International Journal of Zoology Studies
ISSN: 2455-7269
Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 3; Issue 2; March 2018; Page No. 10-17

New records of ascidians (Tunicata, Ascidiacea) from Mandapam coast, Gulf of Mannar, India

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Abstract
An investigation on the ascidian fauna in Mandapam coast, Gulf of Mannar, India was carried out to update the diversity and new record of ascidians. Out of 38 species of ascidians belonging to 14 genera and 7 families recorded, *Rhodosoma turcicum*, *Symplegma brakenhielmi*, *Microcosmus helleri*, *M. squamiger*, *Eudistoma amplum*, *Polyclinum madrasensis*, *Didemnum perlucidum*, *Diplosoma gelatinosa*, and *Lissoclinum patella* were new to this station and two species of *Polyclinum* were new to science. Three species such as *Polyandrocarpa zorritensis*, *Diplosoma gelatinosa* and *D. translucidum* were first time report to India. The number of species was high in pre-monsoon, followed by summer, post-monsoon and monsoon seasons. This study represents a first step in documenting the ascidian communities in Mandapam coast and provides a baseline of local diversity of ascidians against which it will be possible to determine whether the diversity of ascidians change over time. Similar studies with detailed taxonomic investigations in other parts of North east coast of India would yield rich haul of ascidians in Indian coast.

Keywords: ascidians, diversity, Indian peninsula, Mandapam, tunicate

1. Introduction
Ascidians are excellent materials for several experimental studies like tissue regeneration [1, 2], immunology [3], budding [4], colony organization [5], embryology [6, 7] and bioaccumulation of elements [8]. Ascidians are signature species for novel compounds with various activities such as anti-tumour [9, 10], anti-inflammatory [11] and anti-leukemic [12]. Ascidians are consumed by human beings and often by marine animals as food in many parts of the world [13]. Some ascidians could also be used as potential indicators of coastal water quality [14]. Though they are filter feeders, they can be used for monitoring of heavy metals [15, 8]. Mandapam is situated in Gulf of Mannar which is one of the marine hotspot areas in India. This station is provided with natural substrates (small stones, embedded rocks, coral pieces, molluscan shells, etc) and artificial substrates (jetty, cement blocks, hull of boats and other fishing harbour installations) which are ideal for settlement of ascidians. Moreover this station experiences two different seasonal patterns, due the influence of the direction and force of the monsoonal winds. Considering the ongoing changes in marine ecosystem, the arrival as well as proliferation of non-indigenous ascidians and seasonality in their recruitment, significant variation in diversity and distribution of ascidians is expected. Developing additional human resources is necessary for the continuity of biodiversity studies and today, no research group is studying ascidians in the Mandapam coast. In light of the above, an investigation was carried out to explore the ascidians fauna in Mandapam coast situated in Gulf of Mannar, India.

2. Materials and Methods
A study was conducted at Mandapam coast (9°28′21″N 79°19′37″E), Gulf of Mannar region, Southeast coast of India for a period of one year from June 2016 to May 2017 covering all the four seasons Pre-monsoon (June-August), Monsoon (September-November), Post-monsoon (December-February) and Summer (March-May). For this study, the sampling area of 10 x 5m was fixed at Mandapam coast. The ascidians in 12 pillars laid down in the sampling area were observed by snorkeling method and few specimens of ascidians were sampled from the study area by adopting various collection methods. Ascidian samples were photographed in situ before being collected. Then the collected samples were narcotized using menthol crystals after treating with a pinch of magnesium chloride for defecation and then preserved in a 10% buffered formalin solution. The preserved samples were brought to laboratory. All possible taxonomical characters were observed under stereo microscope (Micros) and /or compound microscope (Labomed) and then identified by following taxonomic keys and descriptions of [16, 17, 18, 19]. Voucher specimens were deposited in the Zoology Museum of Islamiah College (Autonomous), Vaniyambadi. Water quality parameters such as temperature, salinity, pH and rainfall were also analyzed in-situ using Digital thermometer (Mextech), Refractometer and Digital pH pen (Hanna- HI98107) respectively. Data of annual rainfall (mm) was obtained from Meteorological Department of Tamilnadu.
Percentage occurrence of ascidians was calculated by the following formula.

$$\text{Percentage occurrence} = \frac{\text{Number of individual species}}{\text{Total number of species}} \times 100$$

3. Result
All the ascidians species found during this study at Mandapam waters are listed in Table. 1. We found 38 species of ascidians belonging to 14 genera and 7 families. Among the 38 species, 35 were colonial and 3 solitary. Maximum representation was

from the family Polyclinidae (31.57%), which is followed by Didemnidae (23.68%), Polycitoridae (21.00%), Perophoridae & Pyuridae (7.89%), Styelidae (5.26%) and Rhodosomatidae (02.63%) (Fig.1). The number of species was higher in pre-monsoon (23), followed by summer (20), post-monsoon (17) and monsoon (13) (Table.2).While nine species such as, *Rhodosoma turcicum* (Savigny, 1816) [20], *Symplegma brakenhielmi* Michaelsen 1904 [21], *Microcosmus helleri* Herdman, 1882 [22], *M. squamiger* Michaelsen, 1927 [23], *Eudistoma amplum* (Sluiter, 1909) [24], *Polyclinum madrasensis* Sebastian, 1954 [25], *Didemnum perlucidum* Monniot, F., 1983 [26], *Diplosoma swamiensis* Renganathan, 1986 [38] and *Lissoclinum patella* (Gottschaldt, 1898) [28] were reported for the first time to this station. Three species such as *Polyandrocarpa zorritensis* Van Name, 1931 [29] *Diplosoma gelatinosa* Milne-Edwards, 1841 [30] and *D. translucidum* (Hartmeyer, 1909) [31] were first time reported in India and two species of *Polyclinum* from the family Polyclinidae were new to science. The most abundant species were *Ecteinascidia thurstoni, E. venui, Eudistoma laysani, E. viride, Polyclinum indicum, P. madrasensis, P. nudum* and *Didemnum psammathodes.*

Table 1: Checklist of ascidians in Mandapam coast and their temporal occurrence.

Species	Seasons				
	Occurrence	Pre-Monsoon	Monsoon	Post-Monsoon	Summer
Perophoridae Giard, 1872					
Ecteinascidia thurstoni Herdman, 1906	a	x	-	-	-
E. venui Meenakshi, 2000	a	x	x	-	x
Perophora multiclathrata (Sluiter, 1904)	r	-	-	x	-
Rhodosomatidae Hartmeyer, *1908*					
Rhodosoma turcicum (Savigny, 1816)	r	-	-	x	-
Styelidae Sluiter, *1895*					
Symplegma brakenhielmi Michaelsen, 1904	r	x	-	-	x
Polyandrocarpa zorritensis Van Name, 1931	r	-	-	-	x
Pyuridae Hartmeyer, *1908*					
Microcosmus exasperates Heller, 1878	c	x	x	x	x
M. helleri Herdman, 1882	r	x	-	-	-
M. squamiger Michaelsen, 1927	c	x	x	-	-
Polycitoridae Michaelsen, *1904*					
Eudistoma amplum (Sluiter, 1909)	r	x	-	x	-
Eudistoma constrictum Kott, 1990	c	x	-	-	-
E. gilboviride (Sluiter, 1909)	c	x	-	-	-
E. microlarvum Kott, 1990	r	-	-	-	x
E. laysani (Sluiter, 1900)	a	x	-	x	-
E. ovatum (Herdman, 1886)	c	x	x	x	x
E. pyriforme (Herdman, 1886)	c	x	-	-	-
E. viride Tokioka, 1955	a	-	-	x	-
Polyclinidae Milne-Edwards, 1841					
Synoicum galei Kott, 1992	c	-	-	-	x
Polyclinum fungosum Herdman, 1886	c	-	-	x	x
P. glabrum Sluiter, 1895	c	x	-	-	x
P. indicum Sebastian, 1954	a	x	x	x	x
P. madrasensis Sebastian, 1954	a	x	x	x	x
P. nudum Kott, 1992	a	-	x	-	x
P. saturnium (Savigny, 1816)	r	-	-	-	x
P. solum Kott, 1992	c	-	x	-	x
P. tenuatum Kott, 1992	c	x	-	-	x
Polyclinum n. sp.	c	x	-	x	-
Polyclinum n. sp.	c	x	-	x	-
Aplidiopsis confluata Kott, 1992	c	-	-	-	x
Didemnidae Giard, *1872*					
Tridemnum savignii (Herdman, 1886)	c	x	x	x	x
Didemnum perlucidum Monniot. F., 1983	r	-	x	-	-
Didemnum spadix Kott, 1992	r	x	x	-	-
Didemnum psammathodes (Sluiter, 1895)	a	x	x	x	x
Diplosoma gelatinosa Milne-Edwards, 1841	r	-	-	x	-
D. swamiensis Renganathan, 1986	r	x	-	-	-
D. translucidum (Hartmeyer, 1909)	r	-	-	-	x
*Lissoclinum patella (*Gottschaldt, 1898)	r	-	-	x	-
L. fragile (Van Name, 1902)	c	x	x	x	x

Note: A – Abundant; R – Rare; C – Common; X- Present; - – Absent

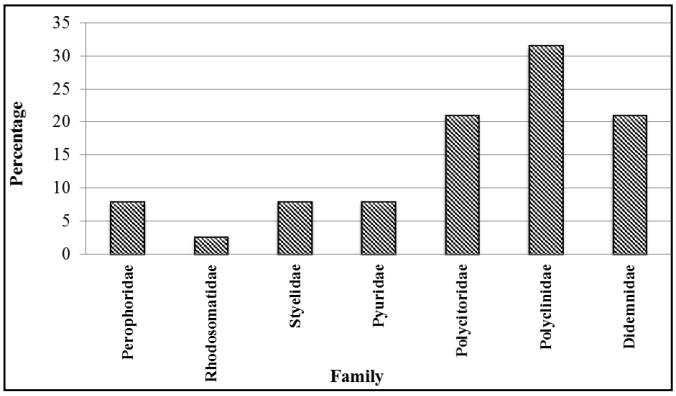

Fig 1: Percentage occurrence of ascidians of various families.

Hydrographical parameters and number of species per season are represented in Table 2. The range of temperature, salinity, pH and rainfall of this station was from 24.4±0.8 to 30.2±1.2^{0}C, 33.1±0.4 to 33.8±0.7 ppt, 7.7±0.1 to 7.9±0.2 and 31 to 235 mm respectively. ANOVA between hydrographical parameters and occurrence of ascidians species showed significant difference (P<0.005).

Table 2: Hydrographical parameters and number of species collected per season

Season	Temperature (^{0}C)	Salinity (%)	pH	Rainfall (mm)	No. of. Species
Pre-Monsoon	27.7±0.4	33.2±0.6	7.7±0.1	96.7	23
Monsoon	26.9±0.6	33.4±0.6	7.8±0.2	118	13
Post-Monsoon	24.4±0.8	33.8±0.7	7.9±0.2	235	17
Summer	30.2±1.2	33.1±0.4	7.7±0.1	31	20
Mean	27.3±0.75	33.12±0.57	7.7±0.15	120.17	18.25

The descriptions of three new records to this station are taxonomically described below:

3. Systematics

3.1.1. *Polyandrocarpa zorritensis* Van Name, 1931 [29]

Class: Ascidiacea
Suborder: Stolidobranchia
Family: Styelidae Sluiter, 1895 [32]
Genus: *Polyandrocarpa* Michaelsen, 1904 [21]
Species: *Polyandrocarpa zorritensis* Van Name, 1931 [29]

Material Examined: The colony of these species is attached to oyster shells, sponges and pillars of jetty at a depth of about 3-5 meters at Mandapam station.
Distribution: Southeast coast, India.
World Distribution: Hawaii, Japan, Mediterranean, southeastern Atlantic or southwestern Pacific, East and West coasts of North America.
Description: *External Appearance:* Zooids are dark brown to black colour and are upto 16-18 mm long and 2.5-3.0 mm in diameter. They are closely attached and are connected at the base of the colony by the presence of their stolon. Individual zooids are sub-cylindrical in shape. The test is thin, leathery and encrust with sand. The oral and atrial siphon are well defined, positioned anteriorly, and have four lobes with two dark bands per lobe.

Internal Structure: Individual zooids are long upto 13-14 mm height. The branchial sac has four folds per side and upto 12 longitudinal vessels are present. Simple tentacles are 24-26 of varying length. The dorsal tubercle is flat, smooth edge with wavy slit shaped transverse opening. The parastigmatic vessel and muscle fibres are present. The stomach is round smooth with 11-12 folds are present. The gonads are oval; polycarps are 7-8 on right, 5-7 on left on each side of endostyle.
Remarks: It is a synonym of *Stolonica zorritensis.* This species resembles the characteristics of *P. maxima* (Sluiter, 1904) [33] in colony shape, branchial tentacles, stomach folds but differs in dorsal tubercle (U or C- shaped), absence of parastigmatic vessel, muscle fibres and pyloric caecum.

3.1.2 *Diplosoma gelatinosa* Milne-Edwards, 1841 [30]

Class: Ascidiacea
Suborder: Aplousobranchia
Family: Didemnidae Giard, 1872 [34]
Genus: *Diplosoma* Macdonald, 1859 [35]
Species: *Diplosoma gelatinosa* Milne-Edwards, 1841 [30]

Material Examined: The colony of these species is attached to oyster shells, sponges and pillars of jetty at a depth of about 2-3 meters at Mandapam station.
Distribution: Gulf of Mannar, Southeast coast, India.
World Distribution: West Indies, West Pacific Ocean.
Description: External Appearance: The colonies are thin, fragile, encrusting sheet upto 5 cm in dimension. Test is soft, transparent and delicate. Zooids are crowded throughout the colony.
Internal Structure: Zooids are about 3-4 mm long. Branchial sac has 6 lobed and the atrial aperture is wide and open. There are 4 rows of about 7 stigmata per row. The abdomen is slightly smaller than the thorax and forms a horizontal rounded gut loop. The stomach is smooth and oval to rectangular shape. Vas deferens is uncoiled. 7-8 testis follicles are present.
Remarks: This species has characteristic by the presence of smooth, gelatinous test, absence of prochloron and number of larval lateral ampullae. The retractor muscle protruding from posterior end of thorax rather than from middle to base of the oesophagus and the extensive thoracic rather than posterior abdominal cloacal cavity. The colony of *D. simile* has also smooth, thin and retractor muscle emerges from posterior end of thorax but differs in number of stigmata and presence of prochloron. *D. virens* is similar to the present species in absence of prochloron, retractor muscle from halfway down oesophageal neck but differs in number of stigmata and colony.

3.1.3 *Diplosoma translucidum* (Hartmeyer, 1909) [31]

Class: Ascidiacea
Suborder: Aplousobranchia
Family: Didemnidae Giard, 1872 [34]
Genus: *Diplosoma* Macdonald, 1859 [35]
Species: *Diplosoma translucidum* (Hartmeyer, 1909) [31]

Material Examined: The colony of these species is attached to oyster shells, sponges and pillars of jetty at a depth of about 2-3 meters at Mandapam station.

Distribution: Southeast coast, India.
World Distribution: Caledonia, Indonesia and Western Australia.
Description: External Appearance: Colonies are fleshy and almost firm sheets over the substrate. In life, they generally are translucent and the zooids are orange, red to brown in colour and are easily visible. In preservative, the colonies are light grey, with white zooids showing through the translucent test. Colonies are 3-4 cm long and 3 cm width. Zooids are difficult to remove from the test of preserved colonies. Common cloacal apertures are present.
Internal Structure: Zooids are about 1 mm long, the thorax and abdomen of same length. The branchial siphon is long, cylindrical with six pointed lobes. The branchial sac is wide, with 4 rows of about 10 stigmata in anterior row and 8 stigmata in posterior row. The atrial aperture is wide, exposing most of the branchial sac directly to cloacal cavity. A moderately long, narrow retractor muscle emerges from posterior end of thorax. The gut loop is horizontal, oesophageal neck is relatively long and the distal half of the abdomen. The vas deferens is curve and has 2 lobes of the testis.
Remarks: This species is characterized by its tough test, relatively small zooids, abdomen posterior to the thorax as described by [19]. *D. velatum* have the same narrow finger like branchial lobes, number of stigmata per row, 2 male follicles, retractor muscle emerges from posterior end of thorax and differentiate in test which is much softer than the present species. Sluiter (1909) [24] and Hartmeyer (1919) [36], observed 6 lobes of long branchial siphon, tough test, 10 rows of stigmata in anterior row are also observed in the present species

New Species
3.1.4 *Polyclinum* n.sp. 1. (Fig. 2)
Class: Ascidiacea
Suborder: Aplousobranchia
Family: Polyclinidae Milne-Edwards, 1842 [37]
Genus: Polyclinum Savigny, 1816 [20]
Species: *Polyclinum* n.sp. 1.

Material Examined: Colonies of this species were collected at two meter depth from the pillars of Jetty installed at Mandapam station.
Distribution: Gulf of Mannar, Southeast coast, India.
Description: *External Appearance*: Colonies are rounded cushions to 4-5 cm in greater extent and up to 1 cm in height. Living colonies are dark pink in colour and light brown in preservative. Colonies are covered with a layer of sand sparsely distributed on the surface. Base of the colony is attached to the substrate. Test is soft and gelatinous with zooids throughout the colony with linear arrangement. Zooids are not loosely attached to the test. No pigment cells on the surface test. Common cloacal openings are sessile and are 3-4 cm apart.
Internal Structure: Zooids are about 6-8 mm long with long cylindrical thorax and three fourth of the total length of the zooid. Small vertically oriented gut loop with small oval shaped posterior abdomen. Long stolonic vessel is attached to the posterior abdomen. Branchial siphon is long with 6 pointed lobes. Atrial lip is long, flat and contains denticulation on its free tip. Atrial lip protrudes from upper border of the aperture and has 7 minute papillae which forms fringe at the tip. Fine longitudinal muscles extend from branchial siphon to about half way down the thorax. Up to 12 rows and about 16 stigmata per row. Branchial papillae are present. A tailed larva has a long trunk which covers the three fourth of the whole larva. Usually 5-8 larva is present in thorax. But 15-20 well matured embryos are also seen in the thorax owing to release.
Remarks: The characteristic feature of this species is large sized thorax, more than half of the total length of zooid with small branchial lobes; oval shaped posterior abdomen. Branchial papillae also present. Fine longitudinal muscle extends only upto the end of thorax. *Polyclinum isipingense* and *P. saturnium* also similar to the present species in many aspects but differ in stigmata per row. *P. gelidus* has also same number of row but differs in the number of stigmata per row and absence of branchial papillae. Since many characteristic features of this species have not bound to already existing species of polyclinum, the present species is reported to be a new species.

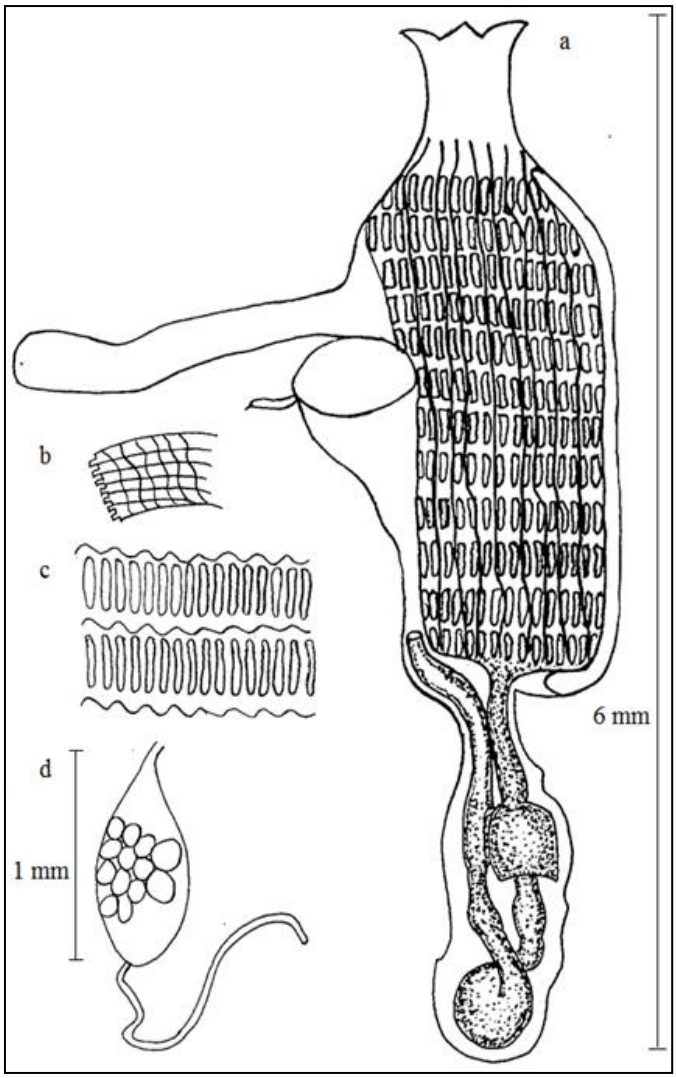

Fig 2: *Polyclinum* n. sp. 1. a) Zooid with abdomen, b) Tip of atrial tongue, c) Portion of branchial sac showing branchial papillae on transverse vessels, d) Posterior abdomen.

3.1.5 *Polyclinum* n.sp. 2. (Fig. 3)
Class: Ascidiacea
Suborder: Aplousobranchia
Family: Polyclinidae Milne-Edwards, 1842 [37]
Genus: Polyclinum Savigny, 1816 [20]
Species: *Polyclinum* n.sp. 2.

Material Examined: Colonies of this species were collected at two meter depth from the pillars of Jetty installed at Mandapam station.
Distribution: Gulf of Mannar, Southeast coast, India.
Description: *External Appearance*: Colonies are globules like, flat-topped, cushion-shaped with 3 cm in greater extent and up to 2 cm width. Sand particles found on the sides of the colony. Colonies are dark brown in preservative. Zooids are crowded, obscuring the form of the system. They are arranged in a single row in upper layer of the test. Test is translucent and gelatinous and fleshy brown in colour. No pigment cells present in the test.
Internal Structure: Zooids are 3-4 mm long with wide thorax, a vertical gut loop in the abdomen and a long pear shaped posterior abdomen connected to abdomen by a small neck. Fleshy brown in preservative. Branchial lobes are broad and well defined. Atrial lip is long arising from the border of aperture. Tip of the atrial lip is pointed and some are fringed. Fine longitudinal muscles extend towards the atrial lip. Branchial sac is wide having 11 rows of stigmata and up to 16 long stigmata per row. The narrow neck connecting to the posterior abdomen, attached in the pole of the gut loop. There are about 20 male follicles present in the pear shaped posterior abdomen. A long stolonic vessel is attached at the bottom of the posterior abdomen. Usually 3-4 larvae seen in branchial cavity. The trunk is ¾ of the total length. It has usual median and lateral ampullae, with wide adhesive papillae. The ectodermal vesicle present in a single row of anterior end of larvae.
Remarks: The characteristic feature of this species are long and wide thorax; well-developed branchial lobes; longitudinal muscle; branchial sac wide up to 16 stigmata per row and pear shaped posterior abdomen. *Polyclinum clarum, P. circulatum* and *P. festum* are similar to the present species but not on number of rows and stigmata. *P. neptunium* is more similar to this species but differs only in atrial lip shape. Since many characteristic features of this species have not bound to already existing species of polyclinum, the present species is reported to be a new species.

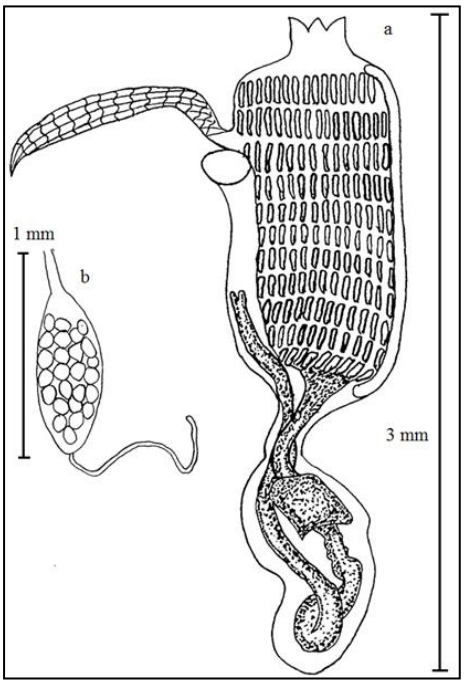

Fig 3: *Polyclinum* n. sp. 2. a) Zooid with abdomen; b) Posterior abdomen of the zooid.

4. Discussion
Among the 38 species of ascidians reported at Mandapam in the present study, 33 species were typically found in Thoothukudi coast [38, 39, 40, 41]. Most of the completely identified ascidians here are known and present around the world. Although the survey was short and covered only one station, the report of 38 species of ascidians including three new to India water, nine new to Mandapam water and two new species shows relatively a high richness when compared to other stations such as Trichendur (15) [42, 38], Rameshwaram (8) [42] and Andaman (2)[43]. The introduction of 3 species to Mandapam water might be through hull fouling of intercoastal trafficking of fishing as well as cargo vessels and coast guard vessels. The movement of recreational and fishing boats had previously revealed to be an important vector for intraregional spread of invaders [44, 45, 46, 47]. There is a growing awareness that ascidians are potential invasive species. This could be justified with the introduction of these species in to the Indian water. Nine species such as, *Rhodosoma turcicum* (Savigny, 1816) [20], *Symplegma brakenhielmi* Michaelsen 1904 [21], *Microcosmus helleri* Herdman, 1882 [22], *M. squamiger* Michaelsen, 1927 [23], *Eudistoma amplum* (Sluiter, 1909) [24],

Polyclinum madrasensis Sebastian, 1954 [25], *Didemnum perlucidum* Monniot, F., 1983 [26], *Diplosoma swamiensis* Renganathan, 1986 [38] and *Lissoclinum patella* (Gottschaldt, 1898) [28] previously reported in Thoothukudi, Chennai, Tiruchendur and Inigo Nagar [40, 41, 48; 49, 42, 50, 39], are recorded at Mandapam station in this study and this might be due to the connectivity between these stations. The presence of two new species could be due to tropical condition, and the Mandapam water is always calm and except in few seasons, there is a plenty of natural and artificial substrata which tend to promote ascidians for their breeding / livelihood [51]. The number of species was higher in pre-monsoon (23), followed by summer (20), post-monsoon (17) and monsoon (13). Pre-monsoon season wa favourable for the recruitment of ascidians due to suitable climatic as well as environmental condition and also due to the water current and available substrata [52]. In monsoon season, the recruitment of ascidian was less as compared to pre-monsoon and this might be due to the changes in environmental parameters such as salinity, pH, temperature, etc. Rocha & Faria (2005) [52] reported that intense hydrodynamics can limit the occurrence of more sensitive ascidians because of turbulence. Monniot (1965) [53] and Hatfield *et al.,* (1992) [54] also emphasized the same. Due to heavy rainfall, there may be a disturbance in sea bed and resulting in increased turbidity, which may affect the ascidian population [53]. Most of the ascidians bred during post monsoon season and degeneration of older colonies and formation of new colonies were observed. Followed by the post monsoon, the recruitment of ascidians raised slowly and this might be due to normal condition of sea water with ideal salinity, temperature, pH, and environment condition. The present study clearly showed temporal pattern within the year of study. In pre-monsoon, both the number and abundance of species were greater than in other seasons. Eight species such as *Microcosmus exasperatus, Eudistoma ovatum, Polyclinum indicum, Polyclinum madrasensis, Polyclinum nudum, Tridemnum savignii, Didemnum psammathodes* and *Lissoclinum fragile* were found to occur throughout the year. Life history traits, longevity and reproduction played a major role to adapt varying environmental and biotic conditions[55]. Some ascidians showed differences in their recruitment during different seasons [56, 57, 58; 55, 59]. Turon (1988) [60] also demonstrated a relationship between reproductive periods and the latitudinal distributions of ascidians.

A clear variation was observed, with the greatest abundance of ascidian species in pre-monsoon, but we do not know if this pattern is repeatable between years. In consequence, the diversity and distribution of species here described may alter from time to time as new colonists invade and exiting colonists change their distribution with the passage of time and changing ecological conditions.

5. Acknowledgement

Our deep sense of gratitude to Department of Biotechnology, Government of India for the financial support (BT/PR6801/AAQ/3/609/2012) and also to our College Secretary and Principal for their motivation.

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