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Short Communication

Morphological and molecular documentation of Jellyfish *Rhopilema hispidum* (Vanhöffen, 1888) (Scyphozoa: Rhizostomatidae) from Mumbai coast, India

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Identification of jellyfish species is challenging, even for taxonomic experts, due to their fragile forms and complex, morphologically-distinct life-history stages. DNA barcoding along with the morphological characters would complement the species identification. In the present study, a jellyfish collected from the Juhu beach, Mumbai coast, Maharashtra, India was identified based on the morphology and molecular markers. Visual morphological characters indicate that the specimen belongs to the genus *Rhopilema*. The partial gene of nuclear Internal Transcribed Spacer 1 was amplified, sequenced and subjected to similarity analysis with the NCBI GenBank database. The analysis showed a similarity of 99 % with the reported *Rhopilema hispidum* and confirmed the species identity. In the Neighbour-joining tree, the present study specimen is clustered with *R. hispidum* reported from Malaysia.

[**Keywords**: Arabian Sea, DNA Barcoding, Internal Transcribed Spacer 1, Taxonomy]

Introduction

True Jellyfishes; the free-swimming medusa-phase of class Scyphozoa (Phylum: Cnidaria) play an important role in the food web of the marine ecosystem[1]. More than 200 species have been reported under this class and, their habitat includes deep sea to shallow coastal areas[2]. They have a wide distribution around the world and prey on smaller phytoplankton, zooplankton, copepods and ichthyoplankton[3,4]. These indicator species change their population size in response to the ecological perturbation. Eutrophication, overfishing, ocean acidification and climate change often cause jellyfish blooms[5-7]. These blooms lead to significant adverse socio-economic effects such as causing harmful and deadly stings, blockage in coastal power plants cooling systems, bio-fouling of fish cages and in trawl catches[8-10]. For the past two decades, jellyfish blooms have been increasingly reported from all around the world, including India[11,12]. Every year, after the monsoon (October to December), jellyfish stings have been reported in Mumbai coast threatening people and tourism[13]. Several studies have reported the occurrence of *Chiropsoides buitendijki*[13], *Chiropsoides quadrigatus*[14] and *Physalis physalis*[15] from Mumbai coast. To better understand and manage jellyfish blooms, such efforts must begin with accurate species identification. However, jellyfish are generally difficult to identify due to lack of distinguishing morphological characters, which are often fragile and get easily damaged during the collection or after preservation[16]. Researchers have used molecular markers along with the morphological characters to distinguish the jellyfishes[17,18]. The present study identified *Rhopilema hispidum* from Mumbai coast using morphology and molecular marker.

Material and Methods

A total of nine individual jellyfishes were collected from Juhu beach, Mumbai, India (19º70' N, 72º49' E) during September 2017. Morphological characters were observed and documented from fresh specimen following the literature by Gul & Morandini[19] and Omori & Kitamura[20]. After morphological examination, tissue samples were collected aseptically from the umbrella (bell margins) portion, preserved in 96 % alcohol and stored at -4 °C until further use. The total genomic DNA was isolated by the CTAB method[19] with slight modifications. Partial sequence of Internal Transcribed Spacer 1 (ITS1) region was amplified using universal primers, ITS1 F: 5'-GTTTCCGTAGGTGAACCTGC-3' and ITS1 R: 5'-GCTGCGTTCTTCATCGATGC-3'[(ref. 21)]. PCR was performed in 25 µl reaction volume containing 50 ng template DNA, 10 pmol of each specific primer, 200 µM of each primer, 1 U of Taq DNA polymerase and 1X Taq buffer with 25 mM $MgCl_2$. The thermocycler was programmed for touchdown PCR as follows: initial denaturation at 95 °C for 5 min, followed by denaturation of 35 cycles of 94 °C for 1 min, annealing starting from 70 °C and decreased the temperature by

1 °C in every cycle for 35 times and extension at 72 °C for 1 min for denaturation, annealing and extension respectively, with final extension at 72 °C for 10 min. The PCR amplification products were purified using the gel extraction kit (Qiagen, Germany) following the manufacturer's protocols. The purified PCR products were sequenced bi-directionally using PCR primers (Eurofins lab, India).

The quality of ITS1 sequence was verified by Phred score of each nucleotide using Finchtv software[22]. Reported ITS1 sequences of *Rhopilema hispidum* and *Rhopilema esculentum* were retrieved from the NCBI database and aligned with the present study sequence using ClustalW program[23]. Genetic distance values using Kimura-2-Parameter (K2P) model were estimated using the MEGA7 software[24]. Neighbor-Joining (NJ) phylogenetic tree was constructed with 1000 bootstrap replicates. *Lobonemoides robustus* (JN202968) is included as an outgroup in the tree.

Results

The collected samples were identified as *R. hispidum* based on the following morphological characters. Exumbrella whitish in colour having a bell with a diameter of 40 to 65 cm, eight rhopalia, brown coloured pointed warts all along the exumbrella, eight marginal lappets and sixteen radial canal systems along exumbrella. Eight distal arms and a single terminal appendage on each arm (Fig. 1).

Only one of the nine specimens could be amplified and sequenced due to the degradation of tissue samples. About 382 bp of ITS1 region was obtained after sequencing. The sequence was submitted to NCBI GenBank with accession no. MK092065. The frequency of nucleotides was A: 22.25, T: 26.18, C: 23.56 and G: 28.01 % with A+T content of 48.43 %. The genetic divergence values (K2P) between conspecific individuals of *R. hispidum* ranged from 0.005 to 0.090 (0.046±0.012; Table 1). The genetic distance values were significantly lower for conspecific individuals than congenerics. Inter-specific divergence value between *R. hispidum* and *R. esculentum* ranged from 0.475 to 0.579 (0.359±0.049).

The Neighbour-Joining tree formed two distinct clusters representing *R. hispidum* and *R. esculentum* with significant bootstrap values. The present study sequence clustered within the clade of *R. hispidum* confirming the species status as *R. hispidum* (Fig. 2).

Discussion

Approximately 34 species of Scyphomedusae have been reported from Indian waters[25]. Most of the records of Scyphomedusae were from the eastern

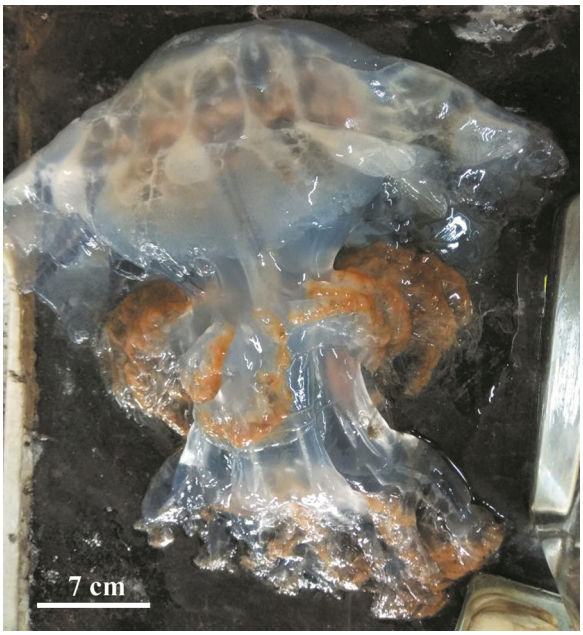

Fig. 1 — *Rhopilema hispidum* collected from Juhu beach, Mumbai coast, India

Table 1 — Genetic divergence values (Kimura 2 Parameter) of ITS1 gene sequence of selected *Rhopilema* species

Sr.No	Species	1	2	3	4	5	6	7	8	9
1	*Rhopilema hispidum (MK092065.1)*		0.021	0.02	0.021	0.063	0.062	0.062	0.062	0.073
2	*R. hispidum* (JN202971)	0.09		0.005	0	0.068	0.067	0.067	0.067	0.075
3	*R. hispidum* (JN202970)	0.084	0.005		0.005	0.067	0.066	0.066	0.066	0.074
4	*R. hispidum* (JN202969)	0.09	0	0.005		0.068	0.067	0.067	0.067	0.075
5	*R. esculentum* (AB377589)	0.481	0.517	0.508	0.517		0.015	0.015	0.015	0.023
6	*R. esculentum* (KR338966)	0.475	0.511	0.502	0.511	0.044		0	0	0.02
7	*R. esculentum* (KR338965)	0.475	0.511	0.502	0.511	0.044	0		0	0.02
8	*R. esculentum* (KR338964)	0.475	0.511	0.502	0.511	0.044	0	0		0.02
9	*R. esculentum* (JN202972)	0.551	0.579	0.57	0.579	0.096	0.079	0.079	0.079	

Below diagonal values are genetic divergences; above diagonal are standard deviations

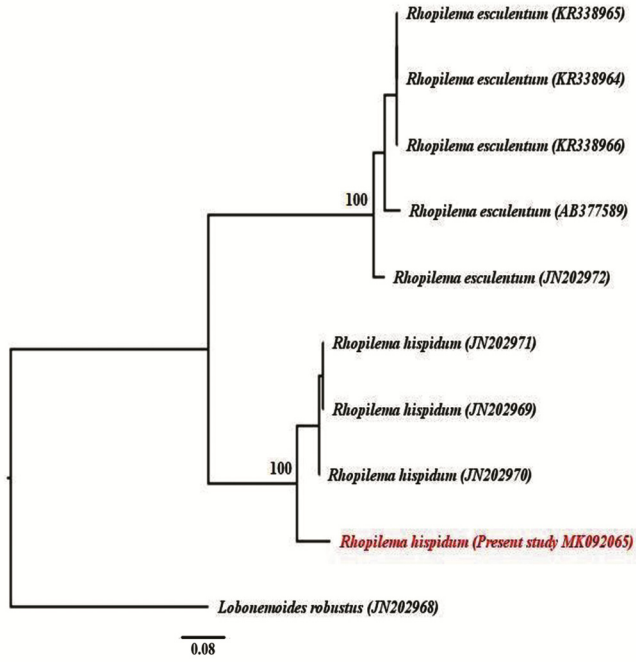

Fig. 2 — Neighbour-joining tree based on ITS1 gene sequences of selected *Rhopilema* species. GenBank accession numbers are given in parentheses (*L. robustus* was included as outgroup)

coast of India and the species identification was primarily based on morphological characters[26,27]. In India, previous studies on jellyfish have focused mostly on aspects of jellyfish stings, envenomation and toxicology of their venoms[28,29]. Previous studies have documented *R. hispidum* from the Indian coast using morphological characters[26,30]. Studies on molecular identification of jellyfish are very limited. In the present study, all samples showed the similar characteristics as reported by Gul & Morandini[18] and Omori & Kitamura[19] thereby confirmed the species. The ITS1 region has been used by as a species-specific molecular marker for identifying species of Scyphomedusa[31,32]. The GC content (51.57 %) of the present study ITS1 is slightly higher than the previous studies[32]. The genetic divergence value observed between *R. hispidum* from Mumbai coast and Malaysian coast (JN202969-71) could be attributed to population genetic divergence. Several studies have reported population genetic structuring among different jelly fish using nuclear and mitochondrial marker[32,33]. In the NJ tree also individuals of different species formed distinct clades. This observation was supported by the divergence values as there was no overlap between the intra-specific and inter-specific variation. The similar observation was previously reported by Rizman-Idid *et al.*[31].

Although *R. hispidum* is moderately venomous, it is edible and important in fisheries. Many Southeast Asian countries harvest them and export for food[34]. In India also, *R. hispidum* catch are processed and exported to the overseas market[35]. However, the jellyfish blooms cause ecological nuisance and their sting can cause dermatitis[36]. Several anthropogenic (coastal developmental projects) and climate change (increased sea surface temperature) could be the factors responsible for increase in the number of jellyfish near Mumbai coast[37]. However, detailed studies are required to investigate the biological, hydrological and geological factors responsible for jellyfish invasion to the Mumbai coast.

Conclusion

The present study demonstrates for the first-time barcoding technique *via* the sequencing of partial ITS1 region of *R. hispidum* to confirm the morphological identification of this species and their presence off the Mumbai coast, Arabian Sea, India. Future studies of this species should aim at conducting genotyping of more individuals using several genetic markers to detect differences between populations in India.

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Conflict of Interest

The authors declare no competing or conflict of interest.

Author Contributions

RCD wrote the manuscript with input from KAK and APK. RCD and KAK conducted the field study. RCD conducted taxonomic identifications. APK and NS supervised this study and provided research materials.

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