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No. 12, Thiruvannamalai Nagar, Saravanampatti - Kalapatti Road, Saravanampatti,
Coimbatore, Tamil Nadu 641035, India
Ph: +91 9385339863 | www.threatenedtaxa.org
Email: sanjay@threatenedtaxa.org

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Caption: Stripe-backed Weasel *Mustela strigidorsa*. Medium—digital, Software—procreate, Device—iPad + Apple pencil © Dhanush Shetty.

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COMMUNICATION

New records of cheilostome Bryozoa from the eastern coast of India encrusting on the exoskeleton of live horseshoe crabs of Indian Sundarbans

Swati Das [1], Maria Susan Sanjay [2], Basudev Tripathy [3], C. Venkatraman [4] & K.A. Subramanian [5]

[1–4] Zoological Survey of India, M- Block, New Alipore, Kolkata, West Bengal 700053, India.
[1,2] Department of Zoology, University of Madras, Chennai, Tamil Nadu 600025, India.
[5] Zoological Survey of India, Southern Regional Centre, 130, Santhome High Road, Chennai, Tamil Nadu 600028, India.
[1] swtds4788@gmail.com (corresponding author), [2] mariasanjay257@gmail.com, [3] tripathyb@gmail.com, [4] cvramanmbs@gmail.com, [5] subbuka.zsi@gmail.com

Abstract: Bryozoans are common commensal on hard surfaces and cover slow-moving animals like molluscans, sea turtles, brachyuran crabs, and horseshoe crabs. A total of six species of bryozoans belonging to four genus under three families of order Cheilostomatida were recorded encrusting on the carapaces of horseshoe crabs collected from Indian Sundarbans along the east coast of India and two among them, viz., *Biflustra savartii* (Audouin, 1826) and *Sinoflustra arabianensis* (Menon & Nair, 1975) are reported for the first time. Additionally, *Jellyella tuberculata* (Bosc, 1802) is reported for the first time from West Bengal coastal waters, previously known only from the Odisha coast of India. Both male and female horseshoe crabs were found to have been encrusted with bryozoan mats, although adequately not known about the life stages of their encrustation.

Keywords: Bryozoa, *Carcinoscorpius rotundicauda*, East coast, Epibionts, Indian Sundarbans, *Tachypleus gigas,* Xiphosura.

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Author details: Swati Das is currently working as a research fellow and pursuing her PhD from Madras University, Chennai. Her research topic is on the ecology of Indian Horseshoe Crabs. Her research interests include ornithology, herpetology, wildlife biology, conservation biology, and wildlife photography. Maria Susan Sanjay is a research fellow working on taxonomy of marine bryozoans and also has research interest in ecological conservation and environmental studies. Dr. Basudev Tripathy is a senior scientist having more than 20 years of research experience in zoological research, especially on marine biology and wildlife conservation. He has published more than 100 peer reviewed scientific papers and books as well as book chapters. He has research projects both on the east and west coast of India. Dr. C. Venkatraman is a senior scientist who conducts regular field surveys in coastal habitats of eastern and western coasts of India. He is also an expert in mammals and birds in different habitats. Dr. K.A. Subramanian specializes in aquatic insect diversity and freshwater biodiversity conservation.

Author contributions: SD, BT and KAS designed the survey, SD conducted field survey and collected specimens, MSS examined and identified the specimens, SD and MSS compiled the information, illustration, and prepared the first draft of the manuscript, KAS and BT did manuscript correction, and all authors contributed to drafting the manuscript.

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INTRODUCTION

Bryozoa is considered a minor phylum placed in between phylum Mollusca and Echinodermata and are ancient, microscopic, sessile, and colonial coelomates inhabiting both marine & freshwater ecosystem (Soja 2006). They can erect or encrust on all types of hard, permanent or ephemeral substrates (Canu & Bassler 1920; Harmer 1926; Osburn 1940; Cook 1968; Ziko & Hamza 1987; Xi-Xing 1992; Key et al. 1996). Although mostly found in the littoral zone, bryozoans have been reported up to 6,000 m depth in the marine realm. Studies on the Indian bryozoan fauna are scarce except for some notable documentation by Annandale (1912) and Thornely (1907, 1916), and after that by Menon (1967), Menon & Nair (1967), Nair (1973), Pillai (1978, 1981), Raveendran et al. (1990), Swami & Karande (1987, 1994), Geetha (1994), Swami & Udayakumar (2010), Soja (2006), Mankeshwar et al. (2015), Tripathy et al. (2016), and Venkatraman et al. (2018). However, very few scientific publications are available on the bryozoan fauna of the east coast of India (Robertson 1921; Shrinivaasu et al. 2015).

The horseshoe crabs are marine chelicerates that migrate to nearshore waters during lunar cycles for spawning. Represented by only four extant species within Xiphosura, two species of horseshoe crabs, *Tachypleus gigas* (Müller, 1785) and *Carcinoscorpius rotundicauda* (Latreille, 1802) are known to occur along the upper east coast of India, co-occurring mainly along the West Bengal and Odisha Coast (Annandale 1909; Roonwal 1944; Debnath 1992; Tripathy et al. 2018). *C. rotundicauda* is the most abundant of the two species in Indian Sundarbans (Saha 1989; Debnath 1992; Tripathy et al. 2018). Xiphosurans serve as host species for a variety of organisms, viz., bryozoans, barnacles, oysters, tunicates, coelenterates, flatworms, annelids, isopods, diatoms, amphipods, gastropods, polychaetes, and green algae (Humm & Wharton 1942; Roonwal 1944; Rao & Rao 1972; Davis & Fried 1977; Mackenzie 1979; Shuster 1982; Jeffries et al. 1989; Saha 1989; Debnath 1992; Key et al. 1996). However, *T. gigas* and *C. rotundicauda* are found mainly infested by bryozoans, barnacles, mussels, oysters, limpets, and polychaetes (Botton 2009). There are scanty records on the epizoic bryozoans reported from exoskeleton of horseshoe crabs. Notable works have been carried out by Pearse (1947), Butler & Cuffey (1991), Allee (1922), Watts (1957), and Key et al. (1996). In India, Rao & Rao (1972), Debnath (1992) and Patil & Anil (2000) reported an unidentified species of *Membranipora* as epizoic bryozoa on both *T. gigas* and *C. rotundicauda*. As such, studies on biological studies on horseshoe crabs are limited and commensalism, symbiosis and parasitism on horseshoe crabs, are scantily known from India. The present work attempted documentation of bryozoan species encrusting on the carapaces of horseshoe crabs for the first time from India.

MATERIALS AND METHODS

Study area

Field surveys have been conducted in the Sagar Island and Patiboni areas of the Indian Sundarbans. The Sagar Island (21.791°N, 88.131°E) is situated at the western part of Indian Sundarbans and is the largest island of the Sundarban deltaic complex (Figure 1). Hoogly river borders north and west with Muriganga River in the east and Bay of Bengal in the south. It is a tidal dominated island and characterized by tidal creeks, mud flats/salt marshes, mangroves and sandy beaches/dunes. The Patiboni in Frezerganj (21.578°N, 88.246°E) is well known for its fishing activities, located eastward to the Sagar Island and having a more sandy substrate at the intertidal zones (Figure 1). The estuarine area of the Sagar Island (Tripathy et al. 2018) and Patiboni area of Frezerganj are considered as potential habitats for both species of horseshoe crabs.

Methods

The present study was conducted from March to December 2019 as part of the first authors doctoral research. Sampling was done during the end of high tide and the beginning of low tide, keeping a gap of two hours during the full moon/new moon period to avail the maximum exposed intertidal zone. *C. rotundicauda* and *T. gigas* were observed carefully on the mudflats and wherever encountered on horseshoe crabs, the bryozoan colonies were scraped off from the exoskeleton (Cephalothorax, telson, appendages, gills, and eyes) using a scalpel blade (Tan et al. 2011). The bryozoan specimens were preserved using 70% ethanol in a glass/plastic container and labelled properly in the field itself. The specimens were brought to the base camp and washed thoroughly with freshwater for automatic removal of any debris. In the base camp laboratory, collected bryozoan specimens were soaked with sodium hypochlorite (0.5%) for eight hours to remove the organic tissue and later soaked in distilled water for four hours (Shrinivaasu et al. 2015) and then dried for identification and thereafter photographed with Nikon

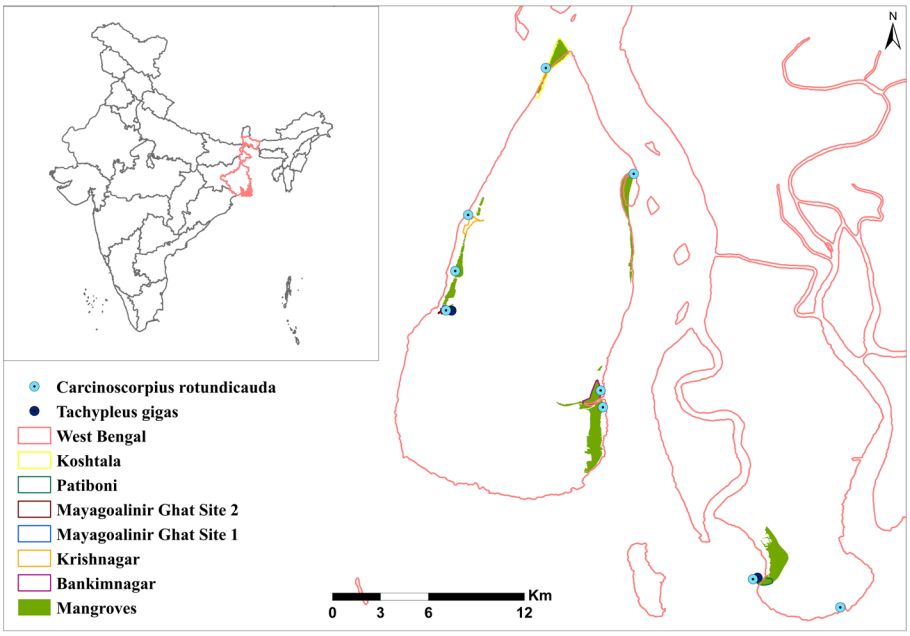

Figure 1. Study area map showing sampling sites of horseshoe crabs.

D7000 with 105 mm VR lens, post-processing with Adobe Photoshop CS6. The specimen was brought to ZSI HQ, Kolkata, for comparing with other museum specimens of the same families and genus, present in the Zoological Survey of India, which is part of the National Zoological Collections. In the field, fouled horseshoe crabs were counted, sexed and measured. After data collection and sampling of bryozoan specimens, horseshoe crabs were released back to the sea. The bryozoan colonies were observed under a stereomicroscope (Leica EZ4), for which the identified colonies were given a gold-palladium coating under vacuum condition and scanning electron micrographs were prepared with a Zeiss Evo 18 special edition SEM, using the "Smart SEM version 5.09" image processing software.

RESULTS

A total of 58 *Carcinoscorpius rotundicauda* (Image 2) and six *Tachypleus gigas* were observed for bryozoan encrustation examination during the study period. Out of 58, 11 *C. rotundicauda* (six male and five female) and five *T. gigas* (four male and one female) were found encrusted with bryozoan mat. A total of six bryozoan species belonging to five genera under three families of order Cheilostomatida were documented encrusting on the exoskeleton of horseshoe crabs from the Indian Sundarbans. The study further confirmed the presence of two bryozoan species, viz., *Biflustra savartii* (Audouin, 1826) and *Sinoflustra arabianensis* (Menon & Nair, 1975), on the carapaces of horseshoe crabs, reported to be recorded for the first time from the Bay of Bengal, previously known from the Arabian sea (Menon & Menon 2006). *Jellyella tuberculata* (Bosc, 1802), previously known only from the Odisha coast of India (Menon & Menon 2006), was reported for the first time from the West Bengal coast during this study.

Systematic Account

Kingdom: Animalia
Phylum: Bryozoa
Class: Gymnolaemata
Order: Cheilostomatida
Suborder: Membraniporina
Superfamily: Membraniporoidea
Family: Membraniporidae
Genus *Biflustra* d'Orbigny, 1852

1. *Biflustra savartii* (Audouin, 1826)

Image 1A

Location: Bankimnagar, Sagar Island, Sundarbans

Substratum: Encrusted on prosoma of *Carcinoscorpius rotundicauda* (A female without telson).

Description: Colony encrusting, forming a unilaminar sheet on the substratum arranged in longitudinal rows. Zooids sub-rectangular or sub-hexogonal, curved and raised distally and angular at the two proximal corners, separated by a raised ridge with a distinct mural rim.

Opesia occupying most of the frontal area, deep and oval, slightly smaller than the frontal membrane, nearly occupying two-thirds of the frontal area.

Distribution: It is a very common species worldwide in the tropical and sub-tropical seas reported from Indonesia and all along the Pacific coast. Earlier, it was reported from Cape Comorin (Menon 1967) and the Mangalore coast (Thornely 1907) in India.

2. *Biflustra hugliensis* (Robertson, 1921)

Image 1B

Location: Patiboni (Frezerganj), Bankimnagar (Sagar Island)

Substratum: Encrusted on prosoma of male *Tachypleus gigas* and female *Carcinoscorpius rotundicauda*.

Description: Colony encrusting, zooecia elongated, aperture occupying three fourths of the front, separated by a delicate calcareous mural rim. Distal portion of the zooid overarching the pre-seeding zooid. Operculum semi-circular, straight at its proximal border, much wider than long. Cryptocyst marginally developed, granular on its surface, serrated coarsely on its inner margin. Ovicells and avicularia are wanting.

Remarks: Earlier, a colony of encrusting *Biflustra hugliensis* was identified from the posterior of the carapace of *Lepidochelys olivacea* (Olive Ridley Sea Turtle) from the Gulf of Kachchh, Gujarat (Frazier et al. 1992).

Distribution: Although a species of tropical and subtropical seas, this species was first identified from the mouth of the Hugli River, Bay of Bengal (Robertson 1921) and subsequently reported from the Gulf of Kachchh, Gujarat (Frazier et al. 1992). Except for these two records, there is no report of this species from anywhere else in India.

Genus: *Jellyella* Taylor & Monks, 1997

3. *Jellyella tuberculata* (Bosc, 1802)

Image 1C

Location: Bankimnagar (Sagar Island) and Patiboni (Frezerganj)

Substratum: Encrusted on ventral side of prosoma of a male *Tachypleus gigas* as well as encrusted on the shell of a mollusc found on the right prosoma of a female *Carcinoscorpius rotundicauda*.

Diagnosis: Colony encrusting, multi-serial. Zooids rectangular to sub-rectangular, quincuncially arrangement, opesia elongate-oval, bordered by a very narrow cryptocystal rim laterally and a cryptocystal shelf proximally; cryptocyst sparsely tubercular. Gymnocyst proximally, starting at the corners of the zooid, then as a thin continuous proximal rim, the gymnocyst arches forward, forming small pockets beneath, especially at the corners; in fully calcified zooids the gymnocystal tubercles can be stoutly developed, completely concealing the proximal cryptocyst.

Distribution: A widely distributed species of the major oceans, this species is reported from North Carolina to Brazil along the Atlantic coast, California to up to Peru along the Pacific coast. Among the Indian Ocean countries, it is reported from Japan and Bangladesh and in India, it has been earlier reported from the coast of Odisha (Menon & Menon 2006).

Family: Electridae

Genus: *Conopeum* Gray, 1848

4. *Conopeum reticulum* (Linnaeus, 1767)

Image 1D

Location: Patiboni (Frezerganj)

Substratum: Encrusted on ventral side of prosoma of a male *Tachypleus gigas*.

Description: Encrusting, colonies appear as whitish patches with uneven growing margin. Zooecia quincuncially arranged, chitinous outline distinct. Shape of zooecia variable, but generally longer than wide, very much elongated in certain cases. Cryptocyst tuberculated, developed all-round the opesia with tubercles projecting into the opesia. The tubercles are more or less of the same length, small tubercles are present in the proximal region of the cryptocyst. In certain Zooids the proximal region of the opesia is broader than the distal region.

Remarks: This species is known to be found on fouling organisms which have been previously identified from the carapace and appendages of the *Neptunus pelagicus* (Swimming Crab) caught in a trawl net in Cochin (Menon 1967).

Distribution: *Conopeum reticulum* is a warm water Indo- Pacific species. This is recorded from Tortuges Island, Florida (Osburn 1950); Indonesia (Harmer 1926); Java, Sumatra, and Myanmar (Marcus 1937). In India, it has been reported from the Arabian Sea along with the Lakshadweep Islands and the Cochin coast (Menon 1967) as well as the Bay of Bengal from Chilka Lake (Annandale 1915).

Family: Sinoflustridae

Genus: *Sinoflustra* Liu & Yang, 1995

5. *Sinoflustra amoyensis* (Robertson, 1921)

Image 1E

Location: Patiboni (Frezerganj) and Bankimnagar

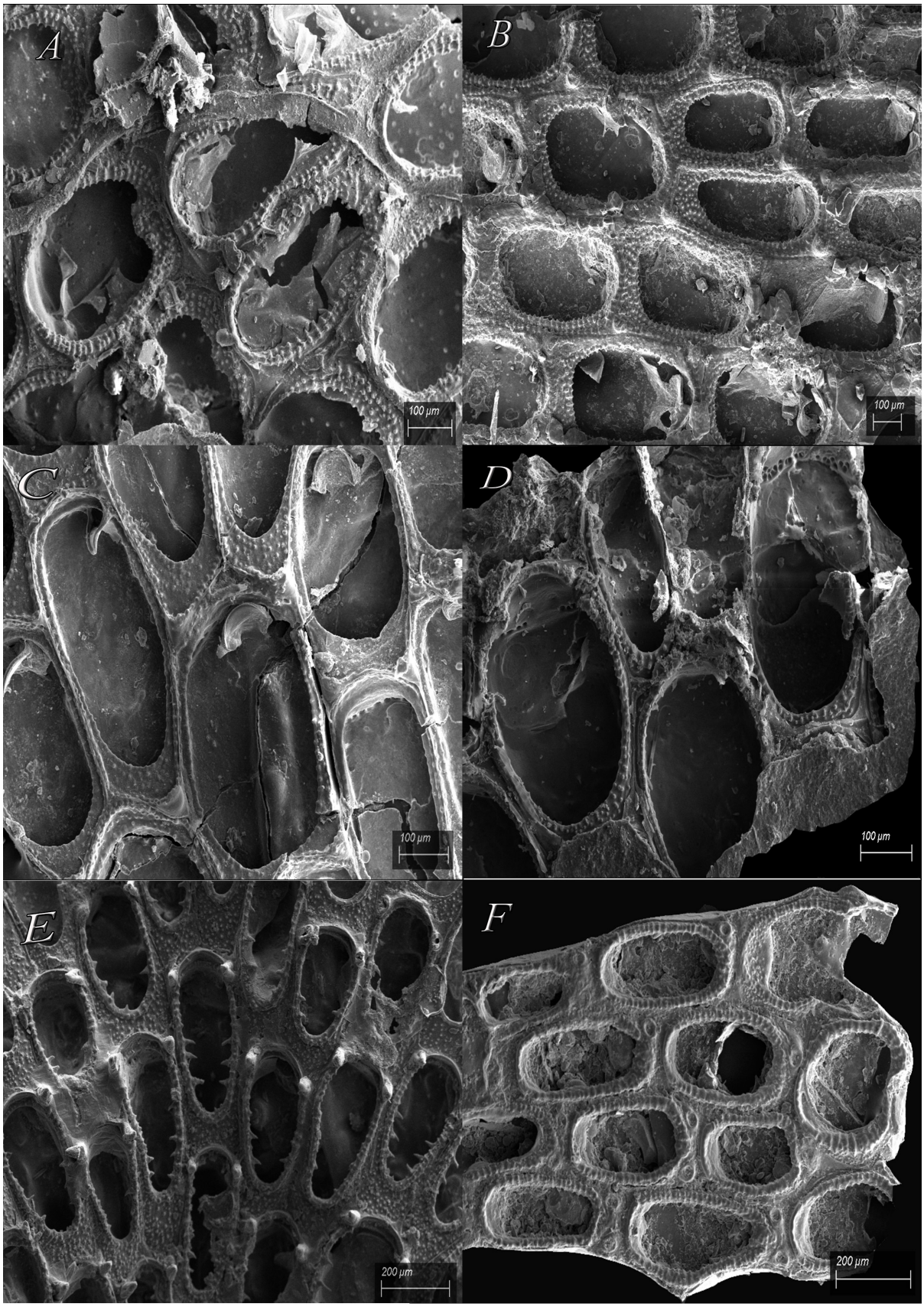

Image 1. A—Biflustra savartii (Audouin 1826) | B—Biflustra hugliensis (Robertson, 1921) | C—Jellyella tuberculata (Bosc, 1802) | D—Conopeum reticulum (Linnaeus, 1767) | E—Sinoflustra amoyensis (Robertson, 1921) | F—Sinoflustra arabianensis (Menon & Nair, 1975).

(Sagar Island)

Substratum: Encrusted on prosoma of a male *Carcinoscorpius rotundicauda* and and also found encrusted on hardened sediments found on the right side of the prosoma of a female *Carcinoscorpius rotundicauda*.

Description: Colony encrusting, white. The zooecia are moderate in size and very delicate and chalk like, zooids elongated rectangular, arranged in quincuncial series, and separated by a distinct fine groove. The mural rim is thin, raised and smooth on its edge. No gymnocyst. Frontal membrane large, occupying the whole of the frontal area. Cryptocyst marginal, narrowest distal to the opesia, developed laterally and proximally, smooth and granular in younger colonies, and granular on its surface in older colonies, with strong cryptocystal spinules. It contains six strong cryptocystal spinules on each side equidistant from each other, on its inner border proximal to the orifice. Opesia elongate and reduced by the cryptocystal spinules. A strong conical spine is present on each distal corner of every zooid.

Distribution: This species has been reported to have its presence since the Pliocene era and distribution range in the Indo-Pacific region. It has been originally collected from Amoy of China; in India, this species has the report of its presence in the Holocene rocks of the west coast of Maharashtra, Ernakulam channel from Cochin, and also from the coast of West Bengal (Menon & Menon 2006).

6. *Sinoflustra arabianensis* (Menon & Nair, 1975)

Image 1F

Location: Patiboni (Frezerganj)

Substratum: Encrusted on the dorsal side of prosoma of a male *Tachypleus gigas*.

Description: Colony encrusting. Grows flat, disk-like structures in the absence of any hindrance. Zooecia elongated, quadrangular the distal portion of the preceding zooecium slightly over arch the proximal portion of the succeeding zooid. Opecia occupying three-fourths of the front, being narrowed distally. Gymnocyst present, slightly extensive proximally. Cryptocyst with spinules, the size of the spinules decrease at the distal portion of the cryptocyst. Ancestrula possesses a pair of branched spines.

Distribution: It has been reported only from Cochin along the coast of the Arabian Sea (Menon 1967). This is the first report from the Bay of Bengal and also from the Indian Sundarbans region.

DISCUSSION AND CONCLUSION

Bryozoans are important macro fouling community in the coastal waters of India. So far, very little is known on the bryozoan species diversity and their association with horseshoe crabs and other organisms with hard surfaces and substratum. In India, the upper eastern coast is a preferred breeding and spawning ground for two species of horseshoe crabs: *Tachypleus gigas* and *Carcinoscorpius rotundicauda.* Both the species are in the data deficient category of the IUCN Red List; however, placed in the Schedule IV category of the Indian Wildlife Protection Act, 1972. The Mangrove Horseshoe Crab *Carcinoscorpius rotundicauda* (Latreille, 1802) is more common on the mudflats of the Indian Sundarbans than the Indian Horseshoe Crab *Tachypleus gigas* (Müller, 1785) although occurring in a sympatric habitat. In the present study, it was observed that adult male and female horseshoe crabs are host for bryozoan mats and the reason could be multiple. As most marine organisms compete for substrate space (Paine 1974; Jackson 1977; Connell & Keough 1985) to attach with suitable host species for their dispersal and gene flow (Wahl 1989), unoccupied and clean, bare exoskeletons of horseshoe crabs may act as an ideal surface for colonization of bryozoan species and probably help them to expand their range of distribution. Currents generated by the movement of hosts, respiration and feeding of the host (Bowers 1968; Wahl 1989; Gili et al. 1993) may help in capture of suspended food particles to the bryozoans. Additionally, host species may also protect bryozoan species from predators like amphipods, annelids, echinoids, isopods, nudibranchs, pycnogonids, and gastropods and in return, bryozoans help the host species via camouflage (Key et al. 1996, 2000; Patil & Anil 2000). As studies elsewhere (Renouf 1932; Cadee 1991) suggest bryozoan encrustation can reduce the effectiveness of the host's organs, hence, it can be inferred that epizoic bryozoans may impair the sight of horseshoe crabs as bryozoan mats were found encrusting on the compound eyes of horseshoe crabs during the present study, although bryozoan growth was also found on the mouth, gills, legs and telson of horseshoe crabs. Therefore, these aspects need further investigation to study the occurrence of any parasitic organisms of Bryozoa, which may impair the movement/function of organs of horseshoe crabs. Overall, the interaction between a horseshoe crab and epizoic bryozoan is found non-symbiotic and facultative (Key et al. 1996) and as epizoic bryozoans have a less negative impact on horseshoe crabs, both co-exist.

Image 2. Field image of bryozoa (pale brown coloured mat) encrusting on the carapace of *Carcinoscorpius rotundicauda*.

Reporting of two species of Bryozoa for the first time from the east coast of India and one new report from the West Bengal coast clearly indicates that further intense surveys will bring more details on Bryozoa and their relationship with horseshoe crabs. Investigations are also required documenting ecological factors that regulate the epizoic bryozoan distribution on horseshoe crabs.

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