

**Report of ciliate-bryozoan-crustacean hyperepibiosis on crab (Decapoda: Brachyura)
from west coast of India, Arabian Sea**

Tapas Chatterjee¹, Igor Dovgal², Leandro M.Vieira³, Arpita Dutta⁴ & Mandar Nanajkar⁵

¹Crescent International School, Bario, Govindpur, Dhanbad 828109, Jharkhand, India. E-mail: drtchatterjee@yahoo.co.in ; drtchatterjee@gmail.com Orcid link: <https://orcid.org/0000-0001-5532-2726>

²A.O. Kovalevsky Institute of Biology of the Southern Seas of Russian Academy of Sciences, 2, Nakhimovave. Sevastopol, 299011, Russia. E-mail: dovgal-1954@mail.ru Orcid link: <https://orcid.org/0000-0002-3876-233X>

³Departamento de Zoologia, Centro de Biociências, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego 1235, Cidade Universitaria, 50670-901 Recife, PE, Brazil. E-mail: leandromanzoni@hotmail.com ; leandro.mvieira@ufpe.br
Orcid link: <https://orcid.org/0000-0001-8661-8861>

⁴CSIR—National Institute of Oceanography, Dona Paula, Goa, 403004, India. E-mail: arpitadutta24009@gmail.com
Orcid link: <https://orcid.org/0000-0002-7331-8760>

⁵CSIR—National Institute of Oceanography, Dona Paula, Goa, 403004, India.

E-mail: mandar@nio.org ; mandar.nanajkar@gmail.com Orcid link: <https://orcid.org/0000-0002-6608-7669>

Abstract

Epibiosis is a common phenomenon, found amongst different taxa of aquatic animals. This relationship could occur as hyperepibiosis, when a basibiont being also an epibiont, providing a stable substrate for the hypersymbiont. Here we reported a ciliate-bryozoan-crustacean hyperepibiosis in Mandovi River mouth, Goa, West coast of India. We provided descriptions and characterization of the crab *Atergatis* sp., serving as basibiont for the bryozoan *Triticella pedicellata* (Alder, 1857), in turn colonized with (hyperepibionts) the ciliates *Paracineta saifulae* (Mereschkowsky, 1877) and *Cothurnia ceramicola* Kahl, 1933. *Paracineta saifulae* and *Cothurnia ceramicola* are reported here for first time from the Indian Ocean.

Key words: Hyperepibiosis, ciliates, bryozoan, crab, Arabian Sea

Running Title: Ciliate-bryozoan-crustacean hyperepibiosis on crab

Introduction

Epibiosis is a facultative association of two organisms: the epibiont and the basibiont (Wahl 1989). The term "epibiont" includes organisms that, during the sessile phase of their life cycle, are attached to surfaces of living substratum, while the basibiont lodges and provides support for the epibiont (Threlkeld *et al.* 1993). There are many benefits but few drawbacks for the organisms involved in the association (Harder 2009).

In marine environment many organisms, from bacteria (Creen *et al.* 2018), protozoa (eg. Precht 1935; Clamp 1973; Jankowski 1981, 2007; Dovgal 1996, 2002; Fernandez-Leborans & Tato-Porto 2000a, b; Fernandez-Leborans *et al.* 2012; Chatterjee *et al.* 2013a, b, 2014, 2018, 2019a, 2020a, b; Clamp *et al.* 2016; Mayén-Estrada & Clamp 2016; Mayén-Estrada & Utz 2018), diatoms (Robinson *et al.* 2016), macroalgae (Littler & Littler 2013), to several metazoans, including sponges, cnidarians, molluscs, rotifers, bryozoans, phoronids, brachiopods, polychaetes, crustaceans, acarines, echinoderms and ascidians (eg. Corriero & Pronzato 1987; Fernandez-Leborans 2003a; Fernandez-Leborans & Gabilondo 2006; McGaw 2006; Ayres-Peres & Mantelatto 2010; Fernandez-Leborans 2003a, 2010; Normant *et al.* 2013; De Smet & Verolet 2016; Ejsmont-Karabin & Karpowicz 2019; Oresland 2019) have adopted epibiotic habit of life at least in one ontogenetic phase.

In India, epibiont ciliates from western Indian coasts were studied by many authors e.g. Santhakumari (1985, 1986a, b), Chatterjee (1996), Dovgal *et al.* (2008, 2009), Chatterjee *et al.* (2013c, 2019b, c; 2020c, d, e).

In some works, in turn, the cases of hyperepibiosis as the form of relation between organisms when an epibiont lodges on another epibiont harbouring on to a host, were discussed (eg. Fernandez-Leborans 2003b, 2013; Mestre *et al.* 2019). Ciliate-bryozoan-crustacean hyperepibiosis of the brachyuran decapods *Goneplax rhomboids* (Linnaeus, 1758) colonized by the bryozoan *Triticella flava* Dalyell, 1884, collected in the north west Mediterranean coast has been studied by Fernandez-Leborans (2003b). Ciliate hyperepibiosis on the hydrozoan *Dicoryne conferta* (Alder, 1856), attached to the shell occupied by hermit crab *Pagurus bernhardus* (Linnaeus, 1758) from the west coast of Scotland was studied by Fernandez-Leborans 2013. Ciliate hyperepibiosis also was noted on the ostracod *Ankylocytheres inuosa* (Rioja, 1942) attached to a crayfish *Procambarus clarkia* (Girard, 1852) in the Iberian Peninsula and Balearic Islands SW Europe (Mestre *et al.* 2019). Suctorian ciliate *Tokophrya fasciculata* (Lopez-Ochoterena, 1964) was observed on stalks of suctorian *T. quadripartita* (Claparede & Lachmann, 1859) and peritrich *Epistylis plicatilis*

Ehrenberg, 1838, in turn colonising the shells of freshwater snails collected from Mexico (Lopez-Ochoterena 1964).

In the present article we report Ciliates-bryozoan-crustacean hyperepibiosis on crab (Decapoda: Brachyura) from the west coast of India, Arabian Sea. Here, we provide characterization of the ciliates *Paracineta saifulae* (Mereschkowsky, 1877) and *Cothurnia ceramicola* Kahl, 1933, that were found as epibionts on bryozoan *Triticella pedicellata* (Alder, 1857), attached to the crab *Atergatis* sp.

Material and Methods

Goa is located on the West coast of India with an area of 3702 sq. km. Our present study area is Mandovi-Zuari River mouth. The Rivers Mandovi and Zuari originate from Western Ghats and flow to the Arabian Sea, and are known as the major two important estuarine systems in Goa. Present study location is situated at 15°28'43.68"N and 73°41'41.28"E (Fig. 1), which is at Mandovi river mouth. The sample was collected out in April 2019. The depth of the sampling location was 20m.

Sediment samples were collected using Van-Veen grab (0.040192 m² area). These samples were passed through a 300µm mesh sieve on board, and preserved with 10% formalin - Rose Bengal solution and stored in polythene bag. Later in the laboratory, the samples were again sieved & washed through 300µm sieve and faunal sample were separated in collection vial (with 5% formalin) for further microscopic analysis. Microscopic observations and photography was carried out for crab and its epibionts (bryozoans and ciliates), using Olympus-SZX10 stereoscopic microscope and upright Microscope Olympus-BX63 with a Camera DP74 and Cell Sense Software. Specimens on the host are deposited in the reference collection of the CSIR—National Institute of Oceanography Taxonomic Reference Centre, Goa, India (CSIR-NIO).

Description of Crab

Atergatis sp. (Fig. 2A) (Phylum - Arthropoda, Subphylum - Crustacea, Class - Malacostraca, Order - Decapoda, Family - Xanthidae, Genus - *Atergatis* De Haan, 1833): One female crab examined, 9 mm carapace width and 6 mm carapace length. Sub quadrilateral carapace, imprecise region, celiped and legs have smooth-hairy surface. The surface of the carapace had brown colour blotch, chelate legs are covered with very fine punctulation, which is clearly faded due to preservatives. The epibranchial angels has an obtuse tooth, carpus of celiped are unarmed. Basal antenna joint shorter, eye stalk thick-short. Anterior margin of the external maxillipeds is almost transverse.

Primary bryozoan epibiont on Crab

Bryozoan epibiont *Triticella pedicellata* attached with crab *Atergatis* sp.

Phylum Bryozoa Ehrenberg, 1831

Class Gymnolaemata Allman, 1856

Order Ctenostomatida Busk, 1852

Family Triticellidae G. O. Sars, 1874

Genus *Triticella* Dalyell, 1848

***Triticella pedicellata* (Alder, 1857)**

(Figs. 2–3)

Triticella pedicellata: Menon, 1972: 622, fig. 45–49.

Triticella pedicellata: Hayward, 1985: 120, fig. 40. (cum syn.)

Description: Colony encrusting on brachyuran crab *Atergatis* sp. (Figs. 2A–B), with adherent convoluted stolons from which arises kenozooids with group, erect zooids (2C). Erect zooids with a long tubular pedicel, translucent cystid, strongly chitinized, 1.5–3.1 mm long, 0.022–0.032 mm wide (Fig. 2D), smooth. Distal zooidal dilatation elongate, almost fusiform, convex proximal, distally truncate, sometimes flattened frontally, about 0.52–0.68 mm long and 0.16–0.20 mm wide (Figs. 2E–F); old zooids with scars and some septae along pedicel, indicating sequential breakages and repairs on it (Fig. 3A). Zooids may be marked by a wrinkled area, with no septum, between pedicel and dilatation. Frenaculum absent. Orifice terminal, circular, with very short collar. Polypide with oval stomach; lophophore with about 11 tentacles, about 0.350 mm long (Fig. 2F). Ovary located at proximal third in zooid dilatation.

Remarks. Menon (1972) reported two *Triticella* from Indian waters: *Triticella koreni* Sars, 1874, found inhabiting on appendages of spinner crab, and *Triticella pedicellata* Alder, 1857, collected on legs of swimming crab. *Triticella koreni* is regarded to be junior synonym of *Triticella flava* Dalyell, 1848 (Hayward 1985). According to Hayward (1985), *Triticella flava* and *T. pedicellata* are distinct in presence of frenaculum (present only in *T. flava*), and number of tentacles (17–20 tentacles in *T. flava* and about 12 tentacles in *T. pedicellata*). Specimens reported as *T. pedicellata* by Menon (1972) are similar to those here described and figured, but he reported slightly shorter pedicel (0.60–1.80 mm long). *Triticella pedicellata* was considered a badly described species (d’Hondt 1983), but despite absence of any morphological differences between Indian specimens and

those described for Britain, we assigned species here described to *T. pedicellata*. *Triticella pedicellata* seems to be part of a species complex due variations reported in that species; thus further molecular genetics studies is needed to solve it. Specimens here reported have dilatations along pedicels, some of those with distinct septum (and scars); they are mainly only in old zooids, indicating breakages and repair of the zooids. Therefore, these dilatations are distinct from those described for *Triticella capsularis* Gordon & Wear, 1999, from New Zealand; *Triticella capsularis* is characterized by having 9–17 tentacles, with longer (up to 10 mm) and wider (0.043 mm) pedicel when compared to *T. pedicellata*. The polypide of *Triticella gracilis* d'Hondt & Hayward, 1981, described from deep waters of Atlantic Ocean, also has few tentacles (10-12 tentacles; acc. d'Hondt 1983), but it is distinct in having shorter zooids (smaller than 0.50 mm long).

Secondary epibionts on crab

Ciliates epibionts on bryozoans *Triticella pedicellata* attached with crab *Atergatis* sp.:

Phylum Ciliophora Doflein, 1901

Class Suctorea Claparède & Lachmann, 1859

Subclass Exogenia Collin, 1912

Order Metacinetida Jankowski, 1978

Family Paracinetidae Jankowski, 1975

Genus *Paracineta* Collin, 1912

***Paracineta saifulae* (Mereschkowsky, 1877)**

(Fig. 3C, 3D-1, 4 A, B)

Brief description: Marine, loricate suctorian with elongate, some asymmetric body, only the bottom half of which cover by stylotheca. The conical stylotheca grades into thin, long pseudostyle, which is 3-4 times the lorica length. The conical part of the stylotheca often have thickened walls, the peculiar bed for body forms as a result. Stylotheca surface crenulated with three to many transverse striations. Capitulate tentacles sometimes retractile, evenly distributed at the anterior surface of body. Macronucleus spherical. There is one contractile vacuole.

Measurements (in μm , based on three individuals): Stylotheca length 20-56 (65 after Mereschkowsky 1877); stylotheca width 24-37 (up to 27 after Mereschkowsky 1877); pseudostyle length 59-269; pseudostyle diameter 3-5; body length 33-61; body width 23-26; macronucleus dimensions 11×16.

Remarks: This species was described under name *Acineta saifulae* by Mereschkowsky (1877) from Onega Bay of the White Sea near the Rem (Russia) on the hydroid *Opercularella grigoriewi* (Mereschkowsky, 1878). Curds (1987) synonymized *A. saifulae* with *Acineta crenata* Fraipont 1878, a species independently described by Fraipont (1878) on the hydroid *Campanula riavolubilis* (Linnaeus, 1758) from Belgian coast of the Northern Sea near Ostend. On the basis that the name *Acineta saifulae* was published earlier (in 1877) than the name *Acineta crenata* (in 1878), Dovgal (2002) concluded that the *Paracineta saifulae* must be considered as senior synonym.

The species was also early mentioned by Collin (1912) under name *Paracineta crenata*, reported from French coast of the Mediterranean Sea near Sète on hydroid *Leuckartia raoctona* (Fleming, 1823) and polychaete *Aphrodite aculeata* Linnaeus, 1761.

Paracineta saifulae was also reported in the Black Sea near Bulgarian coast and in Russian coast near Gelendzhik on artificial substrates (glass slides) (Dovgal 2013).

The ciliate *Paracineta saifulae* was attached on stalk (Fig. 3C) as well on zooidal dilatations (3D-1) of *Triticella pedicellata*. This is the first record of this species from the Arabian Sea and Indian Ocean.

Class Oligohymenophorea de Puytorac *et al.*, 1974

Subclass Peritrichia Stein, 1859

Order Sessilida Kahl, 1933

Family Vaginicolidae de Fromentel, 1874

Genus Cothurnia Ehrenberg, 1831

***Cothurnia ceramicola* Kahl, 1933**

(Fig. 3D-2, E; 4 C, D)

Brief description: Marine loricate peritrich ciliate. Lorica, rounded posteriorly with short external stalk, endostyle and short, broad, longitudinally striated mesostyle. There are one or two zooids in the lorica, which extending up to one third of its length beyond aperture. The pellicula of zooids conspicuously striated. Macronucleus band-like, long.

Measurements (in μm , based on two individuals): Lorica length 65-90 (65-77 after Warren & Paynter 1991); lorica width 18-27 (30 after Warren & Paynter 1991); contracted zooid length 38-37; contracted zooid width 14-17; external stalk length 4-19; external stalk diameter 2; endostyle length 5; endostyle width 5; mesostyle length 3; mesostyle width 4.

Remarks: This species was firstly described as an epibiont of the alga *Ceramium* sp. in the Kiel bay, the Northern Sea (Kahl 1933, 1935). It was also found on bryozoans *Crisia burnea* (Linnaeus, 1758) and *Cribilina punctata* (Hassall, 1841), the polychaete *Spirorbis spirorbis* (Linnaeus, 1758), and the hydrozoan *Gonothyrae aloveni* (Allman, 1859) (Precht 1935). Later this species was also reported by Felinska (1965) attached to algae collected near Plymouth, UK.

The Ciliate *Cothurnia ceramicola* was attached on stalk (Fig. 3E) as well on zooidal dilatations (Fig. 3D-2) of *Triticella pedicellata*. This is the first record of this species from the Arabian Sea and Indian Ocean.

Discussion

The colonial organisms like the bryozoans, hydroids or sessile peritrichs, inhabiting on the body surfaces of molluscs, crustaceans, algal thalli or inanimate substrates (stones) are often colonized by other sessile and vagile organisms representing a peculiar consortium (Duval & Margulis 1995). However, the sessile ciliates are possible most frequently noted among hyperepibionts.

Species of the genus *Triticella* have been widely reported growing on various substrata (Table 1). One-third (4/12) of the *Triticella* species are known to grow on decapods: *T. capsularis*, *T. elongata* (Osburn, 1912), *T. flava* and *T. pedicellata* (Andersen 1962; Jensen & Bender 1972; Abello & Corbera 1996; Gordon & Wear, 1999; Key *et al.* 1999). Compared to other species of this genus, *T. pedicella* seems to occur on a variable range of substrates, such as gastropod shells (Jensen & Bender 1972), and it is also reported on unfamiliar surface viz. sea snakes (Pfaller *et al.* 2012). Despite studies showing association between *Triticella* species and other organisms (Table 1), ciliate-bryozoan-crustacean hyperepibiosis has been studied only by Fernandez-Leborans (2003b), who reported specimens of the brachyuran decapods *Goneplax rhomboids* (Linnaeus, 1758) from north west Mediterranean coast with bryozoans *Triticella flava* as primary epibionts, while secondary epibionts were identified belonging to three ciliates genera (*Zoothamnium*, *Cothurnia* and *Corynophrya*). Ciliate hyperepibiosis was also recorded on the hydrozoan *Dicoryne conferta* (Alder, 1856), attached to the shell occupied by hermit crab *Pagurus bernhardus* (Linnaeus, 1758) from the west coast of Scotland (Fernandez-Leborans 2013); seven epibionts on *Dicoryne conferta* were recorded, including six suctorians: *Trematosoma constricta* (Collin, 1909) (reported as *Conchacineta constricta*), *Paracineta anisostyla* (Fernandez-Leborans & del Arco, 1996) (reported as *Corynophrya anisostyla*), *Actinocyathula homari* (Sand, 1899), *Paracineta saifulae* (Fraipont, 1878) (reported as

Actinocyathula crenata), *Acineta sulcata* Dons, 1927 and *A. corophii* Collin, 1912, and one peritrich species (*Zoothamnium* sp.).

Lagenophrys discoidea Kellicott, 1887 was noted on the ostracod *Ankylocytheres inuosa* (Rioja, 1942) attached to a crayfish *Procambarus clarkia* (Girard, 1852) in the Iberian Peninsula and Balearic Islands SW Europe (Mestre *et al.* 2019). Suctorian ciliate *Tokophrya fasciculata* (Lopez-Ochoterena, 1964) was observed at stalks of suctorian *T. quadripartita* (Claparède & Lachmann, 1859) and peritrich *Epistylis plicatilis* Ehrenberg, 1838, attached to the shells of freshwater snails *Ladislavella elodes* (Say, 1821) (reported as *Lymnaea attenuata* Say, 1829) and *Physa osculans* Haldeman, 1841 collected from Mexico (Lopez-Ochoterena 1964).

There are different views about useful and harmful aspects of epibiont on crustacean hosts: epibionts overload could incur more weight on the crustacean and could reduce its mobility by modifying the hydrodynamic efficiency (equilibrium) of the body and hindering the movement of its appendages. These circumstances could cause crustaceans to be vulnerable to predations (Overstreet 1973). A beneficial aspect for the basibiont could be possible camouflage effect provided by the gregarious epibiont colony (Ingle 1983; Wahl 1989). The sessile epibionts might obtain some benefits due to host mobility, when it passes in other localities with more comfortable conditions for example food availability. The secondary epibionts can use the primary ones (epibiont) as useful substrate, which is raised above bottom (sediment) that allow them to catch or filter the food from different water horizons.

In the present context where the benthic habitat is constantly exposed to hydrodynamics loads, epibiosis demands finding a hard substrate (stable habitat for attachment) which is scarce in the subtidal shelf region having silty-muddy sediment (Connel & Keough 1985; Gili *et al.* 1993), the bryozoans prefer to settle on crab. The present study also notes the predominance of bryozoan *Triticella* on the dorsal and dorso lateral surface of the carapace. Another important attribute deciding the fate of bryozoan epibiont is the host (crab) ecdysis, wherein the epibiont colony has a limited period (time frame) from recruitment to reproduction to have successfully survival strategy (Eggleston 1971). Any mismatch may result in colony failure for bryozoans, which will further affect the ciliate hyperepibionts.

Moreover, the ciliates have to time its colonisation, asexual reproduction and growth within the time frame (period) of bryozoan recruitment and proliferation. Only certain growth stage of bryozoan will cue the settlement of ciliates viz. *Paracineta saifulae* and *Cothurnia ceramicola* as they were absent on the main host carapace (*Atergatis* sp.) noted earlier as well by Fernandez-

Leborans (2013). Bryozoans usually settle one month after newly moulted crab once bacterial biofilm appear as cue (Gili *et al.* 1993). The crab pilosity and pigmentation also plays role in settlement of bryozoans with possible aid in mimetic camouflage (Fernandez-Leborans 2003a, b).

This complex synchronised process has an ecological function for successional host acquisition and certainly is the most conducive survival strategy rather than attachment to inanimate surfaces. Mostly such interactions have positive feedback loop for all the entities involved but many a times the effects can be density dependant (Nanajkar *et al.* 2019). The bryozoans may take advantage of the sloppy feeding behaviour of crab while the ciliates may take advantage from both the hosts and or bacterivory. Crabs are known to harbour anemones and seaweeds as ecto-commensals to deter predators (Cutress & Ross, 1969) similar may be the case of bryozoans, which may have chemo-repellent properties protecting the host. Overall success of recruitment on crab host may be advantageous for bryozoan while the ciliates use the bryozoan as basibiont for feeding and refuge.

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Legends for Figures:

Figure 1. The map of study area.

Figure 2. A–F, Bryozoan *Triticella pedicellata* (Alder, 1857): A, B. Epibiont colony attached on crab *Atergatis* sp. (Crustacea: Brachyura); C. Group of erect zooids; D. Three zooids, two with long tubular pedicel and one with distal zooidal dilatation (at center) with broken stalk; E. Close-up of the distal zooidal dilatation; F. Close-up of the distal zooidal dilatation with extended polypide.

Figure 3. A. Scar and septate condition in stalk of *Triticella pedicellata* old zooids; B. Origin of stalk of *Triticella pedicellata*; C. Epibiont *Paracineta saifulae* on stalk of *Triticella pedicellata*; D. Ciliate epibiont on Bryozoan zooidal dilatation – 1. *Paracineta saifulae*; 2. *Cothurnia ceramicola*; E. Many ciliate *Cothurnia ceramicola* on stalk of *Triticella pedicellata*.

Figure 4. A, B. *Paracineta saifulae* (Mereschkowsky, 1877); C, D. *Cothurnia ceramicola* Kahl, 1933.

Legend for Table:

Table 1. Bryozoan species of *Triticella*, including host/substrate, locality of occurrence, and major references for each species.

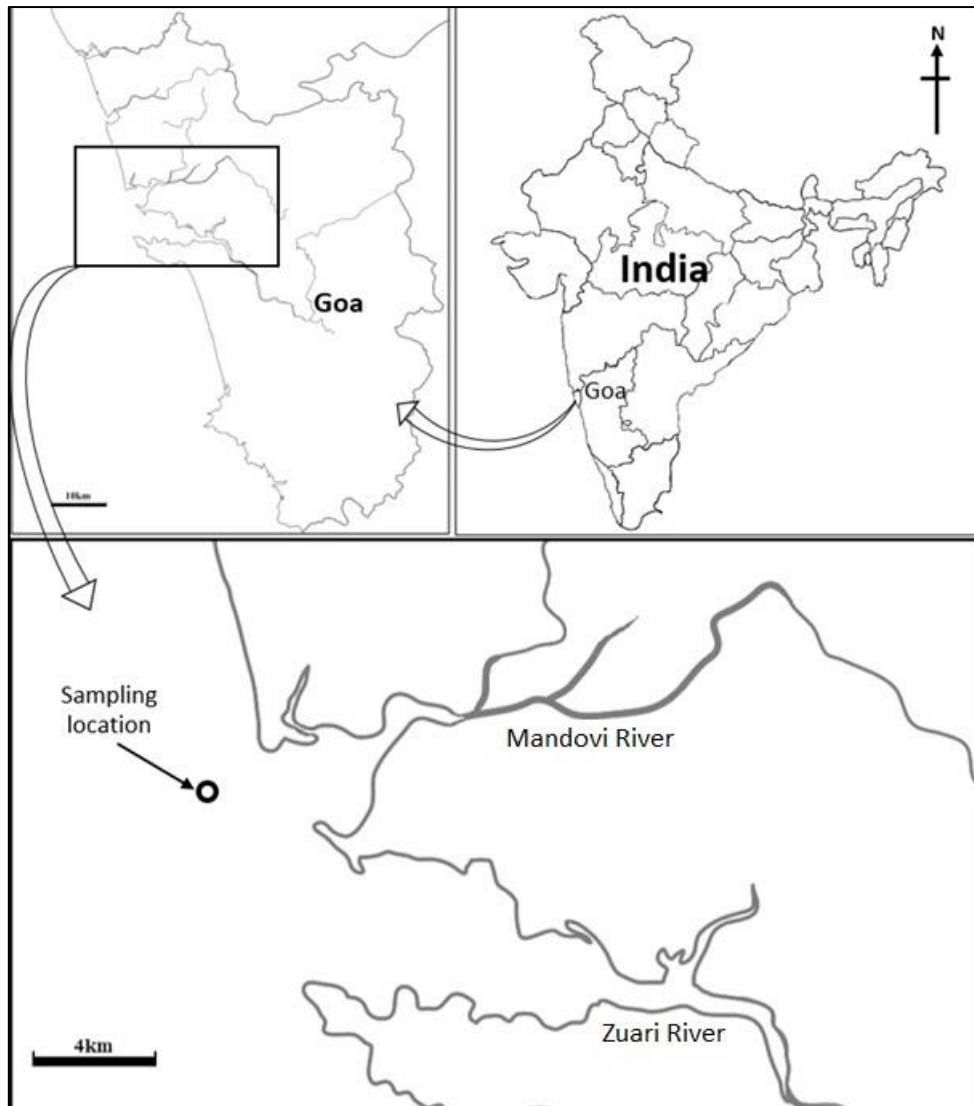


Figure 1

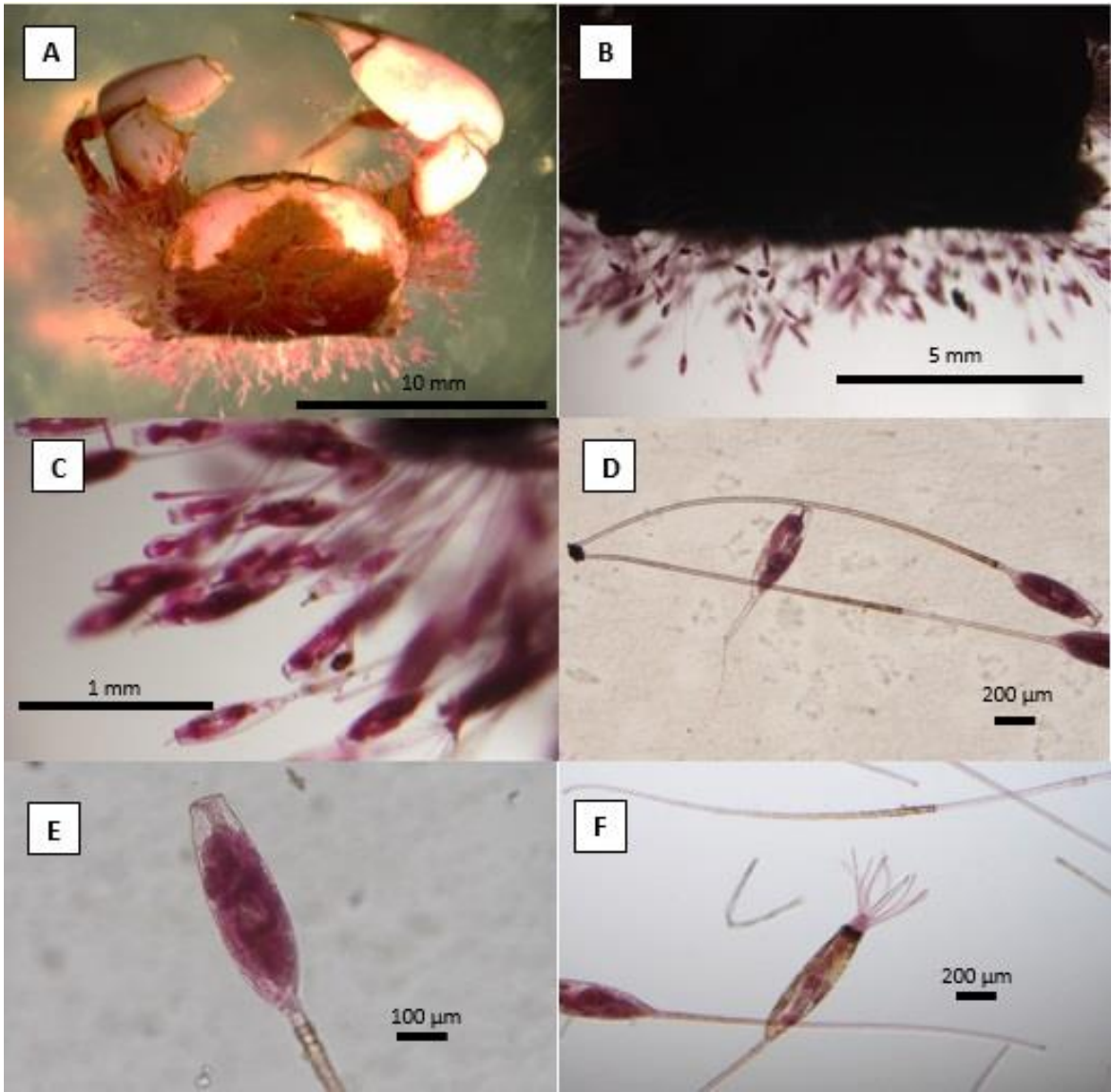


Figure 2

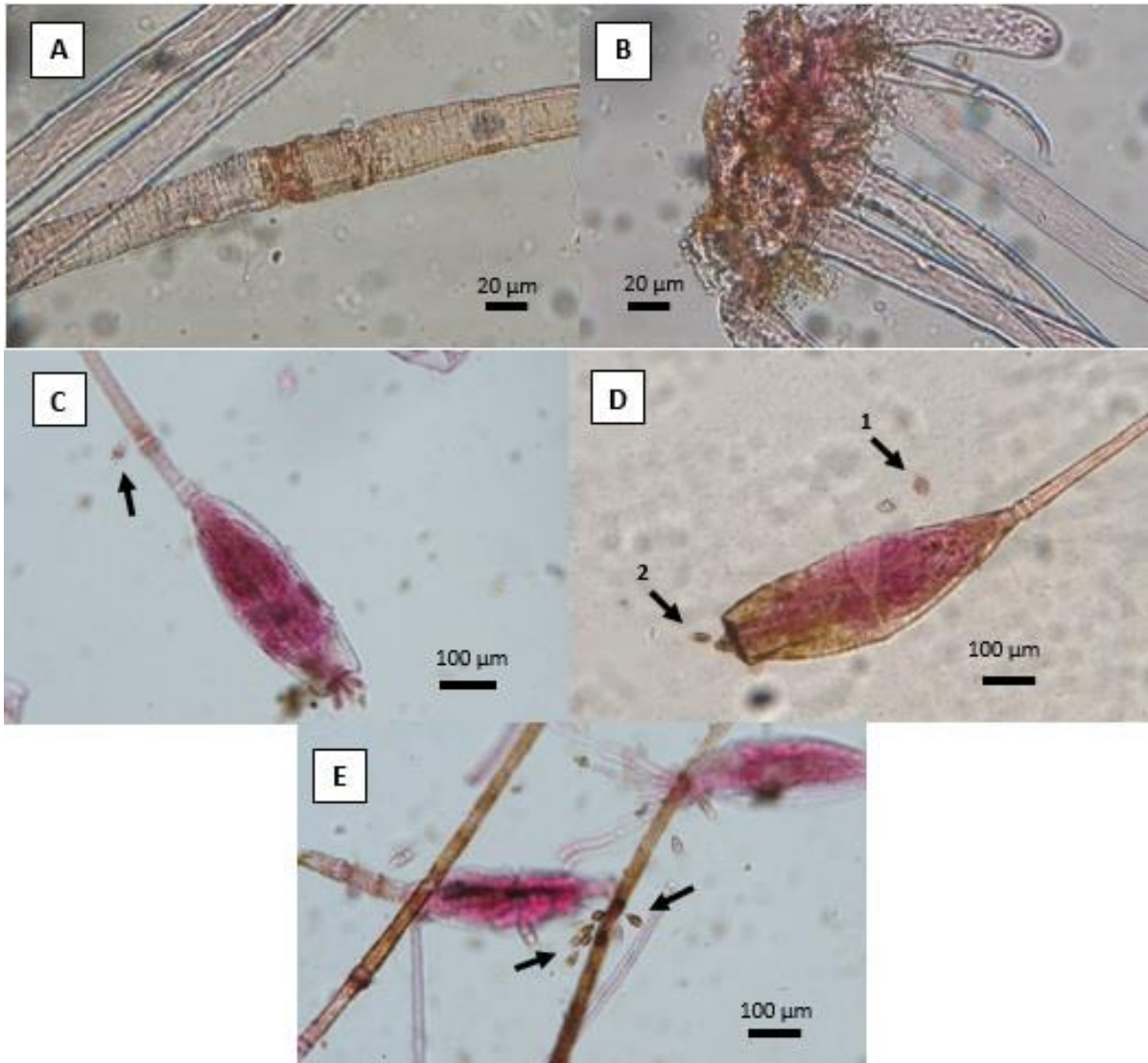


FIGURE 3



FIGURE 4

Table 1. Bryozoan species of *Triticella*, including host/substrate, locality of occurrence, and major references for each species.

Species	Host/substrate	Depth (m)	Ocurrence	References
<i>T. annulata</i> O'Donoghue & O'Donoghue, 1926	Bryozoans; molluscs shell	10–36	NE Pacific	O'Donoghue & O'Donoghue (1926)
<i>T. calveti</i> d'Hondt & Hayward, 1981	Echinoid spines	4149–4327	NE Atlantic	Hayward (1978) (as <i>Triticella</i> cf. <i>pedicelata</i>); d'Hondt & Hayward (1981)
<i>T. capsularis</i> Gordon & Wear, 1999	Paddle crab (Portunidae)	0–3	New Zealand	Gordon & Wear (1999)
<i>T. elongata</i> (Osburn, 1912)	generally associated to different decapod crustaceans; Polychaeta (<i>Chaetopterus</i> sp.)	0–45	NE Atlantic and E Pacific	Osburn (1912), Osburn 1953 Key <i>et al.</i> (1999)
<i>T. flava</i> Dalyell, 1848 ¹	Generally associated to decapod crustaceans	0–1000	NE Atlantic, North Sea, Mediterranean, Adriatic and India	Silén (1935); Menon (1972) (as <i>Triticella koreni</i>); Hayward (1985); Abelló & Corbera 1996; Fernandez-Leborans (2003); di Camillo <i>et al.</i> (2008)
<i>T. fusiformis</i> d'Hondt & Hayward, 1981	Unknown	4287–4301	NE Atlantic	d'Hondt & Hayward (1981)
<i>T. gracilis</i> d'Hondt & Hayward, 1981	Sipunculid worm	2175–2790	NE Atlantic	d'Hondt & Hayward (1981)
<i>T. maiorovae</i> Grischenko & Chernyshev, 2017	Sipunculid worm	3350	N Pacific (Sea of Okhotsk)	Grischenko & Chernyshev (2017)
<i>T. minini</i> Grischenko & Chernyshev, 2015	Spines of echinoid	3307–5362	NW Pacific (adjacent to the Kuril–Kamchatka Trench), and N Pacific (Sea of Okhotsk)	Grischenko & Chernyshev (2015, 2017)
<i>T. nodosa</i> Gordon, 1986	Spines of echinoid	151–297	New Zealand	Gordon (1986)
<i>T. pedicellata</i> (Alder, 1857) ¹	Decapod crustaceans; gastropods shells; hydroids and sea snakes	11–200	NE Atlantic and North Sea, India	Marcus (1940); Osburn 1953; Andersen (1962); Jensen & Bender (1972); Menon (1972); d'Hondt (1983); Pfaller <i>et al.</i> (2012)
<i>T. periphanta</i> Marcus, 1922	Mussel shells; stones	64	New Zealand	Marcus (1922)

¹Species widely reported in different localities, but some of these records still require revision.