



## SIX SPECIES OF ASCIDIANS OF THE FAMILY DIDEMNIDAE AS NEW RECORDS TO GULF OF MANNAR, INDIA

Senthamarai, S.,<sup>1</sup> Meenakshi, V.K.<sup>2\*</sup> and Gomathy, S.<sup>3</sup>

<sup>1</sup>Department of Biological Science, Crescent College of Education, Somasipadi PO, Thiruvannamalai District, Tamilnadu, India.

<sup>2</sup>Department of Zoology, A.P.C. Mahalaxmi College for Women, Thoothukudi - 628002, Tamilnadu, India.

<sup>3</sup>PG and Research Department of Zoology, V.O. Chidambaram College, Thoothukudi - 628008, Tamilnadu, India.

\*Email: vkmeenakshi.apcm@gmail.com

**Abstract:** Biodiversity of colonial ascidians of the family Didemnidae is rich in Indian coastal waters. This paper describes the ascidians of this family collected manually from the intertidal rocky areas, from a depth of 2-6 metres by SCUBA diving and trawl discards of fish and mussel landing centres of the coastal districts of Tamil Nadu along the Gulf of Mannar coast. A total of six species of the family Didemnidae comprising two of the genus - *Didemnum* Savigny, 1816 (*Didemnum moseleyi* and *Didemnum tonga*), one of *Diplosoma* Macdonald, 1859 (*Diplosoma simile*) and three of *Trididemnum* Della Valle, 1881 (*Trididemnum clinides*, *Trididemnum paraclinides* and *Trididemnum savignii*) were identified. Detailed descriptions are given along with Camera Lucida drawings, taxonomic keys and photographs of colonies.

**Key words:** Didemnidae, *Didemnum*, *Diplosoma*, *Trididemnum*, New Record.

### INTRODUCTION

Colonial ascidians of the family Didemnidae are diverse in the tropical water of Indian seas due to favourable features of environment (Personal observation - Meenakshi *et al.*, 2003). They exhibit prolific replication and growth pattern resulting in the formation of extensive colonies over almost all marine underwater structures (Kott, 2001). Though members of this family are encountered in all collections, very few reliable morphological features of the highly contracted small zooids, factors affecting shape, size and colour of colonies; difficulty in ascertaining nature of colonial systems makes accurate identification upto species problematic. The family Didemnidae Giard, 1872 is represented in Indian coastal waters by six genera - *Didemnum* Savigny, 1816; *Diplosoma* Macdonald, 1859; *Leptoclinides* Bjerkan, 1905; *Lissoclinum* Verrill, 1871; *Polysyncraton* Nott, 1892 and *Trididemnum* Della Valle, 1881 (Renganathan, 1981, 1982, 1986; Meenakshi, 2000; Meenakshi *et al.*, 2014;

Senthamarai *et al.*, 2016a). Eight species of the genus *Didemnum* - *D. candidum* Savigny, 1816, *D. chartaceum* Sluiter, 1909, *D. cuculliferum* (Sluiter, 1909), *D. granulatum* Tokioka, 1954, *D. ossium* Kott, 2001, *D. psammathodes* Sluiter, 1895, *D. spongiode* Sluiter, 1909, *D. ternerratum* Kott, 2001; two of each of the genera *Diplosoma* - *D. swamiensis* Renganathan, 1986, *D. virens* (Hartmeyer, 1909), *Leptoclinides* - *L. madara* Tokioka, 1953, *L. rufus* (Sluiter, 1909) and *Lissoclinum* - *L. fragile* Van Name, 1902, *L. punctatum* Kott, 1977; one of the genus *Polysyncraton* - *Polysyncraton millepore* Vasseur, 1969, and three of the genus *Trididemnum* - *T. cerebriforme* Hartmeyer, 1913, *T. nubilum* Kott, 1980 and *T. paracyclops* Kott, 1980 has been previously recorded from this region (Renganathan, 1981, 1982, 1986; Meenakshi, 1997, 2000; Meenakshi *et al.*, 2014; Senthamarai *et al.*, 2016b). Though colonies of Didemnidae are macroscopic and conspicuous, found in almost all fouling communities, very little taxonomic importance has

been given to this group. This paper describes colonial ascidians of the family Didemnidae collected from the coastal districts of Tamil Nadu along the Gulf of Mannar, India.

## MATERIAL AND METHODS

Samples of ascidians were collected from intertidal rocky areas manually, at a depth of 2-6 metres by SCUBA diving and from trawl discards of fish and mussel landing centres of the coastal districts of Tamil Nadu along the Gulf of Mannar coast. They were narcotised with a few crystals of menthol to facilitate complete relaxation of the thorax and preserved in 4% seawater-formalin (Kott, 1985). After one week to ten days the entire colony, zooids, larva were viewed under the dissecting, stereo, binocular microscopes to study the morphological and anatomical features of the collected ascidians and accurately identified up to species level (Meenakshi, 1997). Taxonomic characters studied include shape, size, colour, nature of 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 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 spicule distribution, size, shape and tip of spicule. The results are interpreted with the help of Camera Lucida drawings, taxonomic keys and photographs of colonies. Voucher specimen of each species has been deposited in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Thoothukudi, India.

## RESULTS AND DISCUSSION

Members of the family Didemnidae have a wide geographical distribution. In this study the genus *Didemnum* is represented by two species - *Didemnum moseleyi* (Herdman, 1886), *Didemnum tonga* (Herdman, 1886), *Diplosoma* by one species

*Diplosoma simile* (Sluiter, 1909) and *Trididemnum* by three species - *Trididemnum clinides* Kott, 1977, *Trididemnum paraclinides* Kott, 1982 and *Trididemnum savigni* (Herdman, 1886) as new records to Indian coastal waters.

### Genus *Didemnum* Savigny, 1816

The most reliable characters to distinguish the species of the genus *Didemnum* are nature of colony - encrusting sheet like colonies with several common cloacal apertures or cushion shaped with single opening and extensive cloacal systems, presence of calcareous spicules in test, size and form of spicules, distribution of bladder cells, size of zooids, 4 rows of stigmata, length of branchial siphon, shape of branchial lobes, wide atrial apertures, presence or absence of bifid atrial languet, position, length of retractor muscle, nature of gut loop and coils of vas deferens (Kott, 2001).

#### 1. *Didemnum moseleyi* (Herdman, 1886) (Fig. 1A,B,C)

*Synonymies:* *Leptoclinium moseleyi* Herdman, 1886: 272; *Didemnum moseleyi*: Sluiter, 1909: 45; 1913: 74; Van Name, 1918: 151; Tokioka, 1954: 243; 1955: 44; 1961: 106; 1967: 65; 1970: 52; Kott, 1957: 136; 1962: 328; 1998: 82; 2001: 211.

*Materials examined:* Several colonies were collected from mussel (*Perna viridis*), chank beds, trawl discards, coral reefs and intertidal rocky areas of 38 stations along the Gulf of Mannar coast including Vanthivu Islands during the period from 2000-2003 by V.K. Meenakshi and S. Senthamarai.

*Description: Colony:* The colony studied is 4 x 3.5 cm in extent with 0.5 mm thickness, firm and hard encrusting sheets on the surface of dead coral stones (Fig. 1A). The surface of the colony is smooth taking the shape of the underlying rubbles. The common cloacal apertures are scattered, present on small raised ridges with spicules arranged in a floral pattern passing onto the common cloacal cavity. The branchial apertures of the zooids over the surface of the colony are made conspicuous by the accumulation of spicules on their lobes. The common cloacal cavity is extensive and traversed by the entire thoracic part of the zooids, enclosed in a thin layer of test with fewer spicules. The common cloacal cavity divides the test into two distinct layers, an upper thick layer in level with the thorax of the zooids and a lower

thicker layer enclosing the abdomen and larvae. A very spacious lacuna is present in the thoracic zone. There is a thin superficial bladder cell layer with scattered minute pigment cells. Living colonies are with chocolate brown pigment giving a network like appearance. Spicules are 0.04 to 0.05 mm in diameter, stellate with 7 - 12 rays in optical section. The rays are long and pointed.

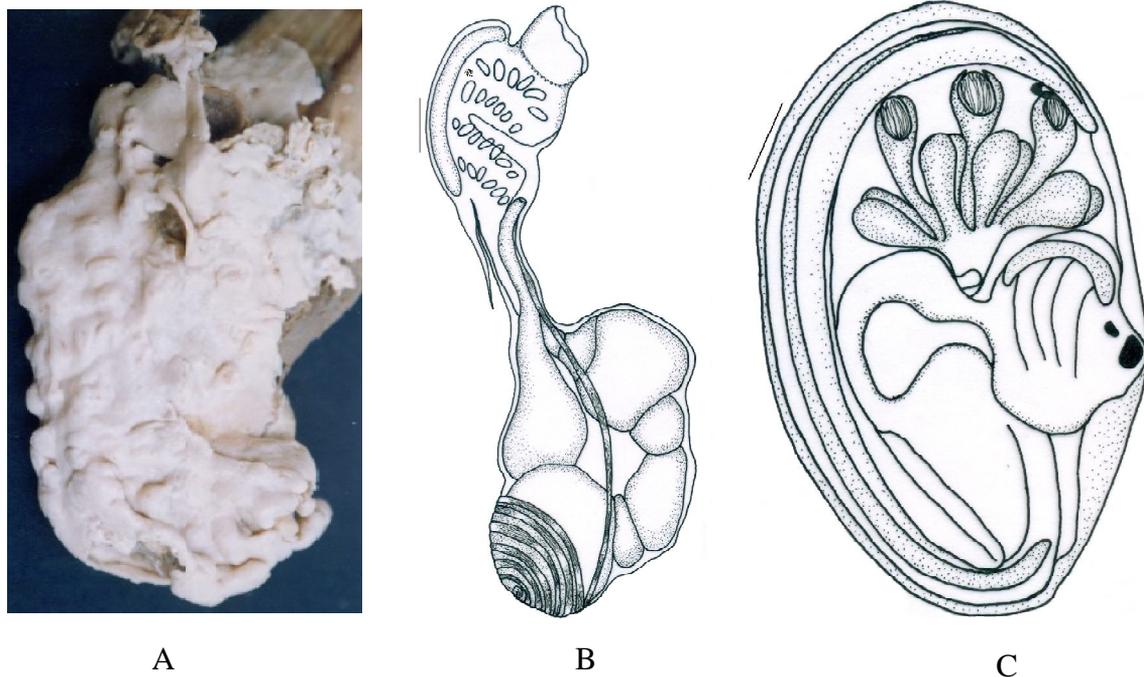
**Zooids:** Zooids are long, light orange yellow in colour with the thorax measuring 0.4 - 0.5 mm and abdomen 0.7 to 0.8 mm (Fig. 1B). Branchial siphon is a short wide cylinder. Branchial lobes are small. Atrial aperture is sessile, wide without an atrial languet. A round thoracic lateral organ is situated between the first and second row of stigmata. Short retractor muscle arises from the anterior end of oesophagus and extends halfway down its length. About 10 -12 branchial tentacles line the base of branchial siphon. Prebranchial area is narrow. There are about 6 stigmata in each of the four rows. Gut loop is flexed distal to stomach and has a long oesophagus, duodenum, large spherical stomach, round posterior stomach and rectum with wide

proximal half and distal narrow part. Anus is smooth, opening into the atrial cavity at the level of fourth row of stigmata. Testis is single surrounded by 7 - 8 coils of vas deferens. Many larvae have been detected in the basal layer of test. They are 0.7 mm long (Fig. 1C). Four pairs of lateral ampullae alternate with 3 adhesive organs. A large otolith and ocelli are present. Tail is wound half way around larval trunk.

**Distribution:** Queensland (Kott, 1962, 2001), Gulf of Suez (Kott, 1957), Indonesia (Sluiter, 1909, 1913), Philippines (Herdman, 1886, Van Name, 1918, Tokioka, 1967, 1970), Palau Is (Tokioka, 1955, 1967), New Caledonia (Tokioka, 1961), Tokara Is (Tokioka, 1954), Fiji (Kott, 1981).

**Current Record:** India

**Remarks:** The present species differs from *Didemnum psammatoide* in the absence of faecal pellets in the test. Zooids of *D. chartaceum* and *D. ossium* have a bifid atrial tongue. The orange coloured colonies of *D. granulatum*, white spongy nature of *D. spongioide* cannot be confused with present species. It differs from *D. candidum* in the thickness of colony, lesser



**Fig. 1.** *Didemnum moseleyi* - A. Colony B. Zooid C. Larva (Scale - 0.1 mm)

number of stigmata per row and presence of pigment cells in test. *D. tonga* can be distinguished by zooid free transparent area of test surrounding common cloacal aperture and absence of pigment cells. Colonies of *D. ternerratum* have cushion shaped elevations and round common cloacal openings whereas *D. cuculliferum* are white encrusting sheets with star shaped common cloacal openings. The present species can be identified by the chocolate brown pigment cells forming a network like appearance on the colony, absence of atrial languet and faecal pellets, nature of common cloacal cavity, arrangement, size of zooids and distribution of spicules.

According to Kott, 2001 *Didemnum moseleyi* is a tropical species which appears to have a distribution range through the Indo-west Pacific covering the Pacific, Indo-Malayan region and Circum Australia. This species was first recorded by Meenakshi *et al.*, 2003 from the Gulf of Mannar. Prior to that only 8 species of the genus - *D. psammotode* (Sluiter, 1895); *D. candidum* Savigny, 1816; *D. chartaceum* Sluiter, 1909; *D. cuculliferum* (Sluiter, 1909); *D. granulatatum* Tokioka, 1954; *D. ossium* Kott, 2001; *D. spongiode* Sluiter, 1909 and *D. ternerratum* Kott, 2001 (Renganathan, 1981; Meenakshi, 1997; Senthamarai *et al.*, 2016b) has been recorded from Indian waters. A review of literature on the closest earlier record of the species and distribution in the eastern Indian Ocean indicates lack of reports from the islands of Gulf of Mannar, Sri Lanka or from Andaman Sea.

## 2. *Didemnum tonga* (Herdman, 1886) (Fig. 2A,B)

**Synonymies:** *Leptoclinum tonga* Herdman, 1886: 269; *Didemnum productum* Monniot, 1995: 323.

**Materials examined:** Two small colonies collected from trawl discards of Kanyakumari and coral reefs of Thoothukudi Harbour area on 28.12.2000 by V.K. Meenakshi.

**Description: Colony:** Colonies are thin, irregular encrusting sheets 2 cm long 1 mm thick with small elevations on the surface. Common cloacal aperture is circular, marked by zooid free transparent area of test. Zooids are arranged on either sides of common cloacal canal. Test is brittle with a thin layer of bladder cells. Spicules are crowded in most part of the test. Living colonies are dull orange in colour while in preservative they are almost white. Spicules

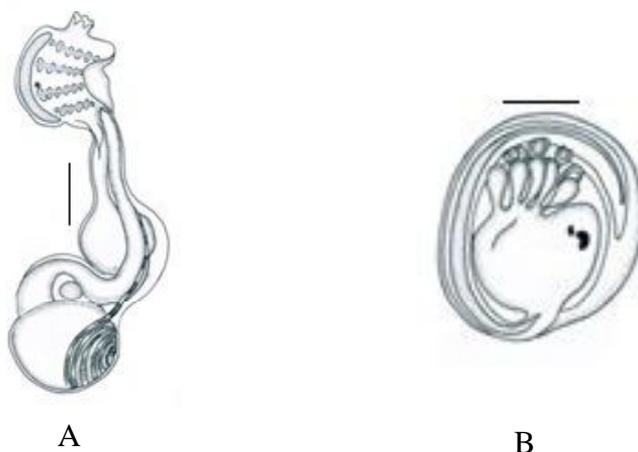
are mostly 0.05 mm in diameter, stellate with 7 - 9 conical blunt end rays which are easily broken.

**Zooids:** The zooids are buff coloured, robust with a thorax measuring 0.3 - 0.4 mm and abdomen 0.5 - 0.6 mm (Fig. 2A). Branchial siphon is short, cylindrical with 6 triangular lobes. Atrial opening is wide exposing the branchial sac. There is no atrial languet. Thoracic lateral organ is situated at the level of third row of stigmata. Retractor muscle is short arising from the posterior end of thorax and is free from the oesophageal neck. 8 - 10 small branchial tentacles are at the base of branchial siphon. Prebranchial area is narrow. There are about six stigmata in each of the four rows. Stigmata of the posterior row are shorter and sometimes difficult to count. Gut loop is wide and twisted. Oesophagus is long. Stomach is large followed by a short posterior stomach, duodenum and narrow rectum. Anus opens into the atrial cavity below the level of fourth row of stigmata. Vas deferens coils 6½ times around the very large male follicle. Incubating larvae were found in the basal test. They are 0.5 mm long with four pairs of deeply separated epidermal ampullae alternating with three adhesive organs (Fig. 2B). An otolith and ocellus are present. The tail is wound more than halfway round the trunk.

**Distribution:** Western Pacific (Herdman, 1886), Marion Reef, Coral Sea (Monniot, 1995).

**Current Record:** India

**Remarks:** This species differs from *D. psammotode* which has abundant faecal pellets in test. Absence of an atrial languet separates it from *D. chartaceum* and *D. ossium*. *D. moseleyi* and *D. granulatatum* has pigment cells in test. Colonies of *D. candidum* do not have a conspicuous bladder cell layer compared to present species. They differ from *D. cuculliferum*, *D. ternerratum* and *D. spongiode* in the nature of colony. Characters used to identify this species are the thin encrusting colonies, brittle test, easily broken rays of spicules and small buff coloured zooids without an atrial languet. Studied material differs from the description of *D. tonga* given by Kott, 2001 after Herdman, 1886 and Monniot, 1995 in the length of branchial siphon, shape of branchial lobes, number of stigmata and coils of vas deferens. Colour of colony and two sorts of spicules observed by Kott, 2001 has not been noted here.



**Fig. 2.** *Didemnum tonga* - A. Zooid B. Larva (Scale - 0.1 mm)

The previous records of this species by Herdman, 1886 and Monniot, 1995 are from Western Pacific collected from a depth of 35 m. In the present study the two small colonies examined were recorded from trawl discards/coral reef of Thoothukudi harbour area (Meenakshi *et al.*, 2003) may be transported on the hull of ships as fouling organisms. In the description given, the minor variations noted from the earlier

reports have been pointed out. This species has not been recorded earlier from the southeast coast of India or from the islands of Gulf of Mannar. The genus *Didemnum* shows great diversity and worldwide distribution. Sincere collection efforts from several new habitats may yield more data on the availability and distribution of the genus.

Key to species of *Didemnum* recorded

1.	Faecal pellets crowded throughout the test .....	<i>D. psammatode</i>
	Faecal pellets not crowded throughout the test .....	2
2.	Atrial languet present .....	3
	Atrial languet absent .....	4
3.	Colonies spongy, zooids and larva ash or black coloured .....	<i>D. chartaceum</i>
	Colonies not spongy, zooids and larva not ash or black coloured .....	<i>D. ossium</i>
4.	Presence of light chocolate brown pigment cells giving network like appearance .....	<i>D. moseleyi</i>
	Absence of light chocolate brown pigment cells giving a network like appearance .....	5
5.	Zooid free transparent area of test surrounded by common cloacal canal .....	<i>D. tonga</i>
	No zooid free transparent area of test surrounded by common cloacal canal ...	6
6.	Star shaped common cloacal opening .....	7
	Rounded common cloacal opening .....	8
7.	Orange coloured thin investing sheet .....	<i>D. granulatum</i>
	White coloured thick encrusting sheet .....	<i>D. cuculliferum</i>
8.	Colonies with cushion shaped elevation and hard test .....	<i>D. ternerratum</i>
	Colonies without cushion shaped elevation and soft test .....	9
9.	Encrusting sheet like colony with larger zooids .....	<i>D. candidum</i>
	Spongy colonies with small zooids .....	<i>D. spongioide</i>

### Genus *Diplosoma* Macdonald, 1859

Members of the genus *Diplosoma* can be identified by colonies with extensive common cloacal cavities, absence of spicules, atrial languet and zooids having large thorax, wide atrial aperture, presence of retractor muscle, squamous epithelium covering the body wall over the abdomen, four rows of stigmata, undivided or with two testis follicles and a straight vas deferens (Kott, 2001).

#### 3. *Diplosoma simile* (Sluiter, 1909) (Fig. 3A,B,C)

*Synonymies:* *Leptoclinum simile* Sluiter, 1909: 77; *Leptoclinum midori* Tokioka, 1954: 11; *Diplosoma virens*: Eldredge, 1967: 228; Millar, 1975: 241; Kott, 1977: 620; Thorne *et al.*, 1977: 575; *Diplosoma midori*: Kott, 1980: 29; *Diplosoma similis*: Kott, 1980: 26; 1981: 191; 1982: 117; Monniot & Monniot, 1987: 62; Monniot, 1994: 9; *Diplosoma simile*: Kott, 1998: 85; 2001: 341.

*Materials examined:* Many colonies collected from intertidal zone, trawl discards, mussel beds, coral reefs and as fouling organisms from various harbour installations, aquaculture cages, barges of 29 stations along the coast of Kanyakumari and Thoothukudi districts in the east coast during 2000 - 2004 by V.K. Meenakshi and S. Senthamarai.

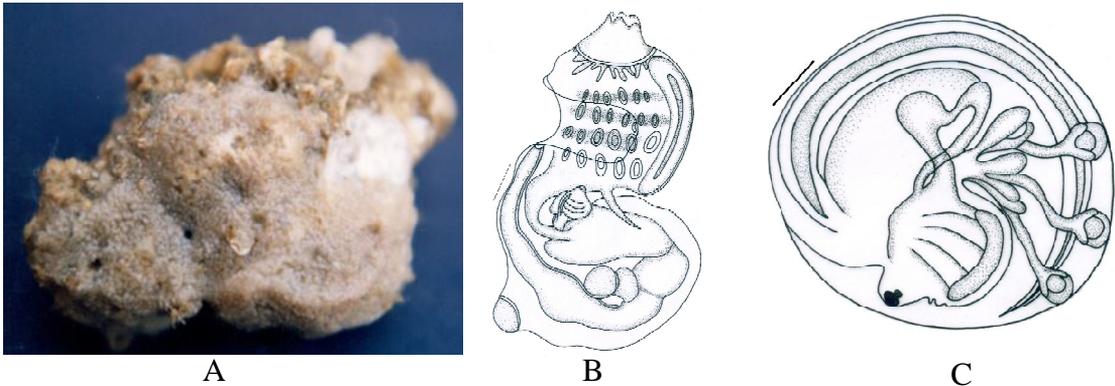
*Description: Colony:* Colonies are large extensive encrusting sheets extending to several centimetres with a thickness of 1.5 mm (Fig. 3A). The colony collected has been found to fill the cracks and crevices of calcareous rocks and extend onto the nearby substratum like shell fragments and pieces of dead corals. Colony surface is smooth. Common cloacal apertures are inconspicuous and sessile. Common cloacal cavity is lined with prochloron. Test is gelatinous, firm, translucent white in colour with a slight tinge of blue. There is a superficial layer of bladder cells. Surface test is comparatively thinner than basal thick test. Zooids are arranged with uniform space between them. Thoraces of zooids cross each other and are surrounded by common cloacal cavity. Abdomen of zooids is embedded in basal test. In some colonies, common cloacal cavity is wide, deep and open; zooids are enclosed in test connectives between surface and basal test. Cloacal cavity and basal test each occupy one third the thickness of colony. Many larvae have been observed in basal test.

*Zooids:* Zooids measure 1.2 to 1.3 mm with the thorax and abdomen of equal length. Branchial siphon is moderately long bordered by six triangular lobes (Fig. 3B). At the base of branchial siphon sphincter muscles are present. Atrial opening is wide exposing a large part of branchial sac into the cloacal cavity. About 20 branchial tentacles of two size alternate with each other. Posterior end of thorax bears a short retractor muscle. Prebranchial area is wide. Thorax is short and wide. There are six oval stigmata in each of the four rows. Oesophageal neck is of moderate length. Abdomen is aligned at right angles to the long axis of thorax. Body wall over the gut loop is covered by black squamous epithelium. Major part of the proximal limb of gut loop is occupied by long elliptical stomach. A short posterior stomach situated at the pole of gut loop is connected to the stomach by a long duodenum. Rectum is wide, opening by a bilobed anus at the level of last row of stigmata. There is a bilobed testis in gut loop and a straight vas deferens. Larva is spherical measuring 0.6 to 0.7 mm in length (Fig. 3C). There are three pairs of slender ectodermal ampullae, each with modified columnar cells forming a swelling at the free end projecting on either side of three median adhesive organs. Tail is wound three quarters of the way round larval trunk.

*Distribution:* Queensland (Kott, 1977, 1980, 1982, 2001), Western Pacific (Kott, 1982; Millar, 1975; Monniot, 1994; Kott, 2001), Central Pacific (Eldredge, 1967; Kott, 1980, 1981), Indonesia (Sluiter, 1909; Hirose *et al.*, 2014), Singapore (Kott, 1982; Su *et al.*, 2013), Japan (Tokioka, 1954), Western Australia, New South Wales, Papua New Guinea, Timor Sea (Kott, 2001), Taiwan (Hirose and Nozawa, 2010).

*Current Record:* India

*Remarks:* *Diplosoma swamiensis* differs from present species in the presence of rounded bodies around testis of zooids and peculiar arrangement of larval ampullae. Though *D. virens* and *D. simile* have prochloron in common cloacal cavity and test connectives; the colonies of *D. virens* are dark green with yellowish zooids and a retractor muscle free from half way down the oesophageal neck. Diagnostic features of *D. simile* are the presence of prochloron, extensive colonies binding dead coral pieces, wide



**Fig. 3.** *Diplosoma simile* - A. Colony B. Zooid C. Larva (Scale - 0.1 mm)

and deep thoracic common cloacal cavities and a retractor muscle free from the posterior end of thorax. Kott, 2001 opines this genus as the least diverse due to difficulty in distinguishing one species from another because of great uniformity in the appearance of the colony and zooids in preservative. This species was first recorded from the Gulf of Mannar water by Meenakshi *et al.*, 2003. Ananthan, 2014 listed the name of this species from the ascidians of Great

Nicobar Biosphere Reserve. Recently Stalin, 2017 included *D. simile* among the photosynthetic ascidians from Andaman and Nicobar Islands. But both these lack taxonomic descriptive characters of the species under study. While attempting to describe the identifying features of the species investigated, those that has been authentically described in detail with nature of colony, zooids and larvae only has been considered.

Key to the species *Diplosoma* recorded

1.	Presence of rounded bodies around the testis .....	<i>D. swamiensis</i>
	Absence of rounded bodies around the testis .....	2
2.	Retractor muscle free from the posterior end of thorax .....	<i>D. simile</i>
	Retractor muscle free from halfway down the oesophageal neck .....	<i>D. virens</i>

**Genus *Trididemnum* Della Valle, 1881**

Characters of the species of genus *Trididemnum* include fleshy, branches and lobes of colony fusing to form spongy colonies enclosing external spaces and wide common cloacal cavities, presence of large stellate, globular or burr like spicules and zooids with three rows of stigmata, atrial aperture on a short laterally or posteriorly directed siphon with the exception of species with prochloron where it is a sessile transverse opening, retractor muscle from posterior end of thorax or oesophageal neck, numerous stigmata, undivided testis and a coiled vas deferens (Kott, 2001).

**4. *Trididemnum clinides* Kott, 1977 (Fig. 4 A,B,C)**

*Synonymies: Trididemnum clinides* Kott, 1977: 617; *Trididemnum viride*: Tokioka, 1967: 87; *Trididemnum* sp. Eldredge, 1967: 184; *Trididemnum*

*clinides* Kott, 1980: 5; 1981: 186; 1982: 109; 1984: 519; 1998: 9; 2001: 260; Monniot & Monniot, 1987: 18; Parry and Kott, 1988: 151.

*Materials examined:* Several colonies collected from mussel beds, trawl discards, intertidal areas, coral reefs and fouling community of 32 stations along the coastal districts of Gulf of Mannar during 2000 - 2003 by V.K. Meenakshi.

*Description: Colony:* Colonies are spongy, extending to a few centimetres with a thickness of 2 mm, produced into few small irregular lobes, otherwise with a flat, smooth upper surface fixed firmly to underlying substratum (Fig. 4A). More than one common cloacal aperture has been observed surrounded by branchial apertures of zooids. Common cloacal cavity is large, horizontal found surrounding the thorax of zooids. Test is soft with a

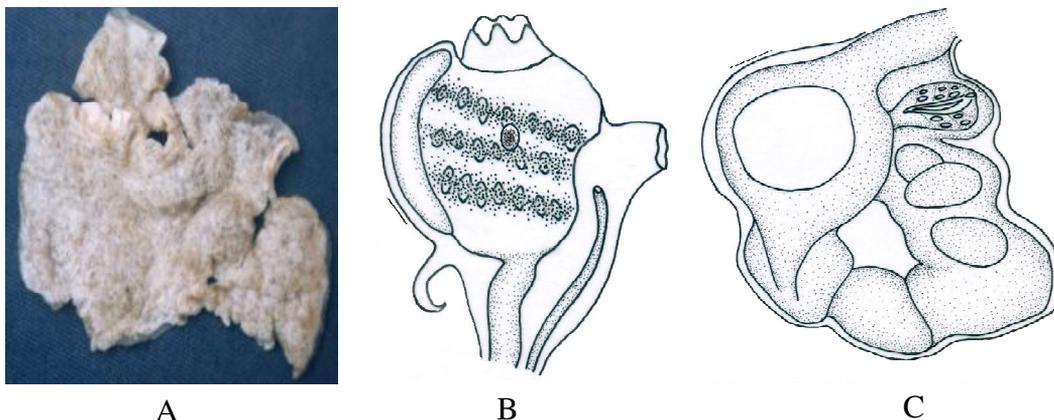
thin superficial layer of bladder cells and a continuous layer of spicules below. In the remaining part of test, distribution of spicule decreases gradually. Spicules are absent from common cloacal cavity. Stellate spicules are 0.04 mm in diameter with 7 - 9 short conical pointed rays. Living and preserved colonies are flesh coloured due to the presence of prochloron and red symbionts embedded in test.

**Zooids:** Zooids measure 1 to 1.2 mm with a thorax 0.4 - 0.5 mm and an abdomen 0.6 - 0.7 mm (Fig. 4 B,C). Branchial siphon is short but wide with six small pointed branchial lobes. Atrial siphon is short arising from the middle of thorax directed laterally with a wavy margin. There is a round lateral thoracic organ present at the level of second row of stigmata. Retractor muscle is short, curved, originating from the posterior end of thorax. Twelve to thirteen short branchial tentacles occupy the base of branchial siphon. Prebranchial area is wide. An endostylar pigment cap has not been noticed. About 6 - 7 oval stigmata are present in each of the three rows. Gut forms a simple relatively short loop with a short oesophagus, spherical stomach, short duodenum, small posterior stomach and a wide rectum narrowing out at the distal end to open by the anus at the level of second row of stigmata. Dorsal side of the gut loop has a single large testis. Vas deferens coils 5 times around it. Larvae are large measuring 0.5 mm with four pairs of ectodermal ampullae alternating with three adhesive organs. An otolith and ocellus are present. Tail is wound more than half way round.

Prochloron cells have been noticed over the larval trunk.

**Distribution:** Queensland (Kott, 1977, 1984, 2001), Philippines (Tokioka, 1967; Kott, 1982), Fiji (Kott, 1980, 1981), Guam (Kott, 1982), Eniwetok (Eldredge, 1967), French Polynesia Monniot & Monniot, 1987), Taiwan (Hirose and Nozawa, 2010). Current Record: India

**Remarks:** Colonies of *Trididemnum cerebriforme* and *T. savignii* do not harbour prochloron in common cloacal cavity and spicules are absent from basal layer of test. Zooids of *T. paracyclops* and *T. nubilum* have sessile atrial aperture. *T. paraclinides* differs from the present species in the presence of large spicules. *T. clinides* is characterised by spongy colonies, large common cloacal cavity, prochloron in test and in arrangement, size of spicules. Specimen studied differs from description of *T. clinides* Kott, 2001 in the presence of large colonies with more than one common cloacal aperture and distribution of spicules. Previous world records of this species are from the South Pacific Ocean, South China Sea. They are reported to occupy cryptic habitats near reef edge according to Kott, 2001. Several colonies examined and described in this paper were recorded from trawl discards, mussel beds, intertidal areas and coral reefs (Meenakshi *et al.*, 2003). Although Abdul Jaffar Ali and Tamil Selvi, 2016 reported this species from the south east coast, the description is short, vague and incomplete lacking systematic comparison with known species.



**Fig. 4.** *Trididemnum clinides* - A. Colony B. Thorax C. Abdomen (Scale - 0.1 mm)

### 5. *Trididemnum paraclinides* Kott, 1982 (Fig. 5A,B,C)

*Synonymies:* *Trididemnum clinides* Kott, 1981: 186; *Trididemnum paraclinides* Kott, 1982: 107; 2001: 276.

*Materials examined:* Number of colonies collected from mussel chank bed, intertidal rocky areas, coral reefs, trawl discards and as fouling organisms from 28 stations of Kanyakumari and Thoothukudi districts during 2000 - 2003 by V.K. Meenakshi.

*Description: Colony:* Fleishy cushion like colonies extending to a size of 3 cm and thickness of 4 - 5 mm with irregular flat topped lobes (Fig. 5A). There are numerous common cloacal apertures on the surface of colony surrounded by branchial apertures. Common cloacal cavity is narrow situated at the level of oesophagus. Prokaryotic symbiotic cells are in the test. There is a thin superficial layer of bladder cells. The test below is soft and contains continuous layer of spicules. In the remaining part of test, distribution of spicule decreases with only few in the basal region. They are absent from superficial layer and common cloacal cavity. Spicules are large about 0.1 mm in diameter, stellate with 8 - 13 conical pointed rays in optical section. Living and preserved colonies are pinkish white to light brown.

*Zooid:* The thorax measures 0.4 - 0.5 mm and abdomen 0.6 - 0.7 mm (Fig. 5B). Branchial siphon

is short with small triangular lobes. Atrial aperture is on a short siphon directed laterally. A lateral organ is situated between the second and third row of stigmata. Retractor muscle is short originating from the posterior end of thorax. 8 - 12 short branchial tentacles could be counted. Prebranchial area is wide. There are about 6 - 7 oval stigmata in each of the three rows. Gut forms a simple vertical loop which is relatively short. Oesophageal neck is long and the stomach is spherical. A short duodenum connects the posterior stomach present at the pole of gut loop with stomach. Rectum is wide, opening by a bifid anus below the level of third row of stigmata. Single large testis is on the dorsal side of the curve of gut loop. Vas deferens coils  $8\frac{1}{2}$  times around the testis. Many larvae are present in the basal layer of test. Larvae are large measuring 0.75 mm. Larval test is embedded with plant cells (Fig. 5C). There are 4 pairs of ampullae alternating with 3 adhesive organs. An otolith and ocellus are present. Tail is wound halfway round the trunk.

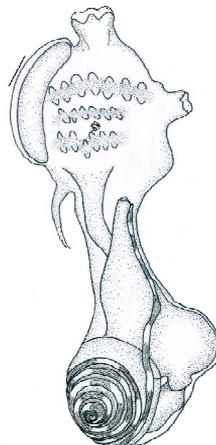
*Distribution:* Western pacific (Kott, 1982), Fiji (Kott, 1981), Indian Ocean (Kott, 2001).

Current Record: India

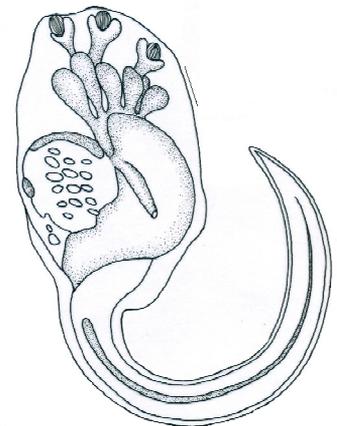
*Remarks:* *Trididemnum paraclinides* resembles *T. cerebriforme* and *T. savignii* in the presence of an atrial siphon but differs from them in having prokaryotic symbionts in the common cloacal cavity.



A



B



C

Fig. 5. *Trididemnum paraclinides* - A. Colony B. Zooid C. Larva (Scale - 0.1 mm)

Zooids of *T. paracyclops* and *T. nubilum* though have prokaryotic symbionts, their atrial aperture is sessile. Large stellate spicules of the present species and the fleshy cushion like colonies differentiate it from that of the spongy colonies of *T. clinides*. Most characteristic features of this species are the nature of the colony, presence of prokaryotes, an atrial siphon, absence of endostylar pigment cap and large spicules.

Earlier records of this species are from the western Pacific and Indian Ocean (Kott, 1981, 1982, 2001). Number of colonies were collected from various habitats of the coastal districts of Gulf of Mannar and recorded by Meenakshi *et al.*, 2003. From the south east coast only three species of the genus - *T. cerebriforme*, *T. nubilum* and *T. paracyclops* has been recorded (Meenakshi, 2000, Senthamarai *et al.*, 2016b). There is no authentic systematic study or records of this species from other locations along the eastern Indian Ocean; hence field collections from different stations are suggested.

#### 6. *Trididemnum savignii* (Herdman, 1886) (Fig. 6A,B,C)

**Synonymies:** *Didemnum savignii* Herdman, 1886: 261; Van Name, 1902: 358; *Trididemnum savignii*: Van Name, 1921: 314; 1924: 23; 1945: 100; Peres, 1949: 184; 1951: 1056; Tokioka, 1953: 197; Kott, 2001: 281.

**Materials examined:** Ten colonies collected from trawl discards, intertidal areas, fouling community and coral reef of 10 stations along Thoothukudi and Ramanathapuram in the east coast during the period 2000 - 2003 by V.K. Meenakshi and S. Senthamarai.

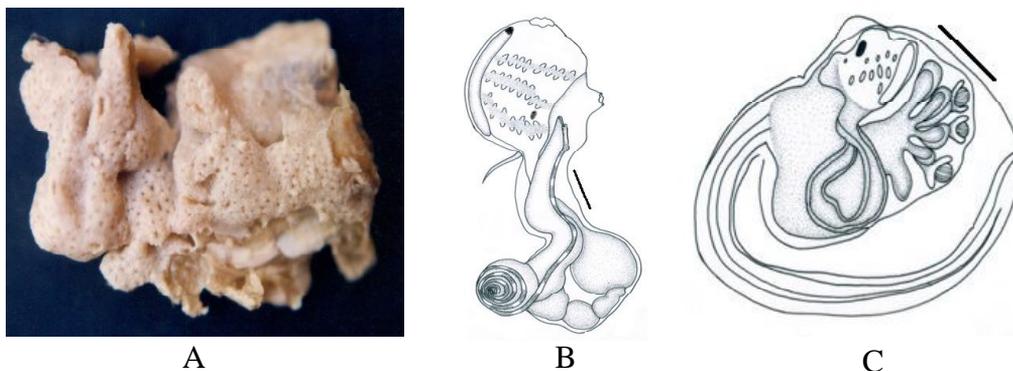
**Description: Colony:** Colonies are very large extending to several centimetres. Texture of the colony is fleshy with a thickness of 3.2 mm (Fig. 6A). Surface is smooth, lobed and slippery. There is a thick superficial layer of bladder cells. Test is divided into a surface, central and basal layer. Central layer is thicker into which some of the contracted zooids are placed. Upper part of this layer is in level with the thorax of zooid and has a thin layer of spicules which is just below the bladder cell layer. Spicules form a thick layer on the basal part of colony whereas it is absent from remainder of lower part. Spicules are stellate, large, 0.14 mm in diameter with 9 - 14 long conical sharply pointed

rays in optical section. Faecal pellets have not been observed in basal test. Extensive common cloacal canals are located below the layer of spicules in level with oesophagus or abdomen. Black pigment cells are present in thoracic layer of test. Living and preserved colonies are light creamy yellow in colour. **Zooids:** Zooids measure 1.5 - 2.0 mm long with a thorax 0.6 mm and abdomen 0.9 mm (Fig. 6B). Branchial siphon is short, cylindrical with 6 small branchial lobes. Atrial siphon is short directed laterally situated in the middle of thorax. Zooids are opaque with dark brown pigmented squamous epithelium covering the thorax and abdomen which is conspicuous anteriorly. An elongate thoracic organ is present between the second and third row of stigmata. Retractor muscle is short originating from posterior end of thorax. There is an endostylar pigment cap. 11 - 12 short branchial tentacles are at the base of branchial siphon. Prebranchial area is wide. There are 7 - 8 elongate stigmata in each of the three rows. Gut loop is curved ventrally with a long oesophagus, spherical stomach and small posterior abdomen at the curve connected by short duodenum. Wide proximal part of rectum narrows out at distal end opening by a round anus at the level of second row of stigmata. There is an undivided testis. Vas deferens coils 5½ to 7½ times. Larva measures 0.7 mm in size (Fig. 6C). There are four pairs of ampullae alternating with 3 adhesive organs. An otolith and ocellus are present. Tail is wound halfway round the larval trunk.

**Distribution:** Indonesia (Sluiter, 1909), West Indies, Florida, Bermuda (Van Name, 1902, 1921, 1924, 1930, 1945), Northern Territory, Western Australia, Queensland (Kott, 2001).

**Current Record:** India.

**Remarks:** Present species resembles *Trididemnum cerebriforme* in the absence of prokaryotic symbionts in common cloacal cavity, nature of colony, superficial bladder cell layer, stellate spicules and zooids with squamous epithelium, small laterally directed atrial siphon and an endostylar pigment cap but differs from it in having characteristic distribution of spicules in subsurface layer above cloacal cavity and absent from basal part. *T. paraclinides* and *T. clinides* though has an atrial aperture on a siphon like the present species differs in the nature,



**Fig. 6.** *Trididemnum savignii* - A. Colony B. Zooid C. Larva (Scale - 0.1 mm)

Key to the species of *Trididemnum* recorded

1.	Presence of prokaryotic symbionts in the common cloacal cavity .....	3
	Absence of prokaryotic symbionts in the common cloacal cavity .....	2
2.	Complex convoluted colonies .....	<i>T. cerebriforme</i>
	Extensive fleshy colonies with smooth slippery surface .....	<i>T. savignii</i>
3.	Atrial aperture sessile .....	4
	Atrial aperture on a siphon .....	5
4.	Endostylar pigment cap present .....	<i>T. paracyclops</i>
	Endostylar pigment cap absent .....	<i>T. nubilum</i>
5.	Large stellate spicules 0.1 mm in diameter .....	<i>T. paraclinides</i>
	Small stellate spicules 0.04 mm in diameter .....	<i>T. clinides</i>

distribution of spicules and presence of prokaryotes in common cloacal cavity. Two other species - *T. paracyclops* and *T. nubilum* which are already reported also have prokaryotic symbionts but their atrial aperture is sessile. Characters to identify this species include the smooth, slippery, fleshy extensive nature of colonies, absence of prokaryotic symbionts in the common cloacal cavity, distribution of spicules, large sized spicules and zooids.

This is a species exhibiting an apparently pan tropical range according to Kott, 2001. From the Gulf of Mannar Coast it was recorded by Meenakshi *et al.*, 2003. Several large colonies collected were examined and described in the present study. Specimen assigned to *T. savignii* by Abdul Jaffar Ali and Tamil Selvi, 2016 from the southeast coast is noted only briefly without the taxonomic details of abdomen, gonads or larva. As pointed out earlier, family Didemnidae is taxonomically the most difficult to identify up to the species level and therefore observation of all the characters of the species are essential to prevent wrong assignments.

#### ACKNOWLEDGEMENTS

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

#### REFERENCES

- Abdul Jaffar Ali, H. and Tamilselvi, M. 2016. Ascidiens in coastal water - A comprehensive inventory of ascidian fauna from the Indian coast. Springer International, Switzerland. pp. 51-130.
- Ananthan, G. 2014. Diversity and Ecology of sedentary ascidiens of the Great Nicobar Biosphere Reserve. First Year Progress Report. pp. 1-103.
- Bjerkman, P. 1905. Ascidiens von dem norwegischen Fischereidampfer 'Michael Sars' in den Jahren 1900-1904 gesammelt. Bergens Museum Arbog Afhandlingar og Arsberetning. 5: 4-29.

- Della Valle, A. 1881. Nouvi contribuzioni alla storia naturale delle ascidie composte del Golfo di Napoli. Atti della Accademia Nazionale Lincei Series. 10 (3): 431-498.
- Eldredge, L.G. 1967. A taxonomic review of the Indo-Pacific didemnid ascidians and descriptions of twenty three central Pacific species. *Micronesica*. 2: 162-261.
- Giard, A. 1872. Recherches sur les ascidies composees ou synascidies. *Archives de Zoology Experimentale et Generale*. 1: 507-704.
- Hartmeyer, R. 1909-11. Ascidien (Continuation of work by Seeliger). pp. 1281-1772. In Bronn, H.G., Klassen und Ordnungen des Tierreichs vol. 3 suppl., part 89-98. (C.F. Winter: Leipzig). (Abstract, repeating lists of species by Schepotieff A, 1911, in *Archives fur Naturgeschichte*. 6: 3-27.
- Hartmeyer, R. 1913. Tunicata. In: L. Schultze, K. Zoologie und anthropologie Ergebnisse einer Forschungsreise in Sudafrika Bd 5, Lfg 2. *Denkschriften der Medizinisch-naturwissenschaftlichen Gesellschaft zu Jena*. 17: 125-144.
- Herdman, W.A. 1886. Report on the Tunicata collected during the voyage of H.M.S. "Challenger" during the years 1873-76. Pt.II, Ascidae compositae. Report on the Scientific Results of the Voyage of HMS Challenger. 14 (38): 1-425.
- Hirose, E. and Nozawa, Y. 2010. Photosymbiotic ascidians from Kenting and Lyudao in Taiwan. *Zoological Studies*. 49 (5): 681-687.
- Hirose, E., Iskandar, B.H. and Wardiatno, Y. 2014. Photosymbiotic ascidians from Pari Island (Thousand Islands, Indonesia). *ZooKeys*. 422: 1-10.
- Kott, P. 1957. The ascidians of Australia II. Aplousobranchiata Lahille: Clavelinidae Forbes & Hanley and Polyclinidae Verrill. *Australian Journal of Marine and Freshwater Research*. 8 (1): 64-110.
- Kott, P. 1962. The ascidians of Australia III. Aplousobranchiata Lahille: Didemnidae Giard. *Australian Journal of Marine and Freshwater Research*. 13 (3): 265-334.
- Kott, P. 1977. Algal supporting didemnid ascidians of the Great Barrier Reef. *Proceedings of the Second International coral reef symposium*. 1: 615-621.
- Kott, P. 1980. Algal-bearing didemnid ascidians in the Indo-west Pacific. *Memoirs of the Queensland Museum*. 20 (1): 1-47.
- Kott, P. 1981. The ascidians of the reef flats of Fiji. *Proceedings of the Linnean Society of New South Wales*. 105 (3): 147-212.
- Kott, P. 1982. Didemnid-algal symbioses: host species in the western Pacific with notes on the symbiosis. *Micronesica*. 18 (1): 95-127.
- Kott, P. 1984. Related species of *Trididemnum* in symbiosis with Cyanophyta. *Proceedings of the Linnean Society of New South Wales*. 107 (4): 515-520.
- Kott, P. 1985. The Australian Ascidiacea. Part I, Phlebobranchia and Stolidobranchia. *Memoirs of Queensland Museum*. 23: 1-440.
- Kott, P. 1998. Tunicata, in Wells, A. & Houston, W.W.K. (eds) *Zoological Catalogue of Australia, Hemichordata, Tunicata, Cephalochordata*. (CSIRO Publishing: Melbourne). 34: 51-292.
- Kott, P. 2001. The Australian Ascidiacea Part 4, Aplousobranchia (3), Didemnidae. *Memoirs of the Queensland Museum*. 47 (1): 1-410.
- Macdonald, J.D. 1859. On the anatomical characters of a remarkable form of compound Tunicata. *Transaction of the Linnean Society of London (Zoology)*. 22: 373-375.
- Meenakshi, V.K. 1997. Biology of a few chosen ascidians. Ph. D., thesis, Manonmaniam Sundaranar University, Tirunelveli. Pp. 40-42.
- Meenakshi, V.K. 2000. *Trididemnum* Della Valle, 1881, an unrecorded genus of colonial ascidian from India. *Journal of the Bombay Natural History Society*. 97 (2): 302-304.
- Meenakshi, V.K., Renganathan, T.K., Senthamarai, S. and Jayalakshmi, J. 2003. Marine Biodiversity – Taxonomy of Indian Ascidians. Final Technical Report of Major Research Project submitted to the Ministry of Environment and Forests, New Delhi. Pp. 1-37.
- Meenakshi, V.K., Senthamarai, S. and Gomathy, S. 2014. *Polysyncraton* Nott, 1892 an unrecorded genus of the family Didemnidae from India. *Journal of Chemical, Biological and Physical Sciences*. 4 (4): 3243-3246.
- Millar, R.H. 1975. Ascidians from the Indo-West Pacific region in the Zoological Museum, Copenhagen (Tunicata: Ascidiacea). *Steenstrupia*. 3 (20): 205-336.
- Monniot, F. 1994. Ascidies de Nouvelle-Caledonie XIV. Le genre *Diplosoma* (Didemnidae) dans le lagon sud. *Bulletin du Museum National d'Histoire Naturelle*. Paris ser. 4. 16 (1): 3-11.
- Monniot, F. 1995. Ascidies de Nouvelle-Caledonie XV Le genre *Didemnum*. *Bulletin du Museum National d'Histoire Naturelle*. Paris ser. 4. 16 (2-4): 299-344.
- Monniot, F. and Monniot, C. 1987. Les ascidies de Polynesie francaise. *Memoirs du Museum National d'Histoire Naturelle* Paris. 136: 1-155.
- Nott, J.T. 1892. On the composite ascidians of the North Shore Reef. *Transactions New Zealand Institute*. 24: 305-344.
- Parry, D.L. and Kott, P. 1988. Cosymbiosis in the Ascidiacea. *Bulletin of Marine Science*. 42 (1): 149-153.

- Peres, J.M. 1949. Contributions a l'etude des Ascidiées de la cote occidentale d'Afrique. Bulletin d l'Institut Francais d'Afrique Noire. 11 (1-2): 159-207.
- Peres, J.M. 1951. Nouvelle contributions a l'etude des Ascidiées de la cote occidentale d'Afrique. Bulletin d l'Institut Francais d'Afrique Noire. 12 (4): 1051-1071.
- Renganathan, T.K. 1981. On the occurrence of a colonial ascidian, *Didemnum psammathodes* (Sluiter, 1895) from India. Current Science. 50 (20): 922.
- Renganathan, T.K. 1982. On the occurrence of a colonial ascidian, *Lissoclinum fragile* (Van Name, 1902) from India. Current Science. 51 (3): 149.
- Renganathan, T.K. 1986. Studies on the ascidians of South India. Ph. D., Thesis. Madurai Kamaraj University, Madurai. Pp. 58-60.
- Savigny, J.C. 1816. Rescherches anatomiques sur les ascidies composees at les ascidies simple. Systeme de la classe des Ascidiées. Memoirs sur les-animaux sans vertebres. 2: 1-239.
- Senthamarai, S., Meenakshi, V.K. and Gomathy, S. 2016a. New records of two species of the genus *Leptoclinides* Bjerkan, 1905 from Indian waters. World Journal of Pharmaceutical and Life Sciences. 2 (2): 196-204.
- Senthamarai, S., Meenakshi, V.K. and Gomathy, S. 2016b. Description of ten species of the family Didemnidae Giard, 1872 from Gulf of Mannar. International Journal of Zoology Studies. 1 (5): 13-24.
- Sluiter, C.P. 1895. Tunicaten. Pp. 163-186. In Semon, R., Zoologische Forschungsreisen in Australien und dem Malagischen Archipel. Denkschriften der Medizinisch-naturwissenschaftlichen Gesellschaft zu Jena. 8: 325-326.
- Sluiter, C.P. 1909. Die Tunicaten der Siboga - Expedition Pt.2. Die merosomen Ascidien. Siboga Expedition. 56: 1-112.
- Sluiter, C.P. 1913. Ascidien von den Aru-Inseln. Abhandlungen herausgegeben von der Senckenbergischen Naturforschenden Gesellschaft. 35: 65-78.
- Stalin, C., Ananthan, G. and Raghunathan, C. 2017. A new record of photosynthetic ascidians from Andaman and Nicobar Islands, India. Indian Journal of GeoMarine Sciences. 46 (11): 2393-2398.
- Su, S.W., Hirose, E., Lee, S.C.S. and Mok, H.K.M. 2013. Photosymbiotic ascidians in Singapore: The turbid waters may reduce the living space. Zookeys. 305: 55-65.
- Thorne, S.W., Newcomb, E.H. and Osmond, C. 1977. Identification of chlorophyll b in extracts of prokaryotic algae by fluorescence spectroscopy. Proceedings of Natural Academy of Sciences USA. 74 (2): 575-578.
- Tokioka, T. 1953. Ascidiens of Sagami Bay. Tokyo: Iwanami Shoten. p. 1-313.
- Tokioka, T. 1954. Contributions to Japanese ascidian fauna VII. Invertebrate fauna of the intertidal zone of the Tokara Island VII Ascidiens. Publications of the Seto Marine Biological Laboratory. 3 (3): 239-264.
- Tokioka, T. 1955. Ascidiens from the Palao Islands (II). Publications of the Seto Marine Biological Laboratory. 5 (1): 43-57.
- Tokioka, T. 1961. Ascidiens collected during the Melanesia Expedition of the Osaka Museum of Natural History. I, Ascidiens presented y Dr. R.L.A.Catala of the Aquarium of Noumea. Publications of the Seto Marine Biological Laboratory. 9 (1): 104-138.
- Tokioka, T. 1967. Pacific Tunicata of the United States National Museum. Bulletin, United States National Museum. 251: 1-242.
- Tokioka, T. 1970. Ascidiens from Mindoro Island, the Philippines. Publications of the Seto Marine Biological Laboratory. 18 (2): 75-107.
- Van Name, W.G. 1902. The Ascidiens of the Bermuda Islands. Trans. Connecticut Academy of Arts and Science. 1: 325-412.
- Van Name, W.G. 1918. Ascidiens from the Philippines and adjacent waters. Bulletin of the United States National Museum. 100 (1): 49-174.
- Van Name, W.G. 1921. Ascidiens of the West Indian region and south eastern United States. Bulletin of the American Museum of Natural History. 44: 283-494.
- Van Name, W.G. 1924. Ascidiens from Curacao. Bijdragen tot de kennis der fauna van Curacao, Resultaten einer Reis van Dr C. J. Van der Horst in 1920. Bijdragen tot der Dierkunde. 23: 23-32.
- Van Name, W.G. 1930. The ascidiens of Porto Rico and the Virgin Islands. Scientific survey of Porto Rico and the Virgin Islands. New York Academy of Sciences: New York. 10 (4): 403-512.
- Van Name, W.G. 1945. The North and South American ascidiens. Bulletin of the American Museum of Natural History. 84: 1-476.
- Vasseur, P. 1969. Deuxieme contribution al'etudedes ascides de Madagascar region de Tulear. Bulletin du Museum National d'Histoire Naturelle ser. 240 (5): 912-933.
- Verrill, A.E. 1871. Descriptions of some imperfectly known and new ascidiens from New England. American Journal of Science. 3 (1): 54-446.

