</em> (Haplotype-2)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td><em>Menippe rumphii</em> (Haplotype-3)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td><em>Menippe rumphii</em> (Haplotype-4)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td><em>Menippe rumphii</em> (Haplotype-5)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td><em>Menippe mercenaria</em></td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><em>Menippe mercenaria</em></td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td><em>Menippe nodifrons</em> (Haplotype-1)</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td><em>Menippe nodifrons</em> (Haplotype-2)</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td><em>Myomenippe hardwickii</em></td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td><em>Chlorodiella cytherea</em></td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td><em>Xantho poressa</em></td>
<td>0.18</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td><em>Paraxanthus barbiger</em></td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td><em>Xantho pilipes</em> (Haplotype-1)</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td><em>Xantho pilipes</em> (Haplotype-2)</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.21</td>
<td>0.21</td>
<td>0.21</td>
<td>0.21</td>
<td>0.21</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td><em>Atergatis integerrimus</em> (Haplotype-1)</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td><em>Atergatis integerrimus</em> (Haplotype-2)</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td></td>
</tr>
</tbody>
</table>

The number of base differences per site from between sequences is shown. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (10000 replicates). *Menippidae* family is separated by vertical black bar. The short codes created with first letter of genus and species are used on upper horizontal matrix bar. Dark highlight: Evolutionary divergence between *Menippe rumphii* and falsely resembling species, Faint highlight: Evolutionary divergence within *Menippe rumphii* haplotype.
labeling the sea food. Serious and sincere efforts are the main backbone for correct species identification and inventorisation. Hence, we tried to search the database for this crab which suggested unavailability of this crab from the west coast of India. We found twenty three occurrences of Menippe rumphi crab from world of which two reported in the Arabian Sea from Oman and Socotra Island, Yemen. To date, one credible occurrence for this crab is available from India reported from Chennai, East coast of India. These above records are reliable; as the specimens are retained after confirm taxonomic identification. Trivedi et al. reported occurrence of this crab from Gulf of Kutch, Gujarat, India. This record is not credible as the photograph publicized by authors is different from the Menippe rumphi suggesting error in taxonomic identification. To confirm and authenticate our record we studied the systematics and taxonomical description of this crab. Lai et al. used morphometric measurements and ratios for discriminating divergence between Portunus pelagicus species complex. In present study, we found morphometric measurements along with ratios as valid and supportive choice in differentiating the species. Classical taxonomy with molecular marker used in this study played important role in identifying the species. For this species, we first time reported the 16S rRNA gene sequence on NCBI database from India which ranks second after the sequence from Singapore (HM637976). NCBI database sequences for all species from the genus Menippe for phylogenetic analysis are used in this study. This step proved to be helpful, as it demonstrated distinct clades for Menippe rumphi haplotypes and other species under this genus. It is apparent from our molecular analysis that the species was Menippe rumphi having monophyletic clade with its haplotype from Singapore. The concept of utilizing classical taxonomy with molecular tools for identification of species is gaining popularity amongst researchers. David et al. identified Paraxanthus barbiger megalopae with the help of classical taxonomy and 16S rRNA gene sequencing. Makinster et al. used PCR amplification of a middle repetitive element for detecting larval stone crabs (Crustacea: Decapoda: Menippidae) in estuarine plankton samples. Shih and Hiroshi have reported nucleotide divergence ranging from 1.46%-2.36% for 16S rRNA gene among the 3 species of Mudflat Crab. In this study, number of base differences per site ranged from 0.0 to 0.21 between sequences of 10 crabs of Brachyuran subsection Heterotremata. In the present study, zero divergence within the Menippe rumphi haplotypes suggested significance of studying the divergence based on the number of base substitutions between the sequences. These results gave vital support to the phylogenetic analysis. At this stage, we studied one gene marker due to the robustness and unambiguous results obtained with 16S rRNA gene marker. There are other markers like COI, 12S, Cyt-B etc available for sequencing and studying phylogenetic relationship. Further scope is open for DNA barcoding and developing more markers for this species.

Conclusion

Record of Menippe rumphi (Fabricius, 1798) from the west coast of India is thus confirmed in the present study.

Acknowledgements

Authors would like to thank authorities of Central Institute of Fisheries Education (ICAR) Mumbai and Vellore Institute of Technology, Vellore India for their support for this work.

References


29. David A, Palma, AT, Veliz D and Pardo L. M. Description, seasonal morphological variation, and molecular identification of Paraxanthus barbiger megalopae obtained from the natural environment. Helgoland Marine Research, 64(2) (2010), 117-123.


