International Journal of Zoology Studies
ISSN: 2455-7269; Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 1; Issue 5; July 2016; Page No. 13-24

Description of ten species of the family Didemnidae Giard, 1872 from Gulf of Mannar

[1] SenthamaraiSakthivel, [*2] Meenakshi Vaidyanathapuram Kesavan, [3] Gomathy Sudalaimuthu

[1] Department of Biological Science, Crescent College of Education, Somasipadi PO, Thiruvannamalai District, Tamilnadu, India
[2, 3] Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin - 628002, Tamilnadu, India

Abstract
The knowledge ofascidian biodiversity especially that of the family Didemnidae is incomplete. Collections were made from intertidal rocky areas, a depth of 2-6 meters, trawl discards and mussel landing centres of the coastal districts - Ramanathapuram, Tuticorin and Kanniyakumari. Ten species of colonial ascidians of the family Didemnidae -six of the genus *Didemnum*; one each of *Diplosoma*, *Lissoclinum* and two of *Tridemnum* were identified and added to the records of Indian waters from the Gulf of Mannar region. All the species are described in detail with original camera lucida drawings, taxonomic keys and illustrated with photographs of colonies, spicules and larvae.

Keywords: Didemnidae, *Didemnum*, *Diplosoma*, *Lissoclinum*, *Tridemnum*, Gulf of Mannar

1. Introduction
The family didemnidae is well represented in Indian waters with considerable species diversity. They are found dispersed from lowest low tides to deep sea. Taxonomy of the different genera, intraspecific variation of the species along with inadequate descriptions makes the study of this family a great challenge. Six genera – *Didemnum* Savigny, 1816 [1], *Diplosoma* Macdonald, 1859 [2], *Leptoclinides* Bjerkan, 1905 [3], *Lissoclinum* Verrill, 1871 [4], *Polysyncraton* Nott, 1892 [5] and *Tridemnum* Della Valle, 1881 [6] of the family didemnidae Giard, 1872 [7] were described from Tuticorin, Melakurumpanai, Manapad and Kooduthalai [8, 9, 10, 11, 12, 13, 14]. Previous records from this region includes two species of each of the genus *Didemnum* – *Didemnum psammathodes* Sluiter, 1895 [15], *Didemnum candidum* Savigny, 1816 [1]; *Leptoclinides*- *Leptoclinides madara* Tokioka, 1953 [16], *Leptoclinides rufus* (Sluiter, 1909) [17]; one species of each of the genus; *Diplosoma* – *Diplosoma swamiensis* Renganathan, 1986 [10]; *Lissoclinum* - *Lissoclinum fragile* Van Name, 1902 [18] *Polysyncraton* – *Polysyncraton millepore* Vasseur, 1969 [19] and *Tridemnum* – *Tridemnum cerebriforme*, Hartmeyer, 1913 [20]. It is evident that an exhaustive survey of the Gulf of Mannar water for the occurrence of the colonial ascidian of the family didemnidae has not been done. The present study is based mainly on samples collected from the coastal district of Ramanathapuram, Tuticorin and Kanniyakumari distributed in a wide geographical area of Gulf of Mannar.

2. Materials and Methods
The important aspects of taxonomical study include field survey, collection, narcotisation, fixation, preservation and identification. Sampling has been done in different station from intertidal rocky areas, a depth of 2-6 meters, mussel landing centers and trawl discards from the Gulf of Mannar along the south east coast of India. The colonies were narcotised with a few crystals of menthol and fixed in a mixture of 40 % formaldehyde and sea water in the ratio 1:10 following the methodology suggested by Kott, 1985 [21]. Based on the observations of morphological and anatomical features the collected ascidians were identified to the species level. The taxonomic characters studied include shape, size, colour, nature of the colony (whether stalked, sessile, cushions, encrustations or with lobulations on the surface), nature of test, test inclusions like sand, algal, pigment cells, faecal pellets, size of zooids, position, length and direction of siphons, mantle musculature, number and nature of branchial tentacles, shape of the opening of dorsal tubercle, number of rows and number per row of stigmata in branchial sac, course of gut loop, length of the oesophagus, shape, size of stomach, number, position, shape and orientation of gonads, larval features like size, shape, number of adhesive organs, ampullae, length of tail and incubation in brood pouch, peribranchial cavity or common test and spicules distribution, size, shape and tip of spicule. Key to identification of Indian ascidians given by Meenakshi, 1997 [9] was used. The entire colony, zooids were observed with dissecting, stereo, binocular microscopes and accurately identified. Diagrams were drawn with the help of Camera Lucida for interpretation of results. Voucher specimen of each species has been deposited in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Thoothukudi.

3. Results and Discussion
Colonial ascidians of the Family Didemnidae are characterised by extensive colonies having minute and simple zooids. Calcareous spicules are present in all the genera except *Diplosoma*. Among the different habitats surveyed for the collection of ascidians, maximum number of species was from the trawl discards. The genus *Didemnum* was represented by six species – *Didemnum chartaceum*, *Didemnum cuculliferum*, *Didemnum granulatum*, *Didemnum ossium*, *Didemnum spongioide*, *Didemnum ternerratum*; compared to two of *Tridemnum* – *Tridemnum nubilum*, *Tridemnum paracyclops*; and one each of *Lissoclinum* – *Lissoclinum punctatum* and *Diplosoma* – *Diplosoma virens*.

3.1 *Didemnum chartaceum* Sluiter, 1909 [17]
[Figure - 1 (A & B), Plate - 1 (A to C)]
Didemnum chartaceum Sluiter, 1909, p. 57 [17]
Didemnum chartaceum: Hastings, 1931, p. 97 [22]

Didemnum chartaceum: Kott, 1981, p. 163, 1998, p. 81 [23]
Didemnum obscurum Monniot, 1995, p. 319 (part: colonies from the barrier reef) [24].
Didemnum obscurum: Monniot & Monniot, 1997, p. 1626 [25].
Didemnum nigrum Monniot & Monniot, 1996, p. 158 [26].
Didemnum chartaceum: Kott, 2001, p. 160 [27].

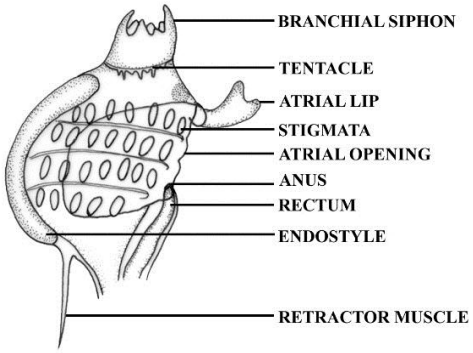
Occurrence
This species was collected from trawl discards of Vedalai, Ramanathapuram District (AS 1502).

Distribution
Australia, Indonesia, Philippines, Fiji, New Caledonia, Arabian Gulf, Andaman Sea, India.

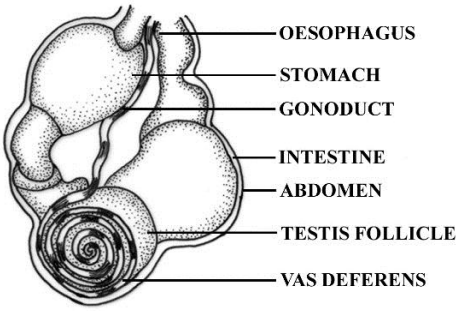
External appearance
Colonies are fleshy, soft and sponge like in appearance. The surface of the colony is produced into many rod or finger like projections. They measure about 6 cm in height and 4 cm width. Outer surface of the colony is very smooth with the presence of thin superficial layer of bladder cells arranged uniformly. Numerous round common cloacal openings are present with 1 mm distance between them. Superficial layer of test is very soft, easily torn and the inner test is fleshy black in colour. Thorax is attached to the outer layer of test, abdomen is embedded in the inner fleshy part in a longitudinal line. Small stellate spicules (0.1 mm - 0.2 mm) are distributed evenly in the test but distribution is lesser around the common cloacal opening. Brown colour pigment cells are sparsely distributed in the test. Algal cells and faecal pellets were not observed.

Internal appearance
Zooids are cylindrical and black coloured. The thorax (0.5 mm) and abdomen (0.6 mm) are of equal length. Branchial siphon short wide with 6 pointed lobes. Atrial opening is wide exposing a large part of branchial sac with a large bifid atrial languet measuring 0.05 mm length. A small retractor muscle is present along the anterior region of the oesophagus. At the base of branchial siphon about 16 small and large tentacles are arranged alternating with each other. Prebranchial area is wide. Thorax has 4 rows of stigmata with 7 in the first 3 rows and only 5 in the 4th row. Stigmata are small rod shaped and evenly spaced. In between the stigmatal rows a thick band of transverse muscles is present. There is a short oesophagus with pear shaped stomach, short duodenum, large post stomach and tubular rectum which connects the anus. It opens into the atrial cavity at the level opposite to the 3rd row of stigmata. Anus is smooth, small and round in shape. There is a testis follicle in gut loop with 4 coils of vas deferens around it. Larvae are embedded in the fleshy test. They are black coloured with 3 funnel shaped adhesive organ and numerous rod shaped ampulla. Tail is wound half way around the head.

Remarks
The present species differs from *Didemnum granulatum* Tokioka, 1954 [28], *Didemnum cuculliferum* (Sluiter, 1909) [17], *Didemnum ternerratum* Kott, 2001 [27], *D. candidum* and *Didemnum spongioide* Sluiter, 1909 [17] in the absence of an atrial languet. It resembles *Didemnum ossium* Kott, 2001 [27] in the presence of atrial languet but differs from it in the nature of the colony and colour of zooids and larva. The identifying features of the present species are the spongy appearance of the colony, ash or black coloured zooids and larva. It resembles *D. chartaceum*: Kott, 2001 [27] in the appearance of the colonies, colour of zooids and larva, presence of atrial languet and several ectodermal ampullae in the larva but differs from it in having larger thorax than the abdomen and more stigmata per row.



A



B

Fig 1: *Didemnum chartaceum* **A**. thorax, **B**. abdomen.
Scale: **A & B** 1cm = 0.05 mm

A **B** **C**

Plate 1: *Didemnum chartaceum* **A.** Colony **B.** Larva **C**. Spicules

3.2 *Didemnum cuculliferum* (Sluiter, 1909) [17]
[Figure - 2 (A to C), Plate - 2 (A to C)]
Diplosomoides cuculliferum Sluiter, 1909, p. 90 [17]
Didemnum cuculliferum: Kott, 1981, p. 164 [23]
Didemnum turritum Michaelsen, 1930, p. 521 [29]
Didemnum nekozita Tokioka, 1967, p. 67 [30]
Didemnum anoi Monniot & Monniot, 1987, p. 25 [31]
Didemnum moseleyi: Eldredge, 1967, p. 210 (part, from Eniwetak) [32]
Didemnum cuculliferum: Kott, 2001, p. 167 [27]

Occurrence
This species was collected from a depth of 2-3 meters, Enayam, Kanniyakumari District (AS 1550).

Distribution
Australia, Indonesia, Palau Islands, Eniwetak, Philippines, French Polynesia, India.

External appearance
Colonies are white thick encrusting sheet measuring 2 cm X 1.3 cm with 3 mm thickness. The outer surface of the colony is elevated and smooth. Star shaped common cloacal opening is present at the elevations. A superficial layer of bladder cells is present. Spicules are distributed uniformly but at the place around the common cloacal opening the number is reduced. Test is very soft and zooids are arranged in circular systems. There are no faecal pellets, pigment cells or algal cells in the test. Spicules are spherical with 7-9 pointed rays in optical section.

Internal appearance
Zooids are yellow in colour measuring about 0.7 mm long with thorax and abdomen. Thorax is elongate and cylindrical. Branchial siphon is wide tube like with 6 pointed triangular lobes. Atrial opening is medium C shaped. Prebranchial area is narrow. Retractor muscle is long, tapering and with a pointed end. It originates from the base of the endostyle, runs attached along oesophagus half way and then hangs freely. There are 4 rows of stigmata with 5 per row. They are long and rod shaped. An atrial languet is absent. Oesophagus is long tube like. Stomach is pear shaped leading to a short duodenum, small post stomach and tubular rectum which leads to the anus. The anus opens at the base of the 4th row of stigmata. Single spherical testis follicle is present at the base of gut loop. There are 5 coils of vas deferens around the testis. Larva is present in the basal test. They measure about 0.6 mm. Three cone shaped ectodermal adhesive organ alternate with 5 pairs of ampulla. Tail is wound half way round the head.

Remarks
D. chartaceum and *D. ossium* differs from the present species in having atrial languet. It resembles *D. granulatum* in the absence of atrial languet but differs from it in the nature and colour of the colony. *D. ternerratum*, *D. candidum* and *D. spongiode* differs from *D. cuculliferum* in having rounded common cloacal opening. The colony of *D. cuculliferum* described by Kott, 2001 [27] differs from the present species in the shape of spicules with 5-7 rod shaped rays with blunt tips, pigment cells and V shaped mass in gut loop compared to the Indian colonies which are thick encrusting with soft test, spicules with 7-9 pointed rays, absence of pigment cells and V shaped mass in gut loop.

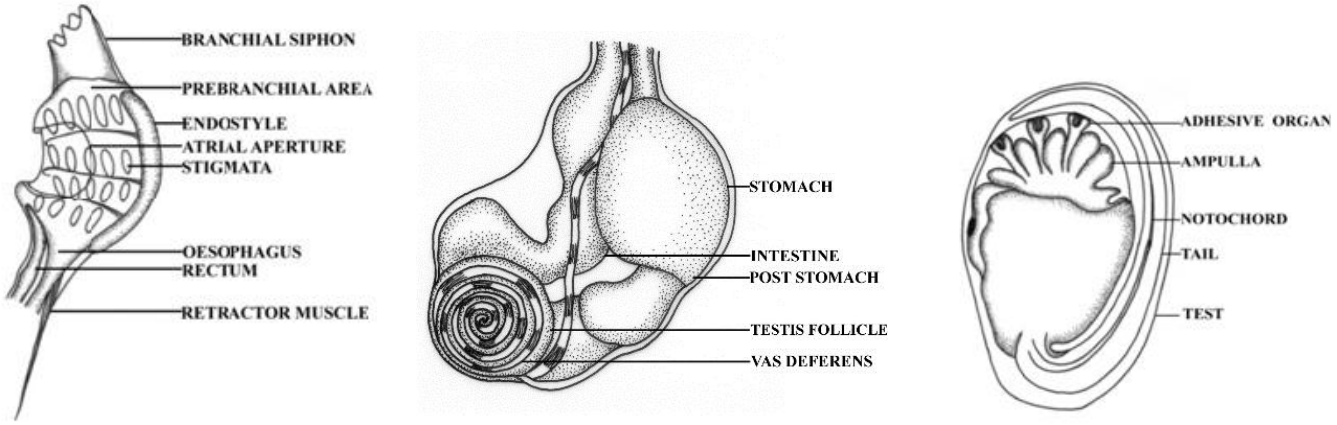


Fig 2: *Didemnum cuculliferum* **A**. thorax, **B**. abdomen, **C**. larva. Scale: **A to C** 1cm = 0.05 mm

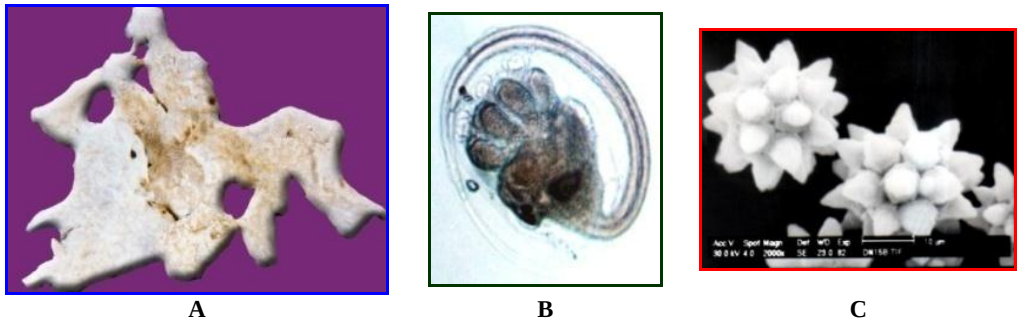


Plate 2: *Didemnum cuculliferum* **A.** Colony **B**. Larva **C**. Spicules

3.3 *Didemnum granulatum* Tokioka, 1954 [28]
[Figure - 3 (A to C), Plate 3 - (A & B)]
Didemnum (*Didemnum*) *moseleyi f. granulatum* Tokioka, 1954, p. 244, 1967, p. 67 [28, 30]
Didemnum moseleyi: Eldredge, 1967, p. 210 (part) [32]
Didemnum granulatum: Kott, 1981, p. 167 [23]
Didemnum granulatum: Monniot & Monniot, 1987, p. 31 [31]
Didemnum pele: Eldredge, 1967, p. 197 [32]
Didemnum granulatum: Kott, 2001, p. 188 [27]

Occurrence
This species was collected from mussel landing center at a depth of 5-6 meters from Enayam puthenthurai, Kanniyakumari District (AS 1345).

Distribution
Australia, Tokara Islands, Fiji, Palau Islands, French Polynesia, Hawaii, India.

External appearance
Colonies are thin investing sheets measuring about 4 cm X 2 cm. Surface of the colony is smooth. The colonies are various shades of orange in life and in preservative. Numerous star shaped common cloacal openings are arranged at a distance of 0.3 mm between them. Zooids are arranged in circular manner. Bladder cell layer is absent. Stellate spicules with 7-9 long pointed conical rays in optical section are sparsely distributed in the test. Test is hard and brittle. Uniformly distributed pigment cells are present. There are no algal cells or faecal pellets. Larva present at the basal layer of test.

Internal appearance
Zooids are yellow in colour, robust measuring 1-1.05 mm length. Branchial sac is wide and rectangular. Branchial aperture is wide fringed with smooth rounded lobes. Atrial aperture is deep, slit like without an atrial languet. Retractor muscle originates at the base of the endostyle as a thin filament. There are 4 rows of stigmata, 6 stigmata in the first 2 rows, five and four in the third and fourth row. They are long and oval in shape. In between the rows of stigmata thick transverse muscle bands are present. At the base of the branchial siphon 10 short tentacles are present. Prebranchial area is narrow. Oesophagus is short. Stomach is large yellow and oval. There is a short post stomach, duodenum and tubular rectum. Anus is a smooth rounded opening situated opposite the level of 4th row of stigmata. No gonads were observed. Larva measures about 0.55 mm in length. They have 3 funnel shaped short ectodermal adhesive organ and 4 ampulla alternating with each other. Tail is wound three fourth the way around the head.

Remarks
D. chartaceum and *D. ossium* have atrial languet hence they cannot be confused with the present species. *D. ternerratum*, *D. candidum* and *D. spongioide* have round common cloacal opening but *D. granulatum* has star shaped common cloacal opening. The colony of *D. cuculliferum* are thick encrusting sheets with white colour. The salient features of the Indian colony are thin investing sheets, star shaped common cloacal opening, hard and brittle test, sparsely distributed stellate spicules, pigment cells in test and yellow coloured zooids. The colony collected from Indian water resembles *D. granulatum*: Kott, 2001 [27] in all respects.

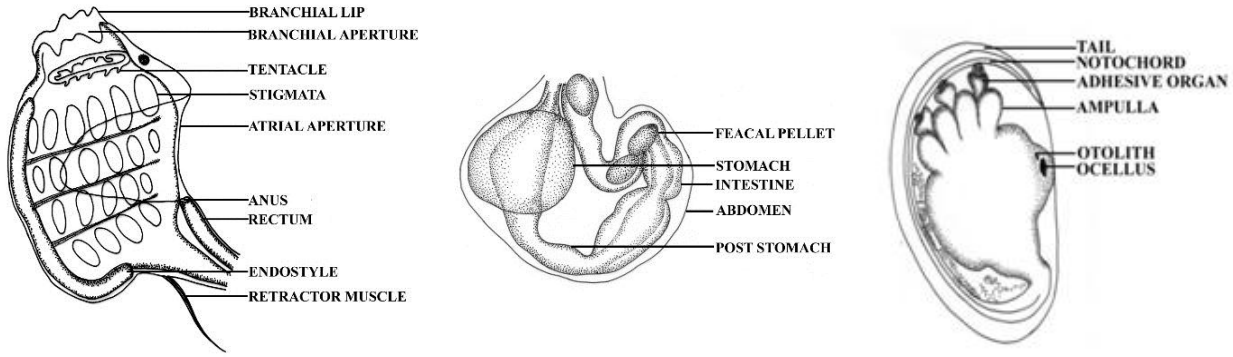


Fig 3: *Didemnum granulatum* **A**. thorax, **B**. abdomen, **C**. larva. Scales: **A to C** 1cm = 0.05 mm.

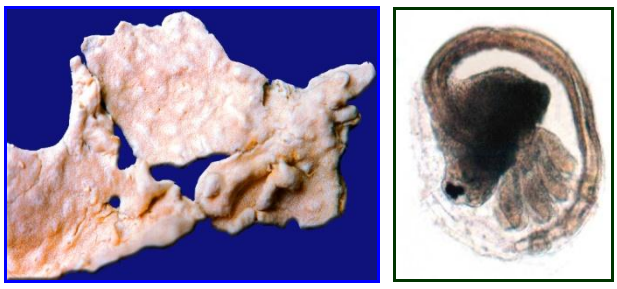


Plate 3: *Didemnum granulatum* **A.** Colony, **B.** Larva.

3.4 *Didemnum ossium* Kott, 2001 [27]
[Figure – 4 (A to C), Plate - 4 (A, B)]
Didemnum (*Didemnum*) *misakiense*: Tokioka, 1967, p. 75 (part, specimens from the Philippines) [30]
Didemnum spongioides: Millar, 1975, p. 232 [33]
Didemnum ligulum: Monniot, 1995, p. 313 [24]
Didemnum ossium Kott, 2001, p. 216 [27]

Occurrence
This species was collected from trawl discards of Veerapandiapattinam, Tuticorin District (AS 418).

Distribution
Western Australia, New Caledonia, Philippines, Northern Territory, India.

External appearance
Colonies are irregular cushions measuring about 3.5 cm in extent, white in life and preservative. An upper spicule-free thick superficial layer of test is present. The remaining part of the test is impregnated sparsely with small stellate spicules. The surface of the colony is slightly raised into many irregular folds. Spicules are 0.025 mm in diameter with 11-13 pointed rays in the equatorial plane. Numerous round common cloacal apertures were observed. Zooids are arranged in a circular manner. Algal cells, pigment cells and faecal pellets are absent. The lacunae are well defined and limited to the thoracic region.

Internal appearance
Thorax measures 0.8 mm and abdomen 0.4 mm. Zooids are robust. The branchial sac is cylindrical in shape. Branchial siphon is long, cylindrical with 6 papillated lobes. Atrial aperture is wide with a bifid atrial tongue of varying size protruding from the anterior rim of the aperture. The atrial aperture exposes a large part of the branchial sac. Prebranchial area is wide. There are about 20 small branchial tentacles at the base of the branchial siphon. 4 rows with 6-10, long, cylindrical stigmata in each row. A long tapering retractor muscle measuring 1 mm extends from the base of the endostlye and hangs freely. The alimentary canal is slightly horizontal and is bent at right angles to the thorax. The oesophagus is long, stomach oval, with a short duodenum, small post stomach and a long tubular rectum. Anus has a round opening situated at the level of 4th row of stigmata. A single oval testis is situated in the gut loop. The vas deferens coil 6 times. Larvae are present in the cavities of test. The trunk is 0.5 mm long and the tail is wound three-fourth the way around it. A distinct circlet of 25 long narrow ectodermal ampullae encircles the anterior end of the larval trunk with its 3 antero-median adhesive organs. An otolith and an ocellus are in the cerebral vesicle.

Remarks
D. granulatum, *D. cuculliferum*, *D. ternerratum*, *D. candidum* and *D. spongiode* differ from the present species in the absence of an atrial languet. *D. chartaceum* resembles *D. ossium* in the presence of an atrial languet but differs in having spongy appearance of colonies, ash or black coloured zooids and larvae. The identifying characters of *D. ossium* are the presence of bifid atrial languet, irregular cushion shaped, white coloured colony, larger thorax, smaller abdomen and 25 long narrow ectodermal ampullae in the larva. *D. ossium*: Kott, 2001 [27] resembles the Indian species in the nature of colonies but differs in the larger size of larva and in having a distinct corolla of 34 long narrow ectodermal ampullae.

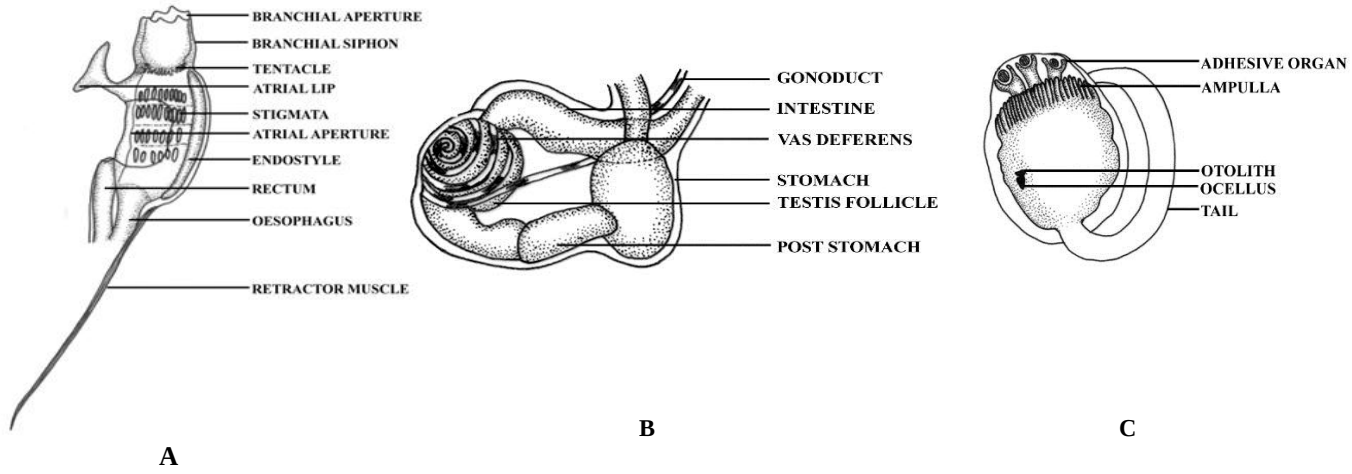


Fig 4: *Didemnum ossium* **A**. thorax, **B**. abdomen, **C**. larva. Scales: **A to C**1cm = 0.12 mm

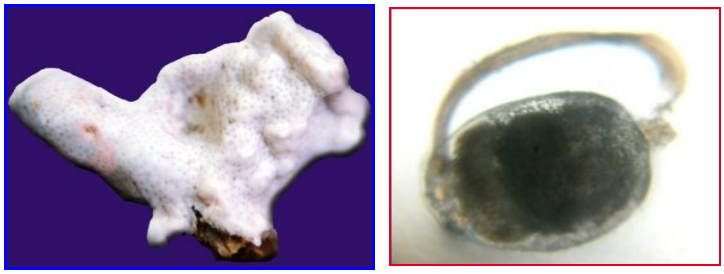


Plate 4: *Didemnum ossium* **A**. Colony **B**. Larva

3.5 *Didemnum spongioide* Sluiter, 1909 [17]
[Figure - 5 (A to C), Plate -5]
Didemnum spongioides Sluiter, 1909, p. 67 [17]
Didemnum spongioides: Monniot, 1995, p. 326 [24]
Didemnum spongioide: Kott, 2001, p. 236 [27]

Occurrence
This species was collected from intertidal area of Keelavaipar, Tuticorin District (AS 746).

Distribution
Australia, Indonesia, New Caledonia, India.

External appearance
Colonies are white, irregular and sponge like in appearance measuring 5 cm X 2 cm. Surface of the test is smooth. The entire colony contains only 2 circular common cloacal openings. Zooids are arranged in a circular manner. Test is soft and zooids are easy to remove. Spicules are distributed uniformly, concentrated in outer layer. In the basal test layer spicules are sparsely distributed. The stellate spicules measure about 0.02-0.03 mm with 9-11 conical, pointed rays in optical section. Superficial layer of bladder cells were not observed. Algal cells, pigment cells and faecal pellets were not present in the colony studied.

Internal appearance
Zooids are yellow in colour measuring 7 mm long with equal thorax and abdomen. The branchial sac is long and cylindrical. Prebranchial area is wide with a short cylindrical branchial siphon having six rounded lobes. Atrial aperture is wide 'C' shaped opening. There is no atrial languet. The long, pointed retractor muscle originates from the branchial sac and extends freely from the base of the endostyle. It measures about 0.25 mm long. At the base of branchial sac and in between the stigmatal rows thick transverse muscle bands are present. The branchial sac has 4 rows of rod shaped stigmata with 6 per row. Oesophagus is long tube like. The stomach is pear shaped which leads into a long duodenum, a short post stomach and a long cylindrical rectum. Anus is smooth rounded opening at the level of 4th row of stigmata. A single testis and a female follicle is present in the gut loop. The vas deferens coils 7 times. Larvae embedded in test are oval measuring 0.35 mm long. 3 median long adhesive organ alternates with 4 long ampullae. The tail is wound three fourth way round the head. Larva contains an oozoid.

Remarks
D. chartaceum and *D. ossium* differs from *D. spongioide* in the presence of a bifid atrial languet.In *D. granulatum* and *D. cuculliferum* the common cloacal opening is star shaped. *D. ternerratum* differs from present species in having cushion shaped colonies with elevations and hard test. *D. candidum* differs from *D. spongioide* in having snowy white encrusting sheet like colony with larger zooids. The salient features of the present species are white coloured, sponge like colonies with smooth surface and small yellow zooid. It resembles *D. spongioide*: Kott, 2001 [27] in all respects except in the presence of stellate spicules with 9-11 conical pointed rays compared to only 7-9 in the Australian specimen and smaller zooids in the Indian species.

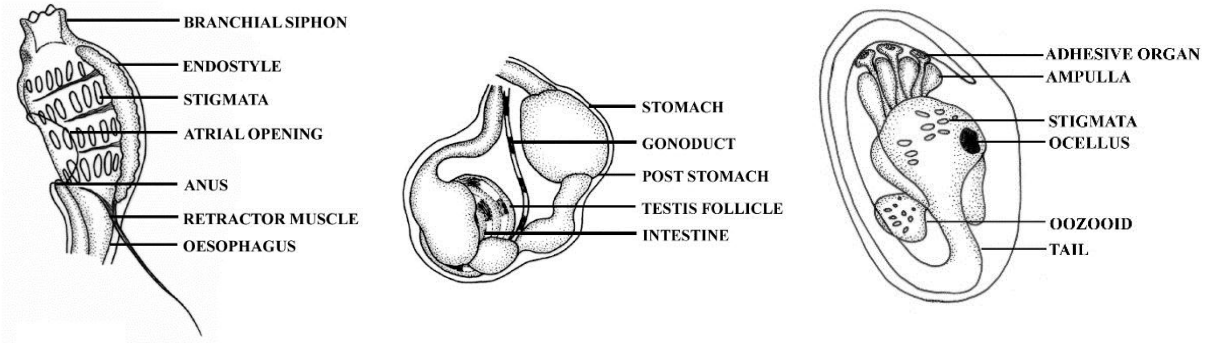


Fig 5: *Didemnum spongioide* **A**. thorax, **B**. abdomen, **C**. larva. Scales **A to C** 1cm = 0.05 mm

Plate 5: *Didemnum spongioide*-Larva

3.6 *Didemnum ternerratum* Kott, 2001 [27]
[Figure - 6, Plate - 6]
Didemnum ternatanum: Kott, 1972, p. 179 [34]
Didemnum roberti: Kott, 1976, p. 68, 1998, p. 83 (part, Bass Strait and Great Australian Bight records) [35, 36]
Didemnum ternerratum Kott, 2001, p. 241 [27]

Occurrence
This species was collected from a depth of 5-6 meters from Tuticorin harbour area, Tuticorin District (AS 733).

Distribution
Australia, India.

External appearance
Colonies are thin, hard, cushion shaped with elevated outer surface measuring 5 cm X 4 cm. In life they are brick red in colour and in preservative the colour fades and turns to orange. The surface test is raised into elevations where the common

cloacal openings are present. Common cloacal opening is circular situated 1 cm apart. Outer surface of the test has a thick bladder cell layer. Spicules are absent in this layer. They are sparsely distributed in the test below the bladder cell layer. Stellate spicules are 0.2 mm in size with very short, numerous conical pointed rays. Test is firm. Zooids are attached to the test and are easy to remove. Faecal pellets are absent in the test but pigment cells and algal cells are present and uniformly distributed.

Internal appearance

Zooids are long, orange in colour measuring 1 mm long. The thorax of the zooids has sparsely distributed algal cells. Branchial siphon is long funnel shaped with 6 triangular lobes. The atrial opening extends from the 2nd to the 4th row of stigmata but does not expose the branchial sac. From the base of branchial sac a long pointed retractor muscle extends along the oesophagus. At the base of branchial siphon a circlet of long tentacles are present. Prebranchial area is wide. Elongate cylindrical branchial sac contains 4 rows of long cylindrical stigmata with 8 in 1st, 7 each in 2nd and 3rd row and 6 in 4th row. In between the stigmatal rows thick muscular band is present. The alimentary canal consists of a long oesophagus, pear shaped stomach, long duodenum, short post stomach and a wide rectum which ends in a smooth rounded anus at the base of the fourth row of stigmata. Single round testis follicle and a female follicle are in the gut loop with 6 coils of vas deferens. In a single colony studied no larvae were observed.

Remarks

D. ternerratum differs from *D. chartaceum* and *D. ossium* in the absence of a bifid atrial languet. The common cloacal openings of *D. granulatum* and *D. cuculliferum* are star shaped compared to the rounded opening in *D. ternerratum*. *D. candidum* and *D. spongioide* differs from the present species in having colonies without cushion shaped elevations and soft test. *D. ternerratum* has special features like thin, hard, cushion shaped elevations on the surface, brick red colour, circular common cloacal opening, orange coloured long zooid and long retractor muscle. *D. ternerratum* Kott, 2001 [27] differs from the Indian specimen in having longer zooids (2 mm) and in the presence of an atrial languet.

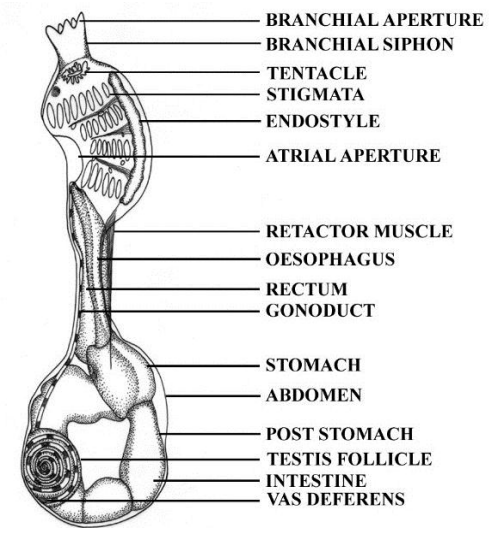


Fig 6: *Didemnum ternerratum* - zooid. Scale 1cm = 0.05 mm

Plate 6: *Didemnum ternerratum*- Colony

Key to the species of *Didemnum* recorded

1. Faecal pellets crowded in the outer layer of test ……..……..……..……..*D. psammathodes*
   Faecal pellets not crowded in the outer layer of test …….……..……..……..…..……2
2. Atrial languet present ……..……3
   Atrial languet absent ……...……..4
3. Colonies spongy in appearance, zooids and larva ash or black coloured .....*D. chartaceum*
   Colonies not spongy in appearance, zooids and larva not ash or black coloured .....*D. ossium*
4. Star shaped common cloaca opening ……..……5
   Rounded common cloacal opening ……6
5. Orange coloured thin investing sheet...……*D. granulatum*
   White coloured thick encrusting sheet..……...*D. cuculliferum*
6. Colonies with cushion shaped elevation and hard test ……...…..……...…..……*D. ternerratum*
   Colonies without cushion shaped elevation and with soft test ……..……...……...……..…….. 7
7. Snowy white encrusting sheet like colony with larger zooids ……...…..……...…..... *D. candidum*
   White coloured sponge like colonies with small zooids ..……...……...……. *D. spongioide.*

3.7 *Diplosoma virens* (Hartmeyer, 1909) [37]

[Figure - 7 (A & B), Plate - 7]

Diplosoma viride Herdman, 1906, p. 341 [38]
Leptoclinum virens Hartmeyer, 1909, p. 145-6 [37]
Leptoclinum virens: Tokioka, 1942, p. 500, 1967, p. 68 [39]
Leptoclinum virens: Kott, 1966, p. 291 [40]
Diplosoma virens: Hastings, 1931, p. 102 [22]
Diplosoma virens: Newcomb & Pugh, 1975, p. 533 [41]
Diplosoma virens: Thinh & Griffiths, 1977, p. 673 [42]
Diplosoma virens: Thinh, 1978, p. 617 [43]
Diplosoma virens: Kott, 1980, p. 22, 1981, p. 193, 1982, p. 114, 1998, p. 85 [44, 23, 45, 36]
Diplosoma virens: Monniot, 1994, p. 10 [46]
Leptoclinum simile Sluiter, 1909, p. 77 (part) [17]
Leptoclinum varium Sluiter, 1909, p. 80 [17]
Leptoclinum calificiforme Sluiter, 1909, p. 82 [17]
Leptoclinum calificiforme: Van Name, 1918, p. 160 [47]
Diplosoma pavonia Monniot & Monniot, 1987, p. 60 [31]
Diplosoma virens: Kott, 2001, p. 347 [27]

Occurrence

This species was collected from trawl discards of Seeniyappa Dharga, Ramanathapuram District (AS 1100).

Distribution

Australia, West Pacific, Fiji, Indonesia, India.

External appearance
Colonies are irregular sheets found attached to the surface of mollusc shell measuring 6.5 cm long, 2.5 cm breadth and 2 mm thickness. At the place of the common cloacal opening conical protrusions are present. Zooids are arranged around the central common cloacal cavity. The test is dark green in colour in life. In preservative it is opaque in nature, transparent and firm. Zooids are embedded in the narrow canals of test traversed by strands connecting them to the basal test. Symbiotic prochloron cells are packed in the test and in the zooids. Pigment cells and faecal pellets were not observed. Long stolonic vessels from the abdominal region of zooid extend through basal test.

Internal appearance
Zooids are difficult to remove from their tough test. They are with 0.55 mm long thorax and 0.45 mm abdomen. In preservative, the zooids are yellowish in colour with a dark yellow stomach. Branchial sac is narrow and long. Branchial siphon is at the anterior end, cylindrical, narrow with a sphincter muscle at the base. The branchial lobes are triangular. About 20 simple branchial tentacles of two sizes are present at the base of the branchial siphon. The prebranchial area is wide. Black pigment spot is present in the body wall near the neural ganglion. Atrial aperture is a 'C' shaped wide opening exposing a part of the branchial sac. There is no atrial languet. Five or six oval or elliptical stigmata measuring 0.05 mm present in each of the four rows. Throughout the zooid prochloron cells are distributed uniformly. A long fine retractor muscle hangs freely halfway down along the oesophageal neck. The abdomen is bent up at right angles to the long axis of the thorax and oesophageal neck. The post pyloric part of the gut is divided into a pear shaped stomach, cylindrical duodenum and long rectum. There were no gonads in the colony studied. Larvae were observed in the cavities of the basal test. The larva measures 2.0 mm in length. The tail is wound half way round. There are 3 median adhesive organs alternating with 4 pairs of ectodermal ampullae.

Remarks
The colonies of *D. swamiensis* Renganathan, 1986 [10] are encrusting, soft gelatinous, semitransparent, white, grey or yellow compared to the present colonies which are irregular sheets, transparent, firm and dark green in colour. The peculiar arrangement of ampullae observed in the larva of *D. swamiensis* has not been seen in the present species. The characteristics of *D. virens* noticed are thin irregular sheets, dark green coloured colonies and yellowish zooids, presence of prochloron in test and zooids, test connectives, variation in the number of lateral ampullae and adhesive organs in the larvae. The species studied differs from *D. virens*: Kott, 2001 [27] in the size and thickness of colonies.

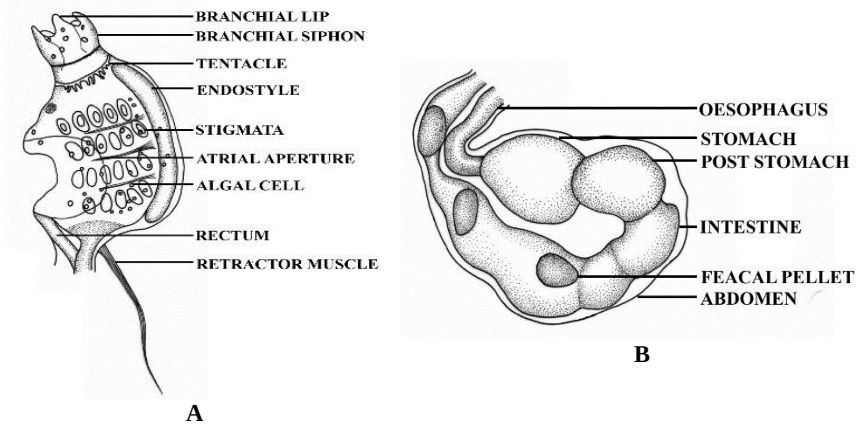


Fig7: *Diplosoma virens* **A**. thorax, **B**. abdomen Scale: **A & B 1cm** = 0.05 mm

Plate 7: *Diplosoma virens* - Colony

Key to the species of *Diplosoma* recorded
1. Encrusting, soft, gelatinous, semi transparent, white, grey or yellow colonies ..…… *D. swamiensis*
2. Thin, irregular sheets, transparent, firm, green colonies ..……..…..……..……..……..…..….. *D. virens*

3.8 *Lissoclinum punctatum* Kott, 1977 [48]
[Figure - 8, Plate - 8]
Lissoclinum punctatum Kott, 1977, p. 620, 1980, p. 20, 1981, p. 198, 1982, p. 114, 1998, p. 88 [48, 44, 23, 45, 36]
Lissoclinum punctatum: Monniot, 1992, p. 575 [49]
Lissoclinum molle: Newcomb & Pugh, 1975, p. 533 [41]
Lissoclinum punctatum: Kott, 2001, p. 318 [27]

Occurrence
This species was collected from trawl discards of Vellapatti, Tuticorin District (AS 382).

Distribution
Australia, West Pacific, Fiji, New Caledonia, Singapore, India.

External appearance
Colonies are very small, spherical or oval (0.4 cm) with a smooth surface attached to sea weeds. They are bright green in

colour because of the presence of prochloron cells. In preservative, the colour fades and the colony becomes transparent. The common cloacal cavity is large. About 10-15 zooids are arranged around the common cloacal cavity. Spicules are not found in places of common cloacal cavity. Prochloron cells are packed in the common cloacal cavity and in the test around the zooids. Test is soft and gelatinous. Zooids are surrounded by spherical spicules. Spicules are not present in other parts of the test. The rays of spicules are rod like having flat ends measuring 0.01 mm.

Internal appearance

Zooids are small about 0.85 mm long with thorax measuring 0.45 mm and abdomen 0.40 mm. branchial sac is long and narrow. The branchial siphon is short, cylindrical with s156+ix tongue shaped lobes. The atrial aperture is a wide opening which exposes most of the branchial sac. There is no atrial languet and retractor muscle. The prebranchial area is wide. There are 5-6 elongated rod like stigmata in each of the 4 rows. A small lateral organ is present half way on the thorax near the side of the endostyle. A simple gut loop consists of a short oesophagus, large oval stomach, short duodenum, small post stomach and a long tubular rectum. The gut loop bends at the region of the oesophagus and lies at right angles to the thorax. Two abdominal buds are present arising from the oesophageal neck. The pharynx of the blastozooids are perforated with stigmata. Gonads and larvae were not present in the colony studied.

Remarks

The colonies of *L. fragile*: Renganathan, 1982 [12] already reported from Indian water are soft thin encrusting white with extensive cloacal cavity. The specimen studied at present has very small, spherical or oval colonies which are bright green in colour. The Indian colonies differ from *L. punctatum* described by Kott, 1977 [48] in the nature of colonies and lesser number of stigmata per row.

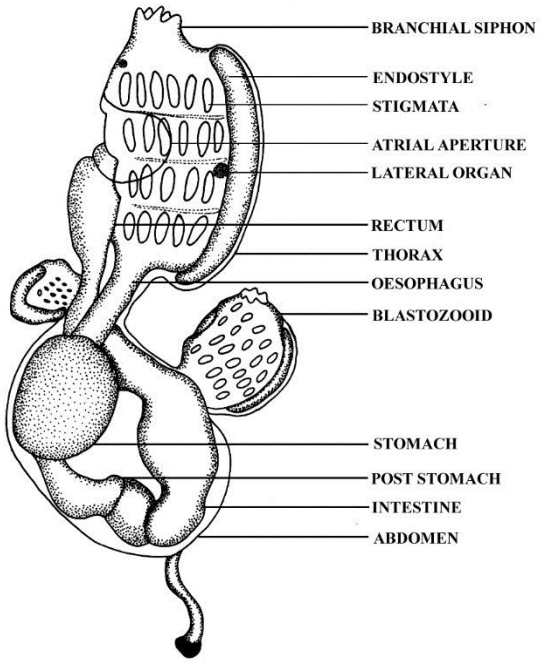


Fig 8: *Lissoclinum punctatum* - zooid Scale 1cm = 0.05 mm

Plate 8: *Lissoclinum punctatum* - Colony

Key to the species of *Lissoclinum* recorded

1. Soft, thin, encrusting, white colonies …..……*L. fragile*

Small, soft, round or oval, bright green colonies … *L. punctatum*

3.9 *Trididemnum nubilum* Kott, 1980 [43]

[Figure - 9 (A to C), Plate - 9 (A & B)]

Trididemnum nubilum Kott, 1980, p. 9, 1981, p. 188, 1982, p. 105, 1998, p. 92 [44, 23, 45, 36]

Trididemnum viride: Tokioka, 1967, p. 87 (part, specimens with spicules with numerous rays) [30]

Trididemnum nubilum: Kott, 2001, p. 274 [27]

Occurrence

This species was collected from trawl discards of Periyapattinam, Ramanathapuram District (AS 1293).

Distribution

Australia, Philippines, Fiji, India.

External appearance

Colonies are small, cushion shaped, measuring 2 cm X 1 cm with 1 mm thickness attached onto the surface of seaweeds. In life, colonies are white in colour, in preservative they become transparent. Circular common cloacal openings surrounded by 15-20 zooids. Zooids are evenly distributed in the test and are easy to remove. Surface of the colony is smooth. Test is very hard and leathery. Spicules stellate, short pointed, conical rays and are evenly distributed throughout the upper layer of test while they are absent from the basal layer. Pigment cells are present. Faecal pellets, algal cells and bladder cells are absent. Thorax of the zooid is attached to the upper test mass.

Internal appearance

Zooids are small, yellowish in colour with 0.2 mm thorax and 0.25 mm abdomen. Thorax is oval in shape. Branchial siphon is short dome shaped with triangular branchial lobes. Atrial opening is a small incision situated at the level of second and third row of stigmata. Retractor muscle is short (0.06 mm) with a pointed tip originating from the middle of the oesophagus. Prebranchial area is narrow. At the base of the branchial sac numerous short pointed tentacles are present. There are three rows of stigmata with 3-5 per row. Stigmata are wide and oval in shape. The size of the stigmata gradually decreases from the first to the third row. Oesophagus is short. Abdomen contains a round stomach, long duodenum, small post stomach and a tubular rectum leading to the smooth round anus. Endostylar pigment cap and pigment cells in the intestinal loop are absent. One male and a female follicle are present in the gut loop. Vas

deferens has 5 coils. Larvae are incubated in the test. They measure 0.4 mm long. There are three median adhesive organs in between the four ectodermal ampullae. Tail is wound round the head three fourth the way.

Remarks

The colonies of *Trididemnum cerebriforme*: Meenakshi, 2000 [14] already reported from Indian water are flat, encrusting, tough, irregular, milky white coloured with patches of green cells on the surface. The zooids have a distinct atrial siphon. *Trididemnum paracyclops* Kott, 1980 [44] have thin, encrusting sheet like colonies with yellow colour and endostylar pigment cap. The identifying characters of the present species are its small, cushion shaped test and white colonies, hard and leathery test, short zooid and oesophagus without an atrial siphon and endostylar pigment cap. The Indian colony differs from *T. nubilum* Kott, 1980 [44] in the nature of the colony and absence of V shaped mass of dark pigment cells in the gut loop.

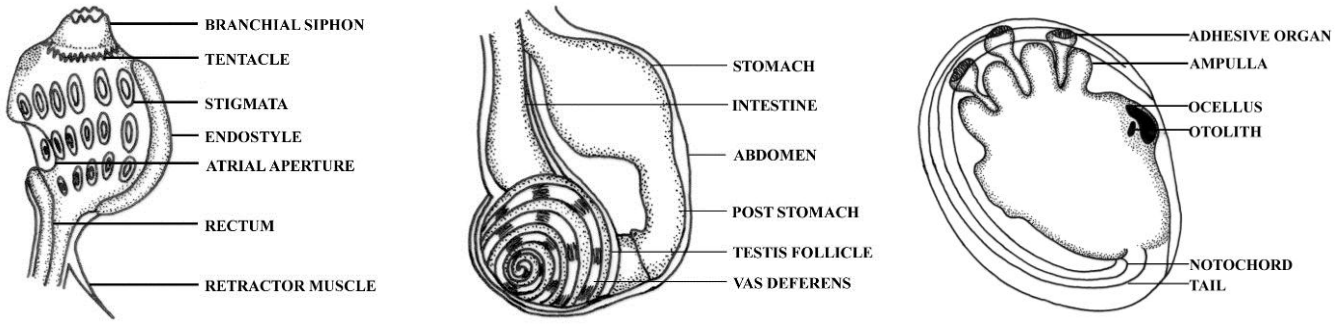
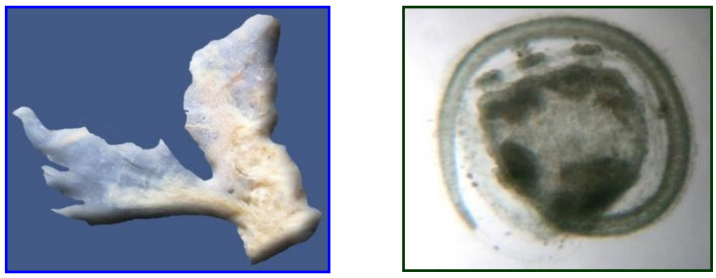


Fig 9: *Trididemnum nubilum* **A**. thorax, **B**. abdomen, **C**. larva. Scales **A to C** 1cm = 0.05 mm



A **B**

Plate 9: *Trididemnum nubilum* **A**. Colony **B**.Larva

3.10 *Trididemnum paracyclops* Kott, 1980 [44]

[Figure - 10, Plate - 10]

Trididemnum paracyclops Kott, 1980, p. 12, 1981, p.188, 1982, p. 111, 1998, p.92 [44, 23, 45, 36]

Trididemnum paracyclops: Monniot, 1991, p. 525 [50]

Trididemnum spiculatum Kott, 1962, p. 281 (part, specimen from Heron Island) [51]

Trididemnum cyclops: Kott, 1977, p. 47 (part, colonies extensive sheets) [48]

Trididemnum cyclops: Monniot & Monniot, 1987, p. 20 (part, larger colonies) [31]

Trididemnum paracyclops: Kott, 2001, p. 276 [27]

Occurrence

This species was collected from trawl discards of Melakadiyapattinam, Kanniyakumari District (AS 1070).

Distribution

Australia, Palau Islands, Guam, Philippines, Fiji, New Caledonia, French Polynesia, India.

External appearance

Colonies are thin, encrusting sheets with a smooth outer surface attached on the surface of molluscan shell. They measure about 2 cm X 1 cm. In life the colonies are yellow but in preservative colour fades and turns to white. Numerous star shaped common cloacal apertures are present spacing 1 mm distance between them. Test is rigid, hard and brittle. An outer spiculeless bladder cell layer is present. Beneath this layer, stellate spicules with rounded tip are distributed evenly. In the basal layer, spicules are sparse. Zooids are evenly distributed in the test. Algal cells are evenly arranged whereas pigment cells and faecal pellets are absent.

Internal appearance

Zooids are long and yellow in colour. The long cylindrical thorax measures 0.6 mm and abdomen 0.8 mm. Thorax is long and cylindrical. The branchial siphon is short with 6 triangular lobes. Prebranchial area is wide. At the base of the branchial siphon 25 short and long tentacles are arranged alternatively. Atrial opening is small 'C' shaped. Endostylar pigment cap is present. The retractor muscle is short, tapering, originating from the middle of the oesophagus. There are 3 rows of long oval stigmata, 6 in the first, 5 each in the middle and posterior rows. Oesophagus is long tube like. The large round stomach leads to a short duodenum, small post stomach and a tubular rectum. Rectum ends by a rounded smooth anus. There were no gonads and larvae in the single colony collected.

Remarks

T. cerebriforme which has already been reported from Indian waters differs from *T. paracyclops* in the nature of colony and in the presence of a short atrial siphon for the zooid. The colonies of *T. nubilum* are small, cushion shaped with smooth surface and hard leathery test. The zooids also lack an endostylar pigment cap. The identifying characters of the present species are star shaped numerous common cloacal openings, yellow coloured colonies and long zooids. The colony from Indian water resembles *T. paracyclops* Kott, 2001 [27] in all respects except for small cushion shaped, smooth, thin encrusting sheet like colonies with a short zooid.

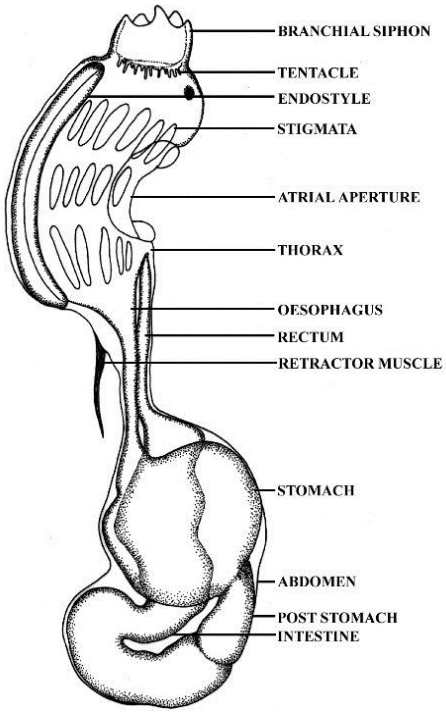
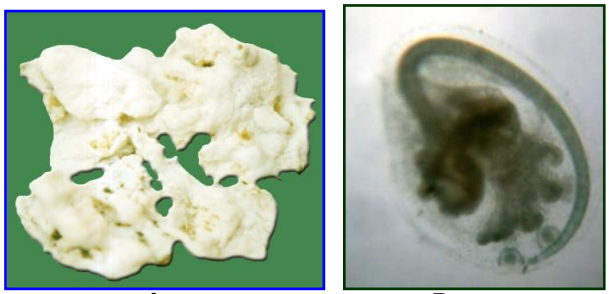


Fig 10: *Trididemnum paracyclops* - Zooid Scales **A to C** 1cm = 0.05mm



A **B**

Plate 10: *Trididemnum paracyclops* **A**. Colony **B**.Larva

Key to the species of *Trididemnum* recorded

1. Endostylar pigment cap present ..…… *T. paracyclops*
   Endostylar pigment cap absent ...……..... 2
2. Atrial siphon present, without retractor muscle …..… ……..……....….. *T. cerebriforme*
   Atrial siphon absent, with retractor muscle ……. ……..…..... *T. nubilum*

4. Acknowledgment

The authors are extremely grateful to Dr. T.K. Renganathan, Professor (Retd.), PG and Research Department of Zoology, V.O. Chidambaram College, Tuticorin for introducing us to the wonderful field of ascidian systematics and to the Ministry of Environment and Forests, New Delhi for financial assistance.

5. References

1. Savigny JC. Rescherches anatomiques sur les ascidies composees at les ascidies simple.Systeme de la classe des Ascidies, in *Memoirs sur les-animaux sans vertebres*, 1816; Part.2, 1-239. (Paris)
2. Macdonald JD. On the anatomical characters of a remarkable form of compound Tunicata. Transaction of the Linnean Society of London (Zoology) 1859; 22:373-375.
3. Bjerkan P. Ascidien von dem norwegischen Fischereidampfer '*Michael Sars'* in den Jahren 1900-1904 gesammelt. Bergens Museum Arbog Afhandlingar og Arsberetning 1905; 5:4-29.
4. Verrill AE. Descriptions of some imperfectly known and new ascidians from New England. American Journal of Science. 1871; 3(1):54-446.
5. Nott JT. On the composite ascidians of the North Shore Reef. Transactions New Zealand Institute 1892; 24:305-344.
6. Della Valle A. Nouvi contribuzioni alla storia naturalle delle ascidia composte del Golfa di Napoli. Atti della Accademia Nazionale Lincei Series 1881; 10(3):431-498.
7. Giard A. Recherches sur les ascidies composes ou synascidies. Archives de Zoology Experimentale et Generale 1872; 1:507-704.
8. Renganathan TK. On the occurrence of a colonial ascidian, *Didemnum psammathodes* (Sluiter, 1895) from India.Current Science 1981; 50(20):9-22.
9. Meenakshi VK. Biology of a few chosen ascidians. Ph. D., thesis, Manonmaniam Sundaranar University, Tirunelveli, 1997.
10. Renganathan TK. Studies on the ascidians of South India. Ph. D., Thesis. Madurai Kamaraj University, Madurai, 1986.
11. Senthamarai S, Meenakshi VK, Gomathy S. New records of two species of the genus *Leptoclinides* Bjerkan, 1905 from Indian waters. World Journal of Pharmaceutical and Life Sciences 2016; 2(2):196-204.
12. Renganathan TK. On the occurrence of a colonial ascidian, *Lissoclinum fragile* (Van Name, 1902) from India.*Current Science* 1982; 51(3):149.
13. Meenakshi VK, Senthamarai S, Gomathy S. *Polysyncraton* Nott, 1892 an unrecorded genus of the family Didemnidae from India. Journal of Chemical, Biological and Physical Sciences. 2014; 4(4):3243-3246.
14. Meenakshi VK. *Trididemnum* Della Valle, 1881, an unrecorded genus of colonial ascidian from India. *Journal of the Bombay Natural History Society*. 2000; 97(2):302-304.
15. Sluiter CP. Tunicaten. In Semon, R. Zoologische Forscungsreisen in Australien und den Malagischem Archipel. Jenaische Denkschriften 1895; 8:163-186.
16. Tokioka T. *Ascidians of Sagami Bay*. Tokyo: Iwanami Shoten1953; 1:313.
17. Sluiter CP. Die Tunicaten der Siboga - Expedition Pt.2. Die merosomen Ascidien. Siboga Expedition 1909; 56:1-112.

18. Van Name WG. The Ascidians of the Bermuda Islands. Trans. Connecticut Acad. Arts Sci 1902; 1:325-412.
19. Vasseur P. Deuxieme contribution al'etudedes ascides de Madagascar region de Tulear. Bulletin du Museum National d'Histoire Naturelle ser 1969; 240(5):912-933.
20. Hartmeyer R, Tunicata. *In*: L. Schultze, Zool. U. anthrop. Ergebnissee Forschungsreise in Sudafrika Bd 5, Lfg 2. Denskschr. med. naturw. Ges. Jena 1913; 17:125-144.
21. Kott P. The Australian Ascidiacea. Part I, Phlebobranchia and Stolidobranchia. Memoirs of Queensland Museum 1985; 23:1-440.
22. Hastings AB. Tunicata. Scientific Reports of the Great Barrier Reef Expedition 1931; 4(3):69-109.
23. Kott P. The ascidians of the reef flats of Fiji. Proceedings of the Linnean Society of New South Wales 1931; 105(3):147-212.
24. Monniot F. Ascidies de Nouvelle-Caledonie XV. Le genre *Didemunm*. Bulletin du Museum National d'Histoire Naturelle, Paris ser, 1995; 416(2-4):299-344.
25. Monniot C, Monniot F. Records of Ascidians from Bahrain, Arabian Gulf with three new species. Journal of Natural History. 1997; 31:1623-1643.
26. Monniot F, Monniot C. New collections of ascidians from the western Pacific and southeastern Asia. Micronesia 1996; 29(2):133-279.
27. Kott P. The Australian Ascidiacea Part 4, Aplousobranchia (3), Didemnidae. Memoirs of the Queensland Museum 2001; 47(1):1-410.
28. Tokioka T. Contributions to Japanese ascidian fauna VII. Invertebrate fauna of the intertidal zone of the Tokara Island VII Ascidians. Publications of the Seto Marine Biological Laboratory 1954; 3(3):239-264.
29. Michaelsen W. Ascidiae Krikobranchiae. Fauna Sudwest Australiens 1930; 5(7):463-558.
30. Tokioka T. Pacific Tunicata of the United States National Museum. Bulletin, United States National Museum 1967; 251:1-242.
31. Monniot C, Monniot F. Les ascidies de Polynesie francaise. Memoirs du Museum National d'Histoire Naturelle, Paris 1987; 136:1-155.
32. Eldredge LG. A taxonomic review of the Indo-Pacific didemnid ascidians and descriptions of twenty three central Pacific species. Micronesia 1967; 2:162-261.
33. Millar RH. Ascidians from the Indo-West Pacific region in the Zoological Museum, Copenhagen (Tunicata: Ascidiacea). Steenstrupia 1975; 3(20):205-336.
34. Kott P. The ascidians of South Australia II, Eastern Sector of the Great Australian Bight and Investigator Strait. Transactions of the Royal Society of South Australia 1972; 96(4):165-196.
35. Kott P. Ascidian fauna of Western Port Bay, Victoria and a comparison with that of Port Phillip Bay. Memoirs of the National Museum, Victoria 1976; 37:53-96.
36. Kott P Tunicata, in Wells A, Houston WWK. (eds) Zoological Catalogue of Australia, Hemichordata, Tunicata, Cephhalochordata. (CSIRO Publishing: Melbourne).1998; 34:51-292.
37. Hartmeyer R. Ascidien (Continuation of work by Seeliger). In Bronn, H.G., *Klassen und Ordnungen des Tierreichs* 3(89-98):1281-1772. Leipzig: C.F. Winter (Abstract, repeating lists of species by Schepotieff A, 1911, in Arch. Naturgesch 1909; 6:3-27).
38. Herdman WA. Report on the Tunicata. *Ceylon Pearl Oyster Fisheries*. Supplimentary reports 1906; 39:295-348.
39. Tokioka T. Ascidians found on the mangrove trees in Iwayama Bay, Palao. Palao Tropical Biological Station Studies 1942; 2(3):497-507.
40. Kott P. Ascidians of northern Australia. University of Queensland Papers, Department of Zoology 1966; 2(15):279-304.
41. Newcomb EH, Pugh TD. Blue-green algae associated with ascidians of the Great Barrier Reef. Nature, 1975; 253:533-534.
42. Thinh LV, Griffiths DJ. Studies of the relationship between the ascidian *Diplosoma virens* and its associated microscopic algae.Australian Journal of Marine and Freshwater Research. 1977; 28:673-681.
43. Thinh LV. Photosynthetic lamellae of *Prochloron* (Prochlorophyta) associated with the ascidian *Diplosoma virens* (Hartmeyer) in the vicinity of Townsville. Australian Journal of Botany. 1978; 26:617-620.
44. Kott P. Algal-bearing didemnid ascidians in the Indo-west Pacific. Memoirs of the Queensland Museum 1980; 20(1):1-47.
45. Kott P. Didemnid-algal symbioses: host species in the western Pacific with notes on the symbiosis. Micronesica 1982; 18(1):95-127.
46. Monniot F. Ascidies de Nouvelle-Caledonie XIV. Le genre *Diplosoma* (Didemnidae) dans le lagonsud. Bulletin du Museum National d'Histoire Naturelle. Paris ser. 4, 1994; 16(1):3-11.
47. Van Name WG. Ascidians from the Philippines and adjacent waters. Bulletin of the United States National Museum 1918; 100(1):49-174.
48. Kott P. Algal supporting didemnid ascidians of the Great Barrier Reef.Proceedings of the Second International coral reef symposium 1977; 1:615-621.
49. Monniot F. Ascidies de Nouvelle-Caledonie XII. Le genre *Lissoclinum* (Didemnidae) dans le lagonsud. Bulletin du Museum National d'Histoire Naturelle. Paris ser. 4, 1992; 14(3-4):565-589.
50. Monniot F. Ascidies de Nouvelle-Caledonie IX. Le genre *Tridemnum*. Bulletin du Museum National d'Histoire Naturelle Paris ser. 4, 1991; 10(3-4):517-529.
51. Kott P. The ascidians of Australia III. Aplousobranchiata Lahille: Didemnidae Giard. Australian Journal of Marine and Freshwater Research. 1962; 13(3):265-334.