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CONFIRMED REPORT OF *APLYSIA ARGUS* RÜPPELL & LEUCKART, 1830 (MOLLUSCA: OPISTHOBRANCHIA: APLYSIIDAE) FROM LAKSHADWEEP, WITH NOTES ON ITS TAXONOMY IN INDIA

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Abstract: The black-ringed *Aplysia* was reported in Indian taxonomic records as *Aplysia dactylomela* Rang, 1828. This paper confirms that the Indian species records of *A. dactylomela* represents *Aplysia argus* Rüppell & Leuckart, 1830, based on morpho-molecular approach.

Key words: Sea slugs, sea hare, Lakshadweep, Cytochrome oxidase I

INTRODUCTION

Aplysids, commonly known as sea hares, are typically large, soft and fleshy bodied heterobranch sea slugs with a pair of broad, blunt oral tentacles, enrolled rhinophores, a pair of lateral tough leathery parapodia, and often a defensive ink gland and an opaline gland. These animals have been recognized in the literature for centuries, with the first verifiable description of a member of the genus *Aplysia* attributed to Pliny in the first century C.E. (Eales, 1960).

Two of the most easily recognizable members of *Aplysia* are the species *Aplysia dactylomela* Rang, 1828 and *Aplysia argus* Rüppell & Leuckart, 1830, both of which possess characteristic black rings on the body. *Aplysia dactylomela* was thought to be a circumtropical species with a broad range in tropical and temperate coastal regions all around the world (Eales, 1960; Bebbington, 1974; Bebbington, 1977), including the Indo-Pacific and the tropical Eastern Pacific (Farran, 1905; Rudman, 1999; Yonow, 2008; Gosliner *et al.,* 2008; Gosliner *et al.,* 2015), the eastern and western Atlantic Ocean (Ortea and Martínez, 1990; Rudman, 1999; Cervera *et al.,* 2004; Valdés *et al.,* 2006), South Africa (Bebbington, 1974), and more recently from several localities in the eastern Mediterranean (Crocetta and Galil, 2012). However, molecular and morphological data revealed that *A. dactylomela* constituted two genetically distinct species, with *A. argus* Rüppell and Leuckart (1828) distributed in the tropical Indo-Pacific, and *A. dactylomela* in the Atlantic Ocean and Mediterranean (Alexander and Valdes, 2013; Valdes *et al.,* 2013).

A review of published work in India shows various records assigned to *A. dactylomela* from several parts of the country such as Tamil Nadu (Satyamurthi, 1952; Sundaran, 1969; Rao, 2003; Balaji *et al.*, 2012; Gopalakrishnan *et al.*, 2012; Venkataraman *et al.*, 2015), Lakshadweep (Apte, 2009; Venkataraman *et al.*, 2015), Gujarat (Narayanan, 1969; Rao, 2003; Apte *et al.,* 2010; Subburaman, 2014; Venkataraman *et al.*, 2015), Andaman and Nicobar Islands (Rao, 2003; Venkataraman *et al.,* 2004; Venkataraman *et al.*, 2015) and from unspecified regions (Apte, 2012; Venkataraman *et al.*, 2012). However, these records have never been verified with molecular data. In this paper we confirm the identity of *Aplysia argus* from Lakshadweep using mitochondrial sequence data.

MATERIALS AND METHODS

Live specimens (40–100 mm) were collected in Agatti, Amini, Androth, Bitra, Chetlet, Kadmat, Kalpeni, Kavaratti, Kilton and Minicoy islands of

Lakshadweep. The specimens were collected from rocky intertidal area and sea grass bed from a depth of 0.2–2 m by hand picking and snorkeling during low tide. The specimens are deposited in the museum collections of the Department of Aquatic Biology and Fisheries, University of Kerala, India (Accession numbers DABF-UOK/GAS 1147-1150). The specimens were photo documented live and tissue samples from the foot region were dissected and preserved in 99% alcohol for molecular analysis. The DNA from one tissue sample was extracted using DNeasy blood & tissue kit of Qiagen (Qiagen, Hilden, Germany). The mitochondrial gene, Cytochrome coxidasesubunit I (COI) was amplified by polymerized chain reaction (PCR) using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198(5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994). The reactions were carried out in a 25 µl reaction volume with Taq PCR master mix (QIAGEN, Hilden, Germany) using the gradient thermal cycler (Eppendorf, Hamburg, Germany).The PCR products were visualized on 1% agarose gels and the most intense products were selected for sequencing. For sequencing the PCR reaction products were purified with USB ExoSAP-IT (Affymetrix Inc., Santa Clara, USA) and sequenced in forward and reverse direction with the PCR primers by the Dideoxy Sanger standard method. Sequencing was performed using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems Inc., Foster City, USA) on an ABI sequencer (Applied Biosystems Inc., Foster City, USA). The edition and alignment of the resulting sequences were performed with BioEdit v.7.0.9.0. (Ibis Biosciences, Carlsbad, USA., Hall, 1999).

The single COI sequence obtained from India was aligned with other sequences of *A. argus* and *A. dactylomela* obtained from GenBank using the software Pro R8 (Kearse *et al*., 2012). Pairwise TrN distances among all specimens were calculated in MEGA 7.0.16 (Kumar *et al.,* 2016). The geographic distribution and structure of haplotypes was visualized by producing a haplotype network using the program PopArt v. 1.7 (Leigh and Bryant, 2015) using the TCS option. Haplotype were color coded by geographic region.

RESULTS AND DISCUSSION

***Aplysia argus* Rüppell & Leuckart, 1830** (Fig. 1)

Phylum: Mollusca
Class: Gastropoda
Subclass: Heterobranchia
Infraclass: Opisthobranchia
Order: Anaspidea
Superfamily: Aplysioidea
Family: Aplysiidae
Synonym: *Aplysia pulmonica* Gould, 1852
Distribution: Widely along Indo-Pacific
Materials Collected: Lakshadweep: Agatti (10°51'N 70°10'E), Amini (11°07N 75°44E), Androth (10°49'N73°38'E), Bitra (11°35'N 72°11'E), Chetlet (10°40'N 72°42'E), Kadmat (11°10'N 72°45'E), Kalpeni (10°04'N 73°38'E), Kavaratti (10°33'N 72°37'E), Kilton (08°18'N 73°03'E) and Minicoy islands (08°19'N 73°04'E).
Size: 40 mm to 112 mm
Habitat: Intertidal rocky areas and sea grass beds upto 0.2–2 m depth.

Identification: Body large, fleshy, yellowish green with numerous scattered white flecks. Network of reticulating brown lines interconnecting to form irregular black rings with white centres on the sides of the body. The rings vary in size and thickness. Parapodial margins highly convoluted. Black rings on the inner surface of parapodia. Internal shell present inside the mantle. Shell small, light yellowish with central calcified region and flexible periphery. Head elongate with large, broad and folded oral tentacles. Rhinophores rolled. A characteristic black patch on the tip of the tail.

Taxonomic notes: This species is identified and listed *Aplysia dactylomela* Rang, 1828 in several records from India, including Tamil Nadu (Satyamurthi, 1952; Sundaran, 1969; Rao, 2003; Balaji *et al*., 2012; Gopalakrishnan *et al*., 2012; Venkataraman *et al*., 2015), Gujarat (Narayanan, 1969; Rao, 2003; Apte *et al*., 2010; Subburaman, 2014; Venkataraman *et al*., 2015), Andaman and Nicobar Islands (Rao, 2003; Venkataraman *et al.,* 2004; Venkataraman *et al*., 2015), Lakshadweep (Apte, 2009; Venkataraman *et al*., 2015), and from unspecified regions (Apte, 2012; Venkataraman *et al*., 2012).

DNA barcoding

Alexander & Valdés (2013) restricted *Aplysia dactylomela* to the Atlantic Ocean and *A. argus* to the Indo-Pacific. The haplotype network (Fig. 2)

Table 1. TrN genetic distance among sequences of *A. dactylomela*(*) and *A. argus*(**), including the single sequence from India, calculated in MEGA 7. The analysis involved 25 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 599 positions in the final dataset.

India**	–																							
KF028649 Reunion**	0	–																						
KF028650 Reunion**	0.005	0.005	–																					
JX560142 Hawaii**	0.005	0.005	0.007	–																				
JX560150 Hawaii**	0.003	0.003	0.005	0.005	–																			
HQ834119 China**	0.003	0.003	0.005	0.005	0.003	–																		
KF041209 Turkey*	0.067	0.067	0.069	0.069	0.067	0.063	–																	
KF041210 Turkey*	0.067	0.067	0.069	0.069	0.067	0.063	0	–																
KF041208 Turkey*	0.067	0.067	0.069	0.069	0.067	0.063	0	0	–															
KF041211 Turkey*	0.067	0.067	0.069	0.069	0.067	0.063	0	0	0	–														
KF028652 Italy*	0.067	0.067	0.069	0.069	0.063	0.063	0.003	0.003	0.003	0.003	–													
KF028651 Italy*	0.067	0.067	0.069	0.069	0.067	0.063	0	0	0	0	0.003	–												
KF028654 Italy*	0.067	0.067	0.069	0.069	0.067	0.063	0	0	0	0	0.003	0	–											
KF028655 Italy*	0.071	0.071	0.073	0.073	0.067	0.067	0.007	0.007	0.007	0.007	0.003	0.007	0.007	–										
KF028653 Italy*	0.063	0.063	0.065	0.065	0.059	0.059	0.021	0.021	0.021	0.021	0.017	0.021	0.021	0.021	–									
KF028656 Italy*	0.063	0.063	0.065	0.065	0.059	0.059	0.021	0.021	0.021	0.021	0.017	0.021	0.021	0.021	0	–								
KF028657 Greece*	0.063	0.063	0.065	0.065	0.059	0.059	0.007	0.007	0.007	0.007	0.003	0.007	0.007	0.007	0.017	0.017	–							
KF028658 Greece*	0.061	0.061	0.063	0.063	0.057	0.057	0.012	0.012	0.012	0.012	0.008	0.012	0.012	0.012	0.008	0.008	0.012	–						
KF028659 Greece*	0.061	0.061	0.063	0.063	0.057	0.057	0.012	0.012	0.012	0.012	0.008	0.012	0.012	0.012	0.008	0.008	0.012	0	–					
KF028660 Canary Is*	0.061	0.061	0.063	0.063	0.057	0.057	0.022	0.022	0.022	0.022	0.019	0.022	0.022	0.022	0.002	0.002	0.019	0.01	0.01	–				
JX560143 Jamaica*	0.061	0.061	0.063	0.063	0.057	0.057	0.012	0.012	0.012	0.012	0.008	0.012	0.012	0.012	0.008	0.008	0.012	0	0	0.01	–			
JX560144 Bahamas*	0.069	0.069	0.071	0.071	0.069	0.065	0.002	0.002	0.002	0.002	0.005	0.002	0.002	0.008	0.023	0.023	0.008	0.014	0.014	0.024	0.014	–		
JX560145 Bahamas*	0.061	0.061	0.063	0.063	0.057	0.057	0.023	0.023	0.023	0.023	0.019	0.023	0.023	0.023	0.002	0.002	0.019	0.01	0.01	0.003	0.01	0.024	–	
AF343427 Florida*	0.067	0.067	0.069	0.069	0.063	0.063	0.017	0.017	0.017	0.017	0.014	0.017	0.017	0.017	0.014	0.014	0.017	0.005	0.005	0.015	0.005	0.019	0.015	–
DQ991927 Florida*	0.059	0.059	0.061	0.061	0.055	0.055	0.017	0.017	0.017	0.017	0.014	0.017	0.017	0.017	0.014	0.014	0.017	0.005	0.005	0.015	0.005	0.019	0.015	0.007

Fig. 1. *Aplysia argus* Rüppell & Leuckart, 1830 in live

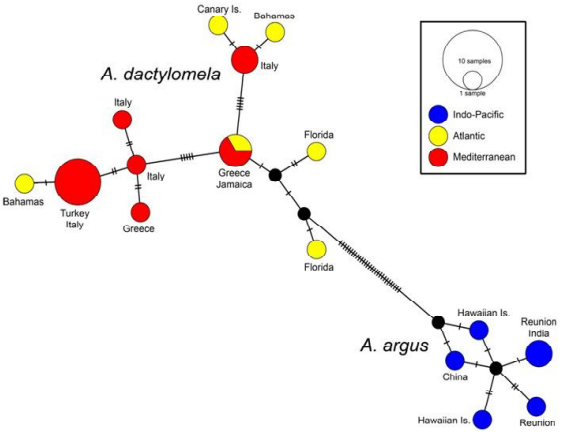

Fig. 2. Haplotype network with the geographic origin of the sequences color coded. Circles are proportional to the number of haplotypes. Black circles correspond to hypothetical haplotypes not detected in the sample. Transverse lines indicate mutational substitutions.

confirmed that the single sequence from India clusters with other Indo-Pacific specimens identified as *A. argus* and therefore this animal is here confidently assigned to this species. The sequence from India is identical to one of the sequences from Reunion Is. (Indian Ocean) obtained by Valdés *et al*. (2013) (Table 1; Fig. 2), however the sample size is too small to determine whether there is any genetic structure within *A. argus*. Available evidence indicates that *A. argus* contains substantially lower levels of genetic diversity than *A. datylomela* (Table 1), even though its geographic range (from the Indian Ocean to the Hawaiian Islands) is much larger.

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