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## SHORT COMMUNICATION

A NEW SPECIES OF SEA SQUIRT RHOPALAEA BILOBATA (ASCIDIACEA: DIAZONIDAE) FROM THE ANDAMAN ISLANDS, INDIA

Jhimli Mondal, C. Raghunathan & Tamal Mondal

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# A NEW SPECIES OF SEA SQUIRT *RHOPALAEA BILOBATA* (ASCIDIACEA: DIAZONIDAE) FROM THE ANDAMAN ISLANDS, INDIA

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**Abstracts:** *Rhopalaea bilobata*, a new species of the class Ascidiacea, under the family Diazonidae has been described from the Andaman Islands of Andaman & Nicobar, India. The species was found in sandy bottoms, reef areas and artificial structures at a depth range of 10–40 m. This species has bilobed anal border, six lobed branchial and atrial siphons, transparent thorax with pigmented spots at the anus, between the two siphons and at the anterior end of endostyle, and ramified basal test. The species is closely related to *R. idoneta, R. macrothorax* and *R. tenuis* in several sets of anatomical and morphological features.

**Keywords:** Anal border, Andaman & Nicobar Islands, Ascidians, Rhopalaea, Tunicata.

Ascidians are invertebrates, sister group to vertebrates (Satoh 2009), that belong to the class Ascidiacea under the subphylum Tunicata. These saclike sessile animals are exclusively marine in nature. About 3000 species of ascidians are found worldwide. The family Diazonidae is recently placed under the order Aplousobranchia (Shenkar et al. 2016) in which the genus *Rhopalaea* belongs. Presently, the genus has 20 accepted species across the world's ocean (Shenkar et al. 2017). Most of the species belonging to this genus are reef associated though some can also be found on artificial reefs (Shenkar 2008, 2013). A total of 11

species under the genus are solitary and nine species are colonial but those do not form the large colonies like the species that belong to the genus *Diazona* (Shenkar 2013). Andaman & Nicobar Islands have extensive reef ecosystems in the continental shelf with a wide range of species abundance. A total of 65 species of ascidians are reported till date from the reefs of these islands (Venkataraman et al. 2012; Ananthan 2014; Ananthan et al. 2015; Mondal et al. 2015, 2017). The present paper describes one new species of *Rhopalaea* and gives its distribution in the Andaman Islands.

#### MATERIALS AND METHODS

Specimens were collected by Self Contained Underwater Breathing Apparatus (SCUBA) diving. The surveys were conducted during October 2013 to December 2015 in various locations of the Andaman Islands (Fig. 1). Specimens were defecated with magnesium sulphate crystals ( $MgSO_4$ · $7H_2O$ ) and then narcotized with menthol crystals, following Meenakshi et al. (2003). After narcotization, specimens were preserved with 4% formaldehyde in seawater. Dissection was carried out under Labomed CZM4 microscope and digitization of detailed anatomy was carried out

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#### Rhopalaea bilobata sp. nov. from Andaman Islands

under Leica M205A DFC 500 stereo zoom microscope. Identification and comparison of anatomical as well as morphological features of the specimens followed by Kott (1990, 2006), Shenkar (2013), Monniot & Monniot (2001), Tokioka (1953, 1971), Monniot (1997), Sluiter (1904), Van Name (1921), Herdman (1880), Kott & Goodbody (2008), Vazquez & Young (1996), and Pérès (1958). The identified specimens were registered in National Zoological Collections and deposited at Zoological Survey of India, Port Blair.

#### RESULTS

Class: Ascidiacea Blainville, 1824 Order: Phlebobranchia Lahille, 1886 Family: Diazonidae Seeliger, 1906 Genus: *Rhopalaea* Philippi, 1843

### Rhopalaea bilobata sp. nov. (Images 1–5)

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#### Material examined

Holotype: ZSI/ANRC-13703, collection of one specimen was made on 22.x.2013 from Wall area (12°03.313'N & 92°57.730'E) located at Havelock Island, South Andaman at 30m depth (Fig. 1), Andaman & Nicobar Islands, India.

Syntypes: ZSI/ANRC-13704. Two specimens were collected on 22.x.2013 from the same location and depth of the holotype specimen and deposited in NZCs after detailed anatomical studies.

Paratypes: ZSI/ANRC-13705 & 13706. One specimen was collected on 16.xii.2013 from Trilby Island (13°24.812'N & 93°04.146'E) of North and Middle Andaman at 18m depth, while one specimen was collected from Pongibalu (11°30.958'N & 92°39.201'E) of South Andaman at 10m depth (Fig. 1).

#### Etymology

The species bears distinctive triangular bilobed anal border.

#### Description

External morphology: Zooids are usually solitary (Image 1a–d), sometimes found in pairs (twice noticed underwater) but do not form large colonies. Zooids are finger like with soft translucent tunic and about 3.5cm (specimens not fully relaxed) - 5.6cm long. As in other species of *Rhopalaea* only the thorax is visible underwater and the abdominal part is concealed in the crevices or sand. Some specimens were collected from artificial



Figure 1. Study areas of Andaman Islands.

substrate like tires and settlement plates.

The tunic delicate anteriorly (Image 2a) but became firm posteriorly (Image 2b), with round horizontal wrinkles on the surface. The tunic of the thoracic region free of epibionts. Basally the tunic produces some branched root-like extensions to adhere to the substrate, sometimes sand embedded in those branches and hard to remove (Image 2c,d). Both branchial and atrial apertures situated at the free anterior end, each with six lobes. Yellow dots present between siphons lobes. Specimens in live condition with a transparent test with a purple circular ring surrounding the branchial siphon and interrupted with ventral and dorsal yellow patches, covering the dorsal tubercle and the anterior end of the endostyle. Along with the endostyle, a yellow line found. Another yellow patch found surrounding the rectum under the anal border. Although, no colours persisted in the preservatives, traces found. A pinkish abdomen (colour faded in the preservatives) clearly seen through the test situated entirely posterior to the pharynx.

Internal structures: Large thorax gradually narrows posteriorly and a narrow oesophageal neck with a club shaped abdomen. One syntype specimen not fully relaxed, the thorax 0.5cm long and abdomen 0.8cm long. In relaxed specimens thorax 1.3–2 cm long and the abdomen 1–1.2 cm. The oesophageal neck very fragile and broke in most of the cases during dissection. Thorax with 10–14 longitudinal muscle bands on each side which arise from both siphons (Image 3a–c). Longitudinal muscles arising from the siphons, run half way of the pharynx and ramify towards the ventral and dorsal border: 6–7 longitudinal muscle bands arise from the branchial siphon and 3–5 from the atrial siphon

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Image 1. *Rhopalaea bilobata* sp. nov. a, b, c & d - in situ image of the species (arrows indicating the atrial siphon). © Tamal Mondal.



Image 2. *Rhopalaea bilobata* sp. nov. a - Thorax enclosed in test; b - Gut loop enclosed in test; c & d - Root like extensions of the test. (Scale -5mm). © Jhimli Mondal.

and 1–2 muscle bands from the region in between the siphons. The branchial siphon with 9–11 circular muscles and the atrial siphon about six (Image 3d). The dorsal tubercle aperture slit-like (slightly curved) in structure (Image 4a). Oral tentacles of two sizes and 18–22 long tentacles alternate with smaller ones.



Image 3. *Rhopalaea bilobata* sp. nov. a, b & c - Thorax with the musculature (Scale - 5mm; 7.80x); d - Musculature of branchial aperture (Scale - 2mm; 11.8x). © Jhimli Mondal.



Image 4. *Rhopalaea bilobata* sp. nov. a - Dorsal tubercle (Scale - 200µm; 95.4x); b - Branchial papillae (Scale - 200µm; 132x); c - Dorsal languets (Scale - 500µm; 41.3x); d - Stigmata (Scale - 200µm; 91.0x). © Jhimli Mondal.

28–32 internal longitudinal vessels present on each side and their number decreases (15–17) towards the oesophagus. Longitudinal vessels supported by papillae (Image 4b,c) and no record of secondary papillae. Three stigmata per mesh (Image 4d), in a relaxed specimen 54 rows of stigmata found (holotype) and 45 rows of stigmata in the syntype (not well relaxed). The dorsal

lamina with 34–36 long triangular languets (Image 4c), starting at the third row of stigmata. The anterior portion of the colour patch along the endostyle wider than along the posterior part.

A vertical gut loop present embedded in the test. The gonads enclosed in the gut loop. Rectum long and opens at the  $10^{th}$ – $13^{th}$  row of stigmata ( $13^{th}$  in not relaxed



Image 5. *Rhopalaea bilobata* sp. nov. a & b - Bilobed anal border with yellow ring (Scale - a, 500μm, 42.4x; b, 500μm); c & d - Bilobed anal border and genital papilla (Arrow) (Scale - c, 500μm, 73.3x; d, 500μm, 63.8x). © Jhimli Mondal.

syntype) in the dorsal side. Anal border bilobed with two slightly indented triangular lips (40–65  $\mu$ m) (Image 5a,b). The opening of oviduct just above the pigmented ring on the base of the anus and the sperm duct with slightly elongated papilla projected little above the oviduct but not beyond the anal border (Image 5c,d). Blood vessels of abdominal tunic continue in the ramifications of the root-like projections of the tunic (Image 2d).

### Distribution

India: Havelock Island, Trilby Island and Pongibalu of Andaman Islands.

#### Discussion

A total of 20 species under the genus *Rhopalaea* were recorded across the world's ocean till now while 11 of the species—*R. abdominalis* (Sluiter, 1898), *R. birkelandi* Tokioka, 1971, *R. cloneyi* Monniot & Monniot, 2001, *R. crassa* (Herdman, 1880), *R. flemingi* (Herdman, 1880), *R. idoneta* Shenkar, 2013, *R. macrothorax* Tokioka, 1953, *R. meridionalis* Kott, 2006, *R. neapolitana* Philippi, 1843, *R. perlucida* Monniot, 1997 and *R. tenuis* (Sluiter, 1904)—are solitary, and nine species—*R. circula* Monniot & Monniot, F. 2003, *R. flesca* (Sluiter, 1904), *R. desme* Monniot, F. 2003, *R. fusca* (Herdman, 1880), *R. hartmeyeri* (Salfi, 1927), *R. orientalis* Pérès, 1958, *R. piru* Monniot & Monniot, 1987, *R. respiciens* Monniot, 1991, *R. sagamiana* Oka, 1927—are colonial (Shenkar,

2013). *Rhopalaea bilobata* sp. nov. has a remarkable resemblance to *R. idoneta* and *R. macrothorax*. The similarity with *R. idoneta* includes the transparent thorax, branching structures for fixation and similar anus shape. The differences include a colourless thorax, the muscular pattern, 4–5 stigmata per mesh, the position of the anus and gonoduct posterior to the branchial sac with the two lobes of the anus deeply divided into lobes, and eight lobed atrial aperture in *R. idoneta* (Shenkar 2013).

The external morphology of the presently described species is quite similar to *R. macrothorax*, also present in the Andaman Islands (Image 6a,b), except for the purple endostyle in *R. macrothorax* which is yellow in *R. bilobata* sp. nov. *R. macrothorax* in our region has 26 oral tentacles, in the range described by other researchers (Tokioka 1953; Monniot & Monniot 2008). Musculature pattern is quite similar (Image 6c,d) as shown by Monniot & Monniot (2008). But *R. macrothorax* has more longitudinal vessels (42) and rows of stigmata (62) than *R. bilobata* sp. nov. The most prominent difference is the anal border and gonoduct openings (Image 6e,f); *R. macrothorax* has a smooth and thick anal border and not triangular; the sperm duct is longer and opens beyond the anal border.

The other two reported species in the region are *R. circula* and *R. fusca* which are colonial and are restricted to the Nicobar group of islands. Apart from



Image 6. *Rhopalaea macrothorax* a, b - Species in in-situ condition; c, d - Thorax with musculature (Scale - c, 2mm, 9.09x; d, 2mm, 8.04x); e, f - Anal border with long sperm duct and oviduct opening (arrow) (Scale -500µm, 50.2x).

being colonial, *R. circula* also has more muscles, rows of stigmata, longitudinal vessels than *R. bilobata* sp. nov. and a different anal and gonadal opening. The pattern of the longitudinal muscles of the new species is similar to *R. fusca* (Monniot & Monniot 2001), but *R. bilobata* sp. nov. has fewer longitudinal vessels and rows of stigmata in comparison with *R. fusca* and it is transparent.

Distinctive differences can be seen with *R. perlucida* which has eight lobed branchial siphon, longitudinal muscles on thorax not ramified towards the dorsal and ventral borders, only eight rows of stigmata along with 50–70 longitudinal vessels and anus located posterior of the third row Monniot 1997). *R. tenuis* has the same basal root-like projections which are also found in the described species, but has fewer longitudinal vessels

(12), 20 longitudinal muscles of which half are originated from the branchial siphon and other half originated from the atrial siphon and four stigmata per mesh (Kott 1990).

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