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# FIRST RECORD OF THE SEA ANEMONE *ANTHOPLEURA BUDDEMEIERI* FAUTIN (CNIDARIA: ACTINIARIA: ACTINIIDAE) FROM THE INDIAN COAST

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**Abstract:** We report the presence of an intertidal sea anemone *Anthopleura buddemeieri* Fautin, 2005 in Indian coast, indicating the extended distribution of this species to Western Indian Ocean. A total of 22 specimens of the species were collected from the rocky shore intertidal habitat of Varkala, southwest coast of India. The sequence data of the mitochondrial gene cytochrome c oxidase 1 (CO1) of the species was also generated during the study.

***Key words:*** Actiniidae, Kerala, Indian Ocean, phylogeny, mitochondrial DNA, distribution

## INTRODUCTION

The sea anemones of India were documented from various coastal and marine habitats of India (Annandale, 1907, 1915; Carlgren, 1925; Panikkar, 1936, 1937a-c, 1939; Parulekar, 1967, 1968, 1969a, b, 1971, 1990; Seshaiya and Cuttress, 1969; Hartog and Vennam, 1993; Madhu and Madhu, 2007; Raghunathan *et al.*, 2014) and the recorded diversity is represented by 54 species belonging to 40 genera and 20 families and majority of the records are from the west coast (Raghunathan *et al.*, 2014). Of late, species such as *Bunodosoma goanense* den Hartog & Vennam, 1993, *Pelocoetes exul* (Annandale, 1907), and *Stephensonactis ornata* Panikkar, 1936, previously known only from Indian subcontinent, were recorded from Singapore, indicating extended distribution of sea anemones in the Indo-Pacific. (Fautin *et al.*, 2015). In this paper we report the presence of an intertidal sea anemone *Anthopleura buddemeieri* in Indian coast, indicating the extended distribution of this species to Western Indian Ocean and provide the sequence data of the mitochondrial gene cytochrome c oxidase 1 (CO1) of the species.

## MATERIALS AND METHODS

Twenty two specimens of sea anemones were collected from the rocky intertidal areas at Varkala beach ($8^0$43’57" N; $76^0$42’20" E), southwest coast of India. All the specimens were collected from the high tide line and were attached firmly to the rock crevices. Some of the animals were photographed *in situ* and others were photographed live in the lab. The animals were fixed in either 5 per cent sea water formalin or in ethanol. The current classification of sea anemone and the taxonomic status of species were cross-checked with the electronic database of Fautin (2011). The voucher specimens are deposited at the Western Ghats Regional Centre of Zoological Survey of India (ZSI/WGRC/IR/V.2549) and at the museum collections of the Department of Aquatic Biology and Fisheries, University of Kerala, India (UOK/AQB/CNI/01-08). Total DNA was extracted from the tentacles and disc of *A. buddemeieri* using DNeasy Blood and Tissue Kit (QIAGEN). The extracted DNA acted as a template for Polymerase Chain Reaction (PCR). Mitochondrial gene cytochrome c oxidase 1 (CO1) was amplified using the universal primers [LCO1490(5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994) in a 25 µl reaction volume with *Taq* PCR master mix (QIAGEN) using the thermal cycler (Eppendorf). The PCR condition included, hot start with 94°C for 1 min, 5 cycles of 94°C for 30 s, annealing at 45°C for 40 s and extension at 72°C for 1 min, 35 cycles of 94°C for 30 s, 51°C for 40 s and final extension at 72°C for 10 min. Polymerase chain reaction products were purified for sequencing with ExoSAP-IT (USB) and sequenced in forward

and reverse direction with the PCR primers by Dideoxy Sanger standard method with BigDye™ Dye Terminator (Applied Biosystems) on an ABI sequencer. The resulting sequence was edited and aligned with BioEdit v.7.0.9.0. (Hall, 1999). Species comparison of the candidate sequence to the most simialr sequences was carried out with the available one from GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Sequence was submitted to the NCBI GenBank in Sequin format according to NCBI's procedure. Phylogenetic analysis and sequence divergence were estimated using the Kimura 2-Parameter distance model of MEGA (Version 6.0) Package (Tamura *et al*., 2013). Maximum likelihood tree was constructed by 1000 bootstrap replicates to provide percentage bootstrap values for branch points. Sequence data are available on GenBank (Acc. No. KM259983).

## RESULTS AND DISCUSSION

SYSTEMATICS

Order ACTINIARIA Hertwig, 1882

Suborder NYNANTHEAE Carlgren, 1899

Infraorder THENARIA Carlgren, 1899

Family ACTINIIDAE Rafinesque, 1815

Genus *Anthopleura* Duchassaing de Fombressin and Michelotti, 1860

***Anthopleura buddemeieri* Fautin, 2005 (Fig. 1 a-c)**

The animal is brown towards the oral disc region, fainter towards the column. The colouration at the bottom and basal disc region appears light brown to fainter orange in live condition. Smaller specimens tan to grey. An important character is the presence of red/pink spots scattered along the column. Size of the spots varies among individuals. In adults, larger spots are visible towards the upper part of the column, which is more prominent and brightly coloured. Spots beneath the oral disc are larger and circular, and size of the spots gradually decreases from top to bottom. Spots at the basal parts are small, irregular and unevenly scattered. The spots at their centre bear verrucae located only at the distal parts. Column tapering towards the bottom, when contracted distal part typically narrows. When retracted animal appears dome- shaped. Pedal discs wider and modified to attach to the cervices on rocks. Oral disc and tentacles vividly coloured and the colouration varies from red, orange, shades of yellow and grey. Tentacles up to 100 and arranged towards the rim of oral disc in three concentric circles.

The anatomical features and morphology of cnidae are in agreement with the original description provided by Fautin (2005).

Measurements: Column length 6.34–18.86mm; column diameter 3.12-10.44 mm; oral disc diameter 4.19-6.95mm; basal disc diameter 4.84-9.55mm.

DNA barcoding: No stop codon, insertions or deletions were found in the amplified sequence, showing that it constitutes functional mitochondrial COI sequence. The amplified sequence (651 bp) was larger than 600-bp, the limit typically observed for NUMTs (nuclear DNA sequences originating from mtDNA). The nucleotide frequency was A (23.35%), C (19.66%), G (23.50%) and T (33.49%). Blast analysis showed 94% similarity with *Anthopleura elegantissima* available in the GenBank (GU443180, GU443181, GU443182 and AF480931), reveals the possibility of the same genera. According to maximum likelihood analysis, the sequence of *A. buddemeieri* of the present study is well clustered to the clade of *A. elegantissima* (Fig. 2). Based on the sequence divergence, sequence of *A. buddemeieri* has lowest genetic distance with *A. elegantissima* (0.027%) followed by *Urticinopsis antarctica* (0.035%) and *Urticina* sp. (0.039%).

*Anthopleura* Duchassaing de Fonbressin & Michelotti, 1860 is a well-known and widely distributed genus of sea anemone with 46 valid species (Fautin, 2011). The species of *Anthopleura* reside in intertidal areas and are commonly used in ecological and ethological research because of their local abundance and diversity (Daly, 2004; Ayre and Grosberg, 2005). The valid species recorded from Indian coastal waters representing the genus include *Anthopleura anjunae* Den Hartog & Vennam, 1993, *Anthopleura asiatica* Uchida & Muramatsu, 1958, *Anthopleura handi* Dunn, 1978, *Anthopleura midori* Uchida & Muramatsu, 1958, *Anthopleura nigrescens* (Verrill, 1928) and *Anthopleura panikkarii* Parulekar, 1968 (Parulekar, 1981, 1990; Tikadar *et al*., 1986; Rao, 1991; Hartog and Vennam, 1993; Datta *et al*., 2008; Raghunathan *et al*., 2014).

This species was first described by Fautin (2005) from Fiji and Papua New Guinea and opined that

this species may be widespread in the tropical western Pacific. Subsequently this species was recorded from Queensland, Australia (Fautin *et al.*, 2008a) and Singapore (Fautin *et al.*, 2008b). This is the first record of *A. buddemeieri* from Indian coast and presence of this species confirms the extended distribution of this species towards the western Indian Ocean. The mitochondrial gene CO1 sequence generated for *A. buddemeieri* clustered with similar sequences of the genus *Anthopleura*. Since the similar sequence of the *A. buddemeieri* is not yet available in the NCBI database, the sequence data generated by us can be used as a baseline data and helpful for researchers in future for identification of *A. buddemeieri* using DNA barcoding. Though mitochondrial CO1 gene is recommended as the universal and standard barcoding marker for most animals (Hebert *et al*., 2004; Radulovici *et al.*, 2010), this will not be fully effective for all anthozoan cnidarians (see McFadden *et al.*, 2011), and therefore use of multiple genes are always recommended for anthozoans for arriving at taxonomic conclusions.

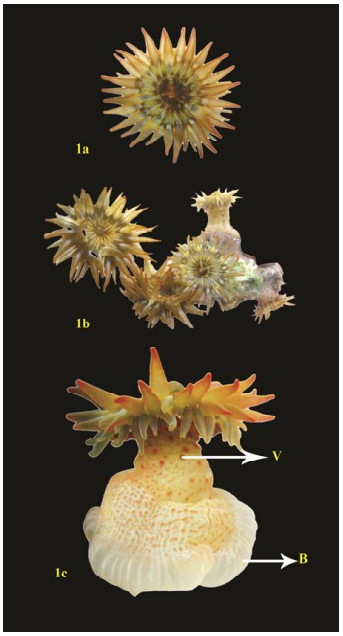


**Fig. 1.** *Anthopleura buddemeieri* Fautin 2005: (a) oral disc with 3 rows of tentacles; (b) colony of sea anemones; (c) live animal showing column, verrucae (v) and basal disc (b)

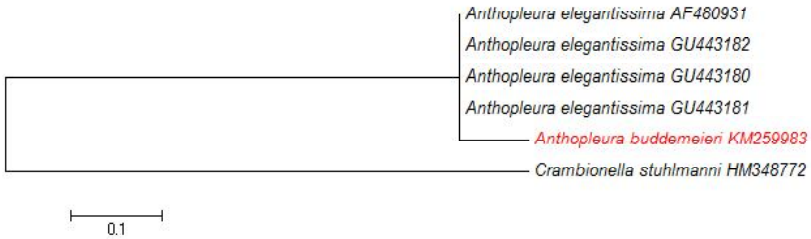


**Fig. 2.** Maximum Likelihood tree of CO1 sequences of species from the genus *Anthopleura*. Red indicates the specimen of the present study

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