



International Journal of Fisheries and Aquatic Studies

E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(3): 409-413
© 2017 IJFAS
www.fisheriesjournal.com
Received: 22-03-2017
Accepted: 23-04-2017

Suneel Kumar Yalla
Research Scholar, Department of
Ocean Studies and Marine
Biology Pondicherry University,
Brookshabad Port Blair,
Andaman and Nicobar Islands

Dr. R Mohanraju
Associate Professor, Department
of Ocean Studies and Marine
Biology Pondicherry University,
Brookshabad Port Blair,
Andaman and Nicobar Islands

New record of *Abdopus aculeatus* (d'Orbigny, 1834) (Cephalopoda: Octopodidae) to Indian waters from the Andaman Island, India and its Molecular characterisation

Suneel Kumar Yalla and Dr. R Mohanraju

Abstract

Abdopus aculeatus are residents of shallow and intertidal reef flats familiar to Indonesian, Australian, Philippine waters. They are commonly referred as Algae octopuses due to their typical resting camouflage that resembles a gastropod shell residing on algae. The present study confirms new record of the present species in Indian waters and depicts its morphology and molecular characterisation. DNA fragments of mitochondrial cytochrome c oxidase sub unit I (COI) gene comprising 654 base pairs are amplified by employing LCO 1490 and HCO 2198 primers. Base frequencies were A= 0.301, C=0.222, G= 0.146, T=0.329, A+T=63.06% and G+C=36.93% respectively. Phylogenetic tree was constructed by maximum likelihood and neighbour –joining tree, genetic distance ranged from 0.00 to 0.08 among individuals. Morphological and molecular studies have confirmed the present specimen as *Abdopus aculeatus*.

Keywords: *Abdopus aculeatus*, morphology, molecular characterisation, new record, Indian waters

Introduction

Octopus genus was first described by Lamarck “1978” based on species type, *Octopus vulgaris*. Octopodidae members share a basic structural plan, diverse morphological characters such as, the presence of eight arms, biserial suckers rows on each arm and an ink sac are the key characters of traditional taxonomy of octopuses. Octopuses belong to the only benthic family of Octopodidae that occupy the benthic region of deep, shallow and intertidal regions. Octopuses greatly vary in their skin colouration, patterning, behaviour and life history and are ubiquitous around the world. A total of 295 species of octopodiformes have been reported from the world oceans (Jereb *et al.*, 2014) [1], among them 60 species are reported from Indian Ocean (Sundaram, 2010) [19]. Studies show that 40 species of octopuses have been reported from India among which 18 species belong to Andaman waters (Venkataraman *et al.*, 2004) [23]. Several genetic studies have been carried out on octopuses and molecular taxonomy (Guzik *et al.*, 2005) [1] has helped in understanding the phylogenetic relationships among the genera. Molecular taxonomy is best understood by analysis the mitochondrial gene cytochrome c oxidase sub unit I (COI) that provides accurate taxonomy. Molecular analysis of the mitochondrial DNA gene, cytochrome c oxidase sub unit I & III (COI & CO III) and cytochrome b (CYTB), and a nuclear DNA elongation factor gene helped in proper identification of the octopuses and established several monophyletic groups which might represent independent genera and support taxonomic changes (Guzik *et al.*, 2005) [3]. The present study provides the new record of a small intertidal octopus from the coast of South Andaman and lays emphasis on morphology and molecular characterisation.

Materials and Methods

Specimen was collected during nocturnal low tide from intertidal region of Burmanallah coast well shown in Fig.1 (Lat 11°33'36.24"N, Long 92°43'49.73"E) of Port Blair in March 2016. Small portion of the tissue was removed from the arm and preserved in 99% ethanol for extraction of DNA. The specimen was fixed in 4% distilled buffered formalin and later transferred to 70% ethanol.

Correspondence
Suneel Kumar Yalla
Research Scholar, Department of
Ocean Studies and Marine
Biology Pondicherry University,
Brookshabad Port Blair,
Andaman and Nicobar Islands

Morphological identification of the species was carried out based on the identification keys as described by Orbigny (1834)^[10]; Roper & Voss (1983)^[13]; and Norman & Finn

(2001)^[8]. The specimen has been registered in National Zoological Collection at ZSI/ANRC -16405 dated 19th December, 2016.

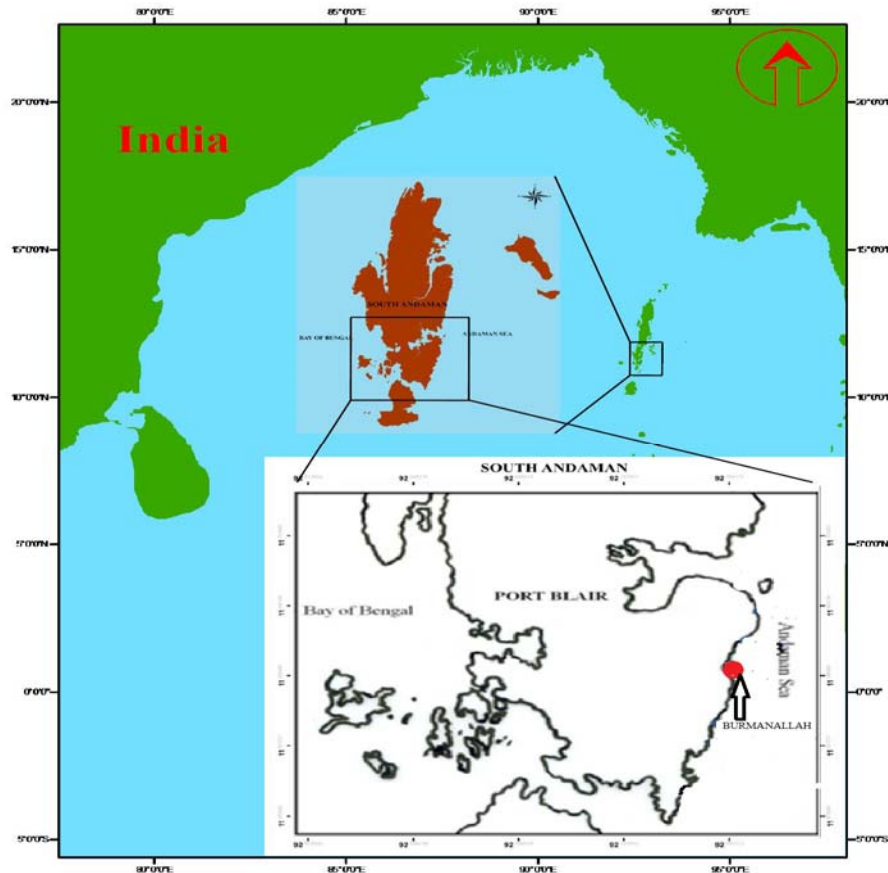


Fig 1: Map showing the study area.

Morphological counts and measurements were recorded as described by Roper and Voss (Roper & Voss 1983)^[13]. Counts and measurements of TL- total length; ML-mantle length; HL-head length; HAL-height arm length; SAL; small arm length; LASC-long arm suckers count; SASC-small arm suckers count; TW- total weight were recorded.

DNA extraction, Polymerase chain reaction and molecular sequencing.

The DNA was extracted following the high universal rapid salt extraction method as described by Salah *et al.* (1997)^[16]. Partial segment of mitochondrial COI gene was amplified using LCO 1490 and HCO 2198 primers (Folmer *et al.*, 1994)^[2]. Polymerase chain reaction was carried in 25 μ l containing 20mM MgCl₂, 16.3 μ l of molecular H₂O, 0.5 μ l 10mM dNTPs, 0.2 μ l of Taq Polymerase and 1.25 μ l of forward and reverse primers (Strugnell *et al.*, 2007). Amplification of COI was performed by employing Applied Biosystems PCR thermal cycler with following conditions: Initial denaturation 3 min at 94 $^{\circ}$ C followed by 40 cycles of denaturation for 40 s at 94 $^{\circ}$ C, annealing for 40 s at 50 $^{\circ}$ C and extension for 1 min at 72 $^{\circ}$ C followed by final extension of 10 min at 72 $^{\circ}$ C. Final PCR product was run in 1% agarose gel electrophoresis and the products were purified with PCR purification kit (Shrimpex GeNo Rime) and sequenced in ABI 3500 DNA Sequencer (Applied Biosystems).

Data Analysis

The sequenced data was aligned using Clustal W (Thompson *et al.*, 1997)^[22] and deposited to the gene bank for retrieving reference number. The extent of genetic similarity between the species was calculated by averaging pair wise comparison of sequence difference across all individuals. The COI sequences of the individual were aligned to yield final sequence of 655 base pair. Pair wise evolutionary distance among the haplotypes was determined by the Kimura 2-parameter method (Kimura, 1980)^[7] using the software program MEGA 6.0 (Tamura *et al.*, 2013)^[21]. The neighbour-joining tree was constructed using mega 6.0 and to verify the robustness of the internal nodes of NJ tree, bootstrap analysis was carried using 1000 pseudo replications.

Results

Systematic

Phylum: MOLLUSCA
 Class: CEPHALOPODA
 Sub-class: COLEOIDEA
 Order: OCTOPODA
 Sub order: INCIRRATA
 Family: OCTOPODIDAE
 Genus: *Abdopus*
Abdopus aculeatus (d'Orbigny, 1834)^[10]
 Common name: Algae octopus

Synonym

Octopus aculeatus d'Orbigny, 1834 [10]

Octopus harmandi Rochebrune, 1882

Morphological description

Specimen in live condition was found to exhibit brown body pattern with a pale medial stripe along with white spots, transverse mantle bar, arms with white spots and the ventral side of the body was white whereas the preserved specimen was pale green (Fig.2). The specimen was of medium in size with following specifications, TL- 25cm, HL-0.8cm, ML- 3.5cm, HRL-20cm, SAL-6cm, LASC-150, SASC-72, TW- 50gm. The body pattern of specimen was brown with pale medial stripe, dorsal mantle with white spots, transverse mantle bar, star-like pattern around the eye, suckers in two rows with moderate size. The current study evidenced autotomy and regeneration of VII arm. Autotomy occurred close to base of 7th arm proximal suckers. Arms were unequal in length. Suckers were muscular and flared with thick

scalloped rim and distinct radial cushions.

DNA Barcoding

The PCR Products of COI gene was successfully sequenced. The sequences composed of A= 0.301, C= 0.222, G= 0.146, T= 0.329, A+T= 63.06% and G+C= 36.93%. The sequence alignment was 654bp and the sequence was compared with four different sequences in order to find the genetic variation, if any. Phylogenetic trees were constructed by maximum likelihood and neighbour joining tree by following Kimura-2 parameter depicted in Figures 2 and 3, respectively, for the comparison of genetic similarities. Both the methods showed similar results indicating that the specimen has no genetic divergence with *A. aculeatus* GQ900726 and *A. aculeatus* HM104254 and only a slight variation with *A. aculeatus* AB430514, *A. aculeatus* LC149616. Genetic distance among the individuals was calculated based on Kimura 2-parametre model (Kimura, 1980) [7], genetic distance fell within the range of 0.00 to 0.08 well depicted in Table.1.

Table 1: Summary of mean K2p distance of different Individuals.

S. No	Name of the Specimen	1	2	3	4	5
1	LT604981 <i>Abdopus aculeatus</i> (Andamans, India).					
2	HM104254 <i>Abdopus aculeatus</i> (Australia).	0.00				
3	GQ900726 <i>Abdopus aculeatus</i> (Indonesia).	0.00	0.00			
4	AB430514 <i>Abdopus aculeatus</i> (Japan).	0.07	0.07	0.07		
5	LC149616 <i>Abdopus sp.</i> (USA).	0.08	0.08	0.08	0.08	

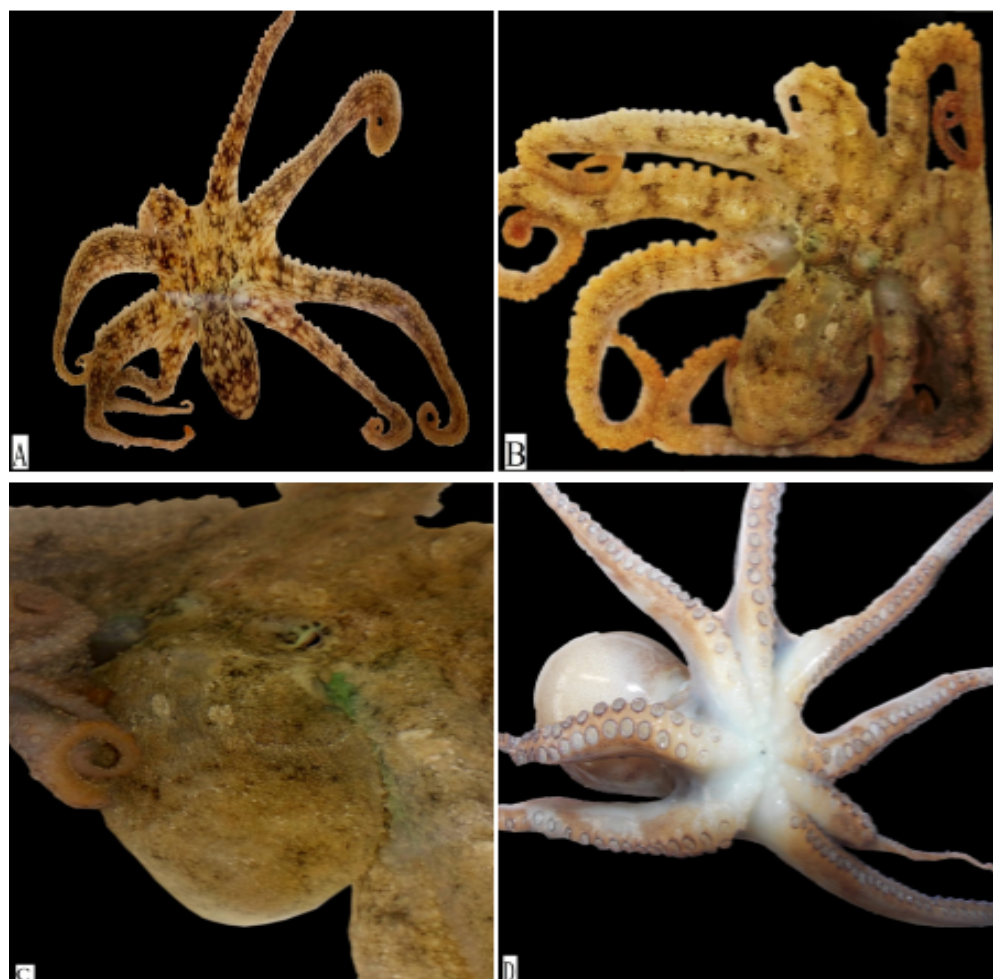


Fig 2: *Abdopus aculeatus* (d'Orbigny, 1834) [10] (A- Dark resting camouflage on dorsal mantle; B- Ochre resting camouflage with transverse mantle bar (dorsal view) ; C-Star like pattern of bars around the eye; D- ventral view and suckers).

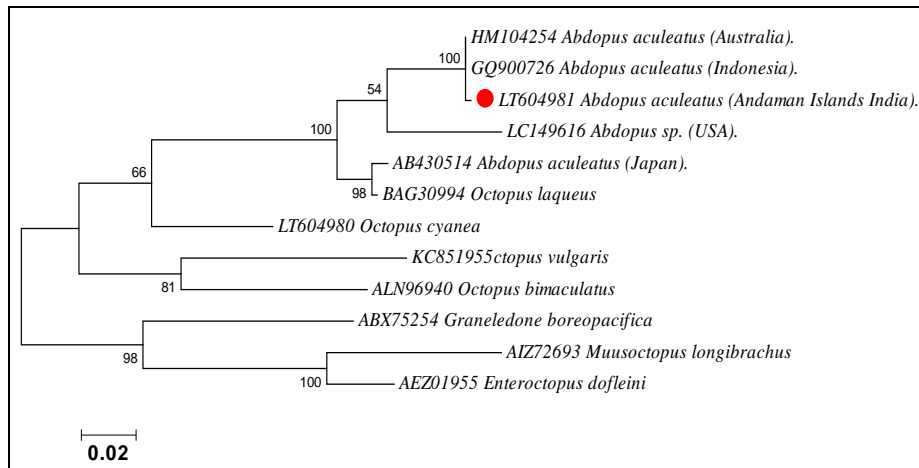


Fig 3: Maximum Likelihood tree NJ using Kimura 2-parameter model derived from analysis of mitochondrial cytochrome oxidase I (COI) gene.

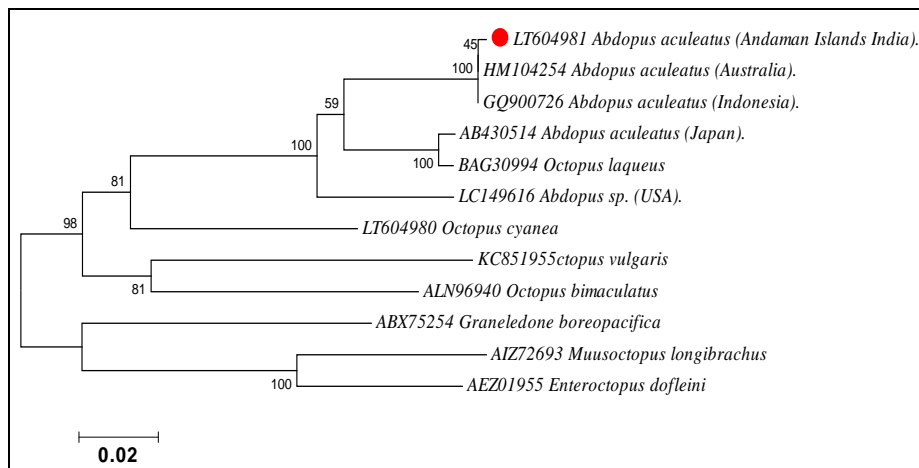


Fig 4: Neighbour-Joining tree (NJ) using Kimura 2-parameter model derived from analysis of mitochondrial cytochrome oxidase I (COI) gene

Discussion

Genus *Abdopus* are intertidal octopuses commonly distributed in Red sea, Phillipines, Tonga Islands, NE Australian region named as *Abdopus abaculus* (Norman & Sweeney, 1997) [9], *Abdopus aculeatus* (d'Orbigny, 1834) [10], *Abdopus capricornicus* (Norman & Finn, 2001) [8], *Abdopus horridus* (d'Orbigny, 1826), *Abdopus tenebricus* (Smith, 1884) [17], *Abdopus tonganus* (Hoyle, 1885) [4], *Abdopus undulates* (Huffard, 2007) [5]. There are about 7 species of the genus *Abdopus* reported worldwide and the occurrence of *Abdopus horridus* was observed in Andaman waters (Venkatraman *et al.* 2004) [23]. The current study reports the presence of *Abdopus aculeatus* from Indian waters adding one more species to *Abdopus* genus from the intertidal habitat. The morphology of the studied specimen was found to resemble closely to *Abdopus aculeatus* (d'Orbigny, 1834) [10] described by Huffard (2007) [6] and Norman and Finn (2001) [8] but the number of suckers were found to be less (150) when compared to the specimen described by Norman and Finn (2001) [8].

DNA barcoding studies of cytochrome oxidase I (COI) gene of *Abdopus aculeatus* LT604981 revealed its genetic similarity with *A. aculeatus* HM104254 (Australia), and with *A. aculeatus* GQ900726 (Indonesia) and slight variance with *A. aculeatus* AB430514 (Japan) and *A. aculeatus* LC149616 (USA) with genetic distances ranging from 0.00 to 0.08 respectively within the genera. The present specimen *Abdopus*

aculeatus LT604981 was genetically compared with other species of its family like *O. Vulgaris*, *O. Laqueus*, *O. bimaculatus* and out group family species like *Graneledone boreopacifica*, *Muusoctopus longibrachus*, *Enteroctopus dofleini*, maximum likelihood phylogenetic tree showed that *Abdopus aculeatus* LT604981 belongs to the family Octopodidae and its genetic similarity with the other members of its family members and dissimilarity with other family. The present study shows that *A. aculeatus* is a sister taxon of *Octopus laqueus* (Fig. 2 & 3) and the morphological and molecular studies confirms the studied specimen as *Abdopus aculeatus* LT604981.

Acknowledgement

The authors express their sincere thanks to Pondicherry University for providing the infrastructure and for the University fellowship awarded to Suneel kumar Yalla.

References

1. Jereb P, Roper CFE, Norman MD, Finn JK. Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Volume Octopods and Vampire squids. FAO species catalogue for fishery purposes. 2014; 4(3):370. Rome, FAO.
2. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan

- invertebrates. *Molecular Marine Biology and Biotechnology*, 1994; 3:294-299.
3. Guzik MT, Norman MD, Crozier RH. Molecular phylogeny of the benthic shallow-water octopuses (Cephalopoda: Octopodinae). *Molecular Phylogenetic and Evolution*, 2005; 37:235-248.
 4. Hoyle WE. Diagnosis of new species of Cephalopoda collected during the cruise of HMS. "Challenger" - I. The Octopoda. *Annals and Magazine of Natural History*, 1885; 5:222-236.
 5. Huffard CL. Ethogram of *Abdopus aculeatus* (d'Orbigny, 1834) (Cephalopoda: Octopodidae): Can behavioural characters inform octopodid taxonomy and systematics? *Journal of Molluscan Studies*. 2007; 73:185-193.
 6. Huffard CL. Four new species of shallow water pygmy octopus (Mollusca: Cephalopoda) from the Kingdom of Tonga. *Molluscan Research*, 2007; 27:147-170.
 7. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 1980; 16:111-20.
 8. Norman MD, Finn Jk. Revision of the *Octopus horridus* species- group, including erection of a new subgenus and description of two member species from the Great Barrier Reef, Australia. *Invertebrate Taxonomy*, 2001; 15:13-35.
 9. Norman MD, Sweeney MJ. The shallow-water octopus (Cephalopoda: Octopodidae) of the Philippines. *Invertebrate Taxonomy*, 1997; 11:89-140.
 10. Orbigny MAd. Voyage dans l' Amerrique meridionale, t. V. Mollusques. In 'Histoire naturelle generale et particuliere Cephalopodes Acetabuliferes vivants et fossiles'. (Eds M. M. Ferussac and M.A. d'Orbigny.) 1834; ix +361. (J.B.Bailliere: Paris).
 11. Orbigny MAd. Tableau methodique de la classe des Céphalopodes: *Annales des Sciences Naturelles*, sér. 1826; 1(7):245-314.
 12. Robson GC. A monograph of the Recent Cephalopoda, Part I: Octopodinae. *British Museum (Natural History)*, London, 1929, 236.
 13. Roper CFE, Voss GL. Guide- lines for taxonomic descriptions of cephalopod species. *Memoirs of the National Museum of Victoria*, 1983; 44:49-63.
 14. Roper CFE, Hochberg FG. Behavior and systematics of cephalopods from Lizard Island, Australia, based on color and body patterns. *Malacologia*, 1988; 29:153-193.
 15. Roper CFE, Sweeney MJ, Nauen CE. FAO species catalogue. Volume 3, Cephalopods of the world: An annotated and illustrated catalogue of species of interest of fisheries. *FAO Fisheries Synopsis*, 1984; 125:1-277.
 16. Salah M, Aljanabi, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*. 1997; 25:4692-4693
 17. Smith EA. Mollusca Report on the zoological collections made in the indo-pacific Ocean during the voyage of HMS 'Alert' *British Museum*, London, 1881-1884; 2:34-116.
 18. Strugnell J, Norman M, Jackson J, Drummond AJ, Cooper A. Molecular phylogeny of coleoid cephalopods (Mollusca: Cephalopoda) using a multigene approach; the effect of data partitioning on resolving phylogenies in a Bayesian framework. *Molecular Phylogenetics and Evolution*, 2005; 37:426-441.
 19. Sundaram S. Octopus Fishery off Indian NW (Maharashtra) Coast. *Fishing Chimes*. 2010; 30:43-45.
 20. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 1993; 10:512-526.
 21. Tamura K, Stecher G, Peterson D, Filipksi A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0 *Molecular Biology and Evolution*, 2013, 10, 1093/molbev/mst197 Advance.
 22. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTALX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 1997; 24:4876-4882.
 23. Venkataraman K, Jeyabaskaran R, Raghuram KP, Alfred JRB. Bibliography and Checklist of Corals and Coral Reef Associated Organisms of India. *Records of The Zoological Survey of India Occasional*. 2004; 226:1-468.