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# Ascidian diversity (Chordata: Tunicata) from Andaman and Nicobar Islands, India

by

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## Abstract

Ascidians are filter-feeding sac-like marine urochordates of great evolutionary, ecological and economic importance. Andaman and Nicobar Islands are one of the most important hot spots of biodiversity in India, while the ascidian diversity of this region is very scanty. Ascidians belonging to 29 species were identified at the Andaman and Nicobar Islands during the field research carried out from March 2014 to April 2015. Eight species (*Didemnum granulatum*, *Didemnum molle*, *Didemnum psammatodes*, *Diplosoma listerianum*, *Lissoclinum fragile*, *Lissoclinum levitum*, *Lissoclinum patella*, *Trididemnum Cyclops*) from the Didemnidae family were found and identified. Various diversity indices, such as the Shannon -Wiener index (H'), Margalef's index (D), Pielou's index (J'), K-dominance curves, Cluster Analysis and Multidimensional Scaling, were used to analyze the diversity, richness and evenness of species, and to compare the diversity between samples and their resemblance in terms of species composition. The maximum species richness was observed in Campbell Bay (2.424) and the minimum in Haddo Wharf (0.910). This finding shows the rich species diversity of ascidian fauna at Andaman and Nicobar Islands.

**Key words:** Ascidians diversity, Andaman and Nicobar islands, cluster analysis, multidimensional scaling

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## Introduction

Coastal waters contain an array of natural and man-made hard substrates that can serve as habitat for sessile fouling communities (Connell & Glasby 1999; Bulleri & Chapman 2010). While man-made structures can increase the amount of space available for fouling organisms, they are frequently associated with decreased water quality due to human activities, such as boat traffic and aquaculture. As a result, sessile communities from urbanized regions may have relatively low diversity, and can be composed mostly of exotic species that have been introduced through ship hulls or ballast water (Piola & Johnston 2008). Ascidians (Tunicata: Ascidiacea) are often dominant members of marine fouling communities from the subtidal zone on both natural and artificial substrates (Lambert & Lambert 2003; Lambert 2007; Shenkar & Loya 2009). They consist of nearly 3000 described species as well as both solitary and colonial forms coming from all marine environments (Monniot et al. 1991; Cameron et al. 2000; Kott 2005). Ascidians inhabit hard substrates such as rocks, pilings, ropes and shells and they feed mostly on fine particles present in water, especially on bacteria and phytoplankton by pumping water into their bodies through the oral siphon and expelling it through the atrial siphon (Inglis et al. 2008). A few species filter more than a few thousand times their own volume every day. Sessile ascidians can accommodate photosynthetic symbionts, for instance, cyanobacteria.

The tunicates comprise a diverse group of flora and fauna, connected by a cellulose-containing tunic that covers their body (Nakashima et al. 2004). With regard to their natural habitat, global shipping and global warming have led to the spread of many ascidians to non-native environments. Some invasive species, the aplousobranch *Didemnum vexillum* in particular, can strongly affect local ecosystems and the aquaculture industry (Lambert & Lambert 2001). Ascidians are particularly successful invaders. Many species show fast growth and maturation rates; they are very fecund and tolerant to changes in temperature, salinity and pollutants (Lambert 2002; 2007; Shenkar & Swalla 2011; Smale & Childs 2012). Generally, colonial tunicates are unpalatable to predators (Vervoort et al. 1998; Pisut et al. 2002) and can replicate and grow rapidly compared to other fouling ascidian species (Bullard et al. 2004; 2007), which gives them the opportunity to invade and substantially change the structure and functioning of the benthic ecosystem (Gittenberger 2007). Ascidians have received much attention in North America and Europe as a consequence of their countless invasions (Lambert 2007; Rius et al. 2008; Dupont et al. 2009). Some colonial species of the family Didemnidae, which host the photosynthetic prokaryote symbiont *Prochloron*, thrive on reef surfaces exposed to high irradiance (Kuhl & Larkum 2002).

The Andaman and Nicobar Islands are a group of 572 islands consisting of islets and rocky outcrops. Of these, 348 are true islands and only 38 islands are inhabited. There are two Marine National Parks, 4 National Parks and 28 Sanctuaries established on the South Andaman Islands (Venkataraman et al. 2012). The Andaman Islands have a vast and well-protected biosphere reserve area. The biosphere reserve encompasses 85% of the Island's area, while the remaining 15% is used for agriculture, forestry and residential areas (Newlon et al. 2003). The first comprehensive work on ascidians in Indian waters was a report on six types of tunicates (Oka 1915). Gravely (1927) reported various ascidians from the Gulf of Mannar (Krusadai Island, Pamban and Rameshwaram). So far, only 50 species have been reported from Andaman and Nicobar Islands (Negi 1996). Since very little literature and research studies are currently available on the ascidian communities from Andaman and Nicobar Islands as well as from the rest of India, it is necessary to survey the ascidian communities in this region. As the biosphere reserve provides habitat for a wide diversity of organisms, it is important to understand the ascidian communities of these Islands. Comprehensive data on these species will help researchers to differentiate between endemic and exotic species in the future. The present study aimed at investigating the diversity of the abundant ascidian communities in the region of Andaman and Nicobar Islands.

## Materials and methods

### Collection and identification

Sampling was carried out in 14 areas of the Andaman and Nicobar Islands (6°–14°N and 91°–94°E) from March 2014 to April 2015 in order to determine the variability in their diversity. Figure 1 shows the map with sampling sites. Samples were collected at a depth of 5 to 9 m from Andaman and Nicobar Islands using the Self Contained Underwater Breathing Apparatus (SCUBA) and were transferred into a jar containing menthol crystal for narcotization. They were then rinsed with water to remove menthol crystal from animals bodies and preserved in seawater mixed with formalin (10%). Taxonomic identification was carried out based on the identification keys provided by Goodbody (1962) and Kott (1985).

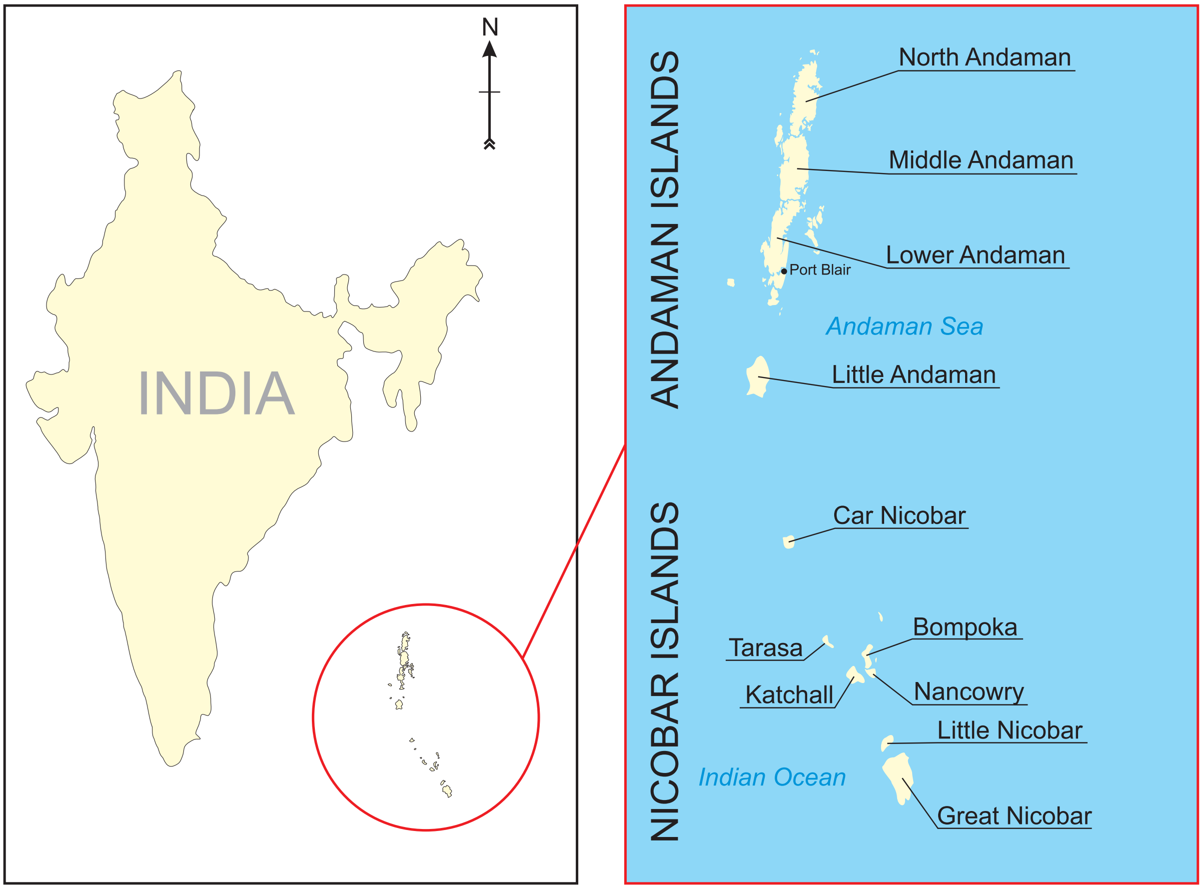


**Figure 1**

Map showing the sampling sites on Andaman and Nicobar Islands

### Diversity indices

Diversity indices such as the Shannon-Wiener diversity index (H') (Shannon & Weaver 1964), Margalef's index (D), Pielou's index (J'), K-dominance curves were used to analyze the variation in diversity. Cluster Analysis was carried out to determine the similarity between different groups of ascidians. All statistical analyses were performed using the PRIMER software.

## Results

A total of 29 species belonging to 7 families, such as Didemnidae, Clavelinidae, Polyclinidae, Ascidiidae, Diazonidae, Pyuridae and Styelidae were collected and identified from Andaman and Nicobar Islands during this study. The suborder Aplousobranchia belonging to the family Didemnidae (with the genus *Didemnum*) was represented by the largest number of species (Table 1).

Each species was identified during the survey based on the taxonomic key characteristics:

### Suborders

#### Aplousobranchia

Gut loop, gonad and heart always entirely posterior to branchial sac.

#### Phlebobranchia

Gut loop, gonads and heart never entirely posterior to branchial sac.

#### Stolidobranchia

The body is not divided; the heart, gut and gonads are in the body wall at the side of a large branchial sac that occupies the whole length of the animal. The body wall musculature well developed, internal longitudinal branchial vessels present, usually numerous and not supported by branchial papillae.

**Table 1**

List of species collected from Andaman and Nicobar Islands during the study period

| Order | Suborder | Family | Genera | Species |
|---|---|---|---|---|
| Enterogona | Aplousobranchia | Didemnidae | *Didemnum* | *Didemnum granulatum* |
| | | | *Didemnum* | *Didemnum molle* |
| | | | *Didemnum* | *Didemnum psammatodes* |
| | | | *Diplosoma* | *Diplosoma listerianum* |
| | | | *Lissoclinum* | *Lissoclinum fragile* |
| | | | *Lissoclinum* | *Lissoclinum levitum* |
| | | | *Lissoclinum* | *Lissoclinum patella* |
| | | | *Trididemnum* | *Trididemnum cyclops* |
| | | Clavelinidae | *Clavelina* | *Clavelina picta* |
| | | | *Clavelina* | *Clavelina robusta* |
| | | Polyclinidae | *Aplidium* | *Aplidium conicum* |
| | | | *Aplidium* | *Aplidium elegans* |
| | | | *Aplidium* | *Aplidium fuscum* |
| | | | *Eudistoma* | *Eudistoma gilboviride* |
| | | | *Eudistoma* | *Eudistoma viride* |
| | Phlebobranchia | Ascidiidae | *Ascidia* | *Ascidia virginea* |
| | | | *Phallusia* | *Phallusia arabica* |
| | | | *Phallusia* | *Phallusia fumigata* |
| | | | *Phallusia* | *Phallusia ingeria* |
| | | | *Phallusia* | *Phallusia julinea* |
| | | Diazonide | *Rhopalaea* | *Rhopalaea crassa* |
| Pleurogona | Stolidobranchia | Pyuridae | *Halocynthia* | *Halocynthia papillosa* |
| | | | *Herdmania* | *Herdmania momus* |
| | | Styelidea | *Botrylloides* | *Botrylloides leachi* |
| | | | *Botrylloides* | *Botrylloides pizoni* |
| | | | *Eusynstyela* | *Eusynstyela latericius* |
| | | | *Polycarpa* | *Polycarpa pigmentata* |
| | | | *Styela* | *Styela gibbsii* |
| | | | *Styela* | *Styela plicata* |

## Families

### Didemnidae

The cloacal system always present; atrial aperture seldom 6 lobed; zooids embedded; colonies form thin encrusting sheets; common test has usually calcareous spicules; body divided into thorax and abdomen; 3 or 4 rows of branchial stigmata; gonads beside gut loop; larvae in common test below zooids.

### Clavelinidae

1. Zooids partially or completely embedded in common test.

2. Colonial or solitary, colonies not regular and rope-like; no short, narrow, cylindrical neck separates zooids from the common test mass.

### Polyclinidae

1. Polyclinidae are one of the most diverse families of Aplousobranchia. All have embedded zooids with 6-lobed or (occasionally) 8-lobed branchial apertures and smooth-rimmed atrial apertures, usually with a tongue from the upper rim of the opening or from the body wall anterior to the opening.

2. Zooids may be arranged with their atrial openings into sometimes extensively branched cloacal canals or in circular systems around simple cloacal cavities.

3. Colonies vary from small cushions to large irregular and sometimes lobed masses, or stalked heads, and many species have sand or other particles embedded in the test.

### Ascidiidae

Gonads enclosed in the primary gut loop; solitary; gut loop on the left side of pharynx; stigmata straight; continuous dorsal lamina.

### Diazonidae

1. The anal border is broken into about 8 rounded lobes.
2. Zooids solitary or at most 2 embedded in common test.

### Pyuridae

1. Test bristles branched and with secondary spines.
2. Siphons with 4 lobes; stigmata straight; no large renal sac.

### Styelidae

1. Gastric caecum, long, curved; stomach barrel shaped.
2. Systems, usually crowded, do not surround protruding areas of well- vascularized test.
3. Zooids are arranged in "leachii type" systems.

Ascidians collected from Andaman and Nicobar Islands at Haddo Wharf (HW), Wandoor Beach (WB), Burmanella (BN), Carbyn Cove (CC), Rangat (RG), North Passage Island (NPI), Button Island (NBI), Laxman Beach (LB), Campbell Bay (CB), Pigeon Islands (PI), Bquary Beach (BB), Afra Bay (AB) and Army Camp (AC) were analyzed for their diversity. The comparison of samples collected at the study sites revealed the following sequence of diversity: Burmanella (3.31) > Campbell Bay (3.214) > Bquary Beach (3.186) > Laxman Beach (3.083) > Wandoor Beach (3.03) > North Button Islands (2.88) > Army Camp (2.82) > Long Islands (2.71) > Carbyn Cove (2.53) > Pigeon Island (2.267) > North Passage Islands (2.24) > Rangat (1.936) and the lowest diversity was observed in Haddo Wharf (1.58). The maximum species richness (2.424) was recorded in Campbell Bay followed by Bquary Beach (2.289), Army Camp (2.233), Laxman Beach (2.199), Burmanella (2.14), Wandoor Beach (2.127), North Button Islands (1.861), Afra Bay (1.803), Long Islands (1.674), Carbyn Cove (1.501), North Passage Islands (1.294) and Rangat (1.082), while the minimum richness was determined in Haddo Wharf (0.91) (Figs 2, 3 & 4). The evenness values ranged from 0.942 to 0.991 in Army Camp and Afra Bay, respectively.


**Figure 2**

Shannon-Wiener diversity (H') of ascidians recorded at the sampling sites during the study period


**Figure 3**

Margalef' s richness index (D) of ascidians recorded at the sampling sites during the study period


**Figure 4**

Pielou's evenness index (J') of ascidians recorded at the sampling sites during the study period

### K- dominance curves

K-dominance curves with cumulative abundance or biomass were ranked and plotted against the species rank in order to estimate the relative dominance of the species along transects and at different depths. The present study reports a relatively low diversity of ascidians in the study area. The sequence of the sampling sites according to decreasing species diversity is as follows: BN (*Didemnum granulatum*, *Didemnum psammatodes* and *Botrylloides leachi*), CB (*Eudistoma gilboviride*, *Eudistoma viride* and *Lissoclinum fragile*), BB (*Phallusia fumigata*, *Phallusia ingeria* and *Ascidia virginea*), LB (*Polycarpa pigmentata, Styela gibbsii, Styela plicata* and *Botrylloides pizoni*) and WB (*Diplosoma listerianum*, *Lissoclinum levitum*, *Lissoclinum patella* and *Clavelina picta*); the intermediate diversity level was found at AC (*Phallusia arabica* and *Phallusia julinea*), NBI (*Eusynstyela latericius*, *Herdmania momus* and *Aplidium fuscum*), AB (*Clavelina robusta*), CC (*Didemnum molle*), LI (*Halocynthia papillosa*) and the lowest diversity was found at HW(*Rhopalaea crassa*), RG (*Aplidium elegans*), NPI (*Trididemnum cyclops*)and PI (*Aplidium conicum*) (Fig. 5). If the curves of intermediate ascidian species diversity were significantly separated by partial types of dominance curves, all other transects (narrow lines) would follow a similar pattern.

### Cluster Analysis

In order to determine the species similarity and dissimilarity, the data obtained from all the sites were included in the cluster analysis and MDS (non-metric multidimensional scaling). The dendrogram from the cluster analysis showed that the sites WB and LB, BB and BN, PI and LI were grouped at the highest levels of similarity (Fig. 6). To confirm these groupings, the MDS plot was prepared. The ordination plots were used to determine the similarity of diversity between all sites at Andaman and Nicobar Islands. The present findings reveal that species at different sites are grouped into three groups: the first group includes the sites of NPI, AC, CB and CC, the second group includes WB, LB, NB, BB and BN and the third group includes PI, LI, RG and AB. Within these groups, three sub-groups were distinguished, which include WB and LB, BB and BN, PI and LI. This shows that the similarity between the diversity of these sites is high. The site HW was excluded from the group, which indicates lower diversity due to environmental pollution (Fig. 7).

## Discussion

Although the class Ascidiacea has been of scientific interest for the last decade (Pourquie 2001), there are large regions around the world where very little research on ascidians has been carried out, resulting in very little data available. In addition, there is a clear trend of arrival and spread of non-indigenous species that put the endemic fauna at risk. Both of these issues indicate the need for additional research in the field of ascidian biodiversity and biogeography (Shenkar & Swalla 2011). In geographical areas where taxonomists have long been active, there are usually large numbers of species identified. On the other hand, along the

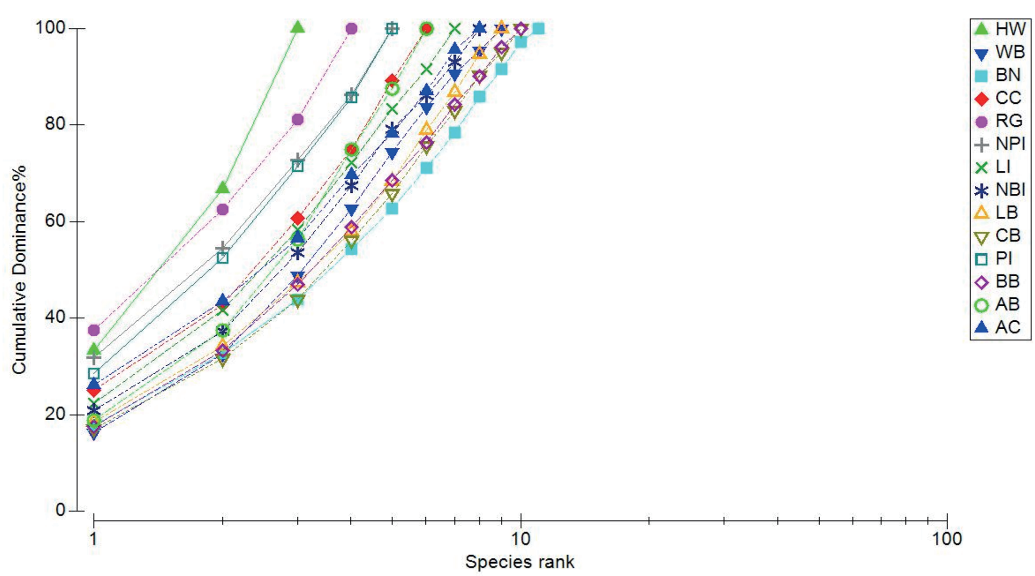


**Figure 5**

K-dominance curves drawn for ascidians identified at the sampling sites

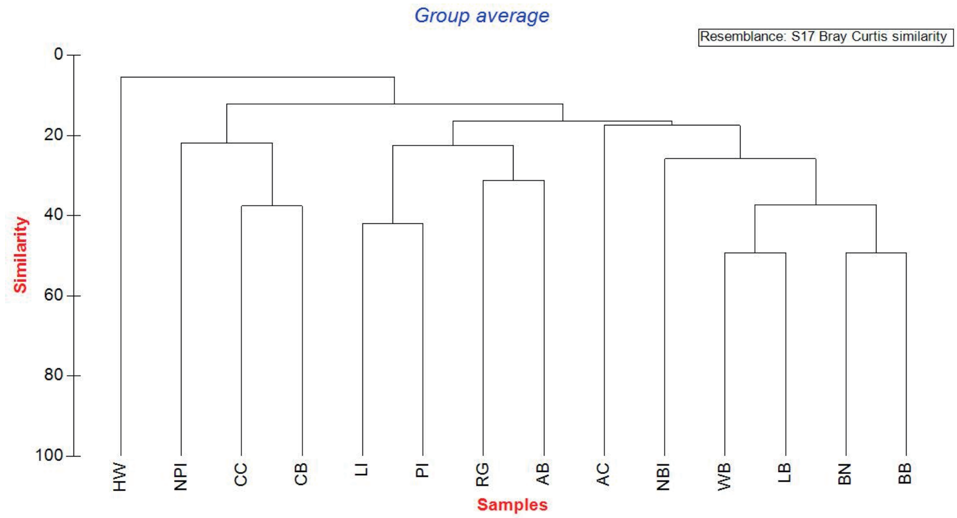


**Figure 6**

Dendrogram drawn for ascidians identified at the sampling sites

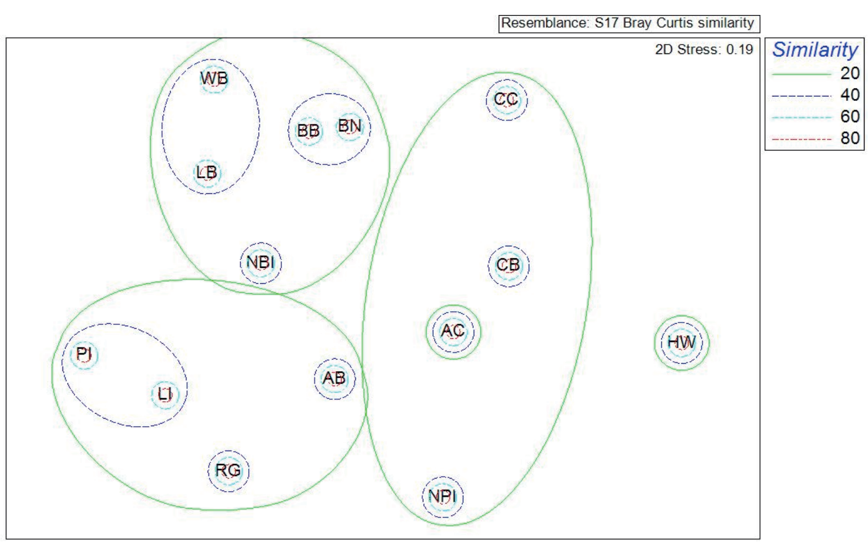


**Figure 7**

MDS plot drawn for ascidians identified at the sampling sites

coasts of South America, the Indian Ocean, and the Eastern Atlantic, there are vast areas with only scarce information about the occurrence of ascidians, and in few cases the only information comes from studies that may be out of date and are not representative of the current diversity in these areas (Van Name 1945; Millar 1965). The data obtained from the present research on the ascidian biodiversity may play an important role in the field of ascidians.

A review of literature on corals, fishes, mollusks, crabs, sea anemones and plankton of Andaman and Nicobar Islands is widely available. Despite being a diverse group in the region, ascidians have not been thoroughly surveyed due to their cryptic nature. The occurrence of ascidians is extremely short-lived, which is why the study was planned to survey the ascidian diversity among different sampling locations at the Andaman and Nicobar Islands. In total, 29 species of ascidians were recorded. They belong to 1 class, 2 orders, 3 suborders, 7 families and 16 genera and were collected in relation to abundance and seasonal variability. Karthikeyan (2010) reported 25 species of ascidians from the region of Palk Bay. More than 400 species of ascidians have been reported in Indian coastal waters by various researchers at different places (Oka 1915; Das 1938; 1945; Sebastian 1952; 1954–56; Renganathan 1981–1984; Abdul Jaffar Ali & Sivakumar 2007: Abdul Jaffar Ali et al. 2009; 2011). A total of 303 species of ascidians were reported from the Indian waters (Meenakshi et al. 2008), including seven species from the Andaman and Nicobar Islands (Venkataraman et al. 2012). Jhimli Mondal (2015) reported about 32 species from the Andaman Islands. But this was a very limited study of ascidians from Andaman and Nicobar Islands considering their abundance and diversity. Of the 29 species identified in the present study, 11 species are simple forms and 18 are colonial forms. The solitary forms are dominant (especially in the deepest waters) and colonial forms are relatively more abundant in shallow waters and on horizontal and vertical substrates (Jackson 1977; Buss 1979). Also in this study, solitary ascidians were found to be most abundant in offshore areas and the abundance of colonial ascidians was observed in intertidal and shallow waters. Clampitt (1970, 1973) and Harman (1972) reported that the substrate is a significant ecological factor influencing the distribution of ascidians.

In the present study, the maximum number of species was recorded at the beaches of Burmanella, Bquarry and Wandoor. This may be due to the availability and stability of various substrates suitable for ascidians like pebbles, permanent barges, oyster bed, boulders, submerged concrete structures, blocks etc. The importance of substrate for the settlement of marine organisms has been reported by many workers (Nielson 1950; Clampitt 1973; Nair & Thamby 1980; Patterson Edward & Ayyakkannu 1992; Baskera Sangeevi 2001). The minimum number of ascidians was recorded at the Haddo Wharf harbor. According to Hedgpeth (1957), storms often radically alter the topography of a sandy beach, so that it varies from day to day, season to season and year to year. This extreme substrate volatility prevents the attachment of sedentary forms in the intertidal zone and the tidal ranges are small, offshore bars often develop, which then alter the animal population of the beach. Young (1989) also reported that the habitat stability appears to be extremely important for the survival of these animals, and excessive pollution has adverse effects on both adults and juveniles.

The minimum value (1.585) of the species diversity (H) was recorded during a monsoon season compared to a post-monsoon season at Haddo Wharf (HW) sites. The maximum (3.315) diversity was observed in summer followed by a pre-monsoon season at Burmanella (BN). Karthikeyan (2010) also reported that the diversity of ascidians was low during monsoon seasons and high during pre-monsoon seasons at the coast of Palk Bay. Most ascidians are sensitive to stress and lower salinity (Goodbody 1961). The ascidians can tolerate low salinities at high temperatures rather than at low temperatures (Sabbadin 1955; Runnstron 1929; Knaben 1952; Dybern 1969) and as a result, many marine organisms cannot survived in high tidal areas (Millar 1953; 1971; Dybern 1963; 1969).

Margalef's richness index (D) was higher (2.424) in Campbell Bay during the summer season and lower (0.910) in Haddo Wharf during the monsoon season. Tamilselvi et al. (2012) reported that Margalef's species richness index reflects the suitability of habitats for organisms and a relatively stable community due to the stability and availability of substrates. Similar seasonal variations were reported earlier by Oka (1915), Gravely (1927), Das (1936, 1945), Meenakshi and Renganathan (1997, 1999), Meenakshi (1997, 2005, 2008), and Krishnan et al. (1989) in Indian waters.

The lowest value (0.942) of Pielou's evenness index (J') was recorded in the Army Camp during the pre-monsoon season and the maximum (0.991) in Afra Bay during summer. Similar findings were reported by several authors: Karthikeyan (2010), Clampitt (1970, 1973), Harman (1972), Wise & Molles (1979), who reported that substrate is a significant ecological factor influencing the distribution of organisms. Temperature (Namaguchi et al. 1997), salinity (Vazquez & Young 1996; 2000), light (Forward et al. 2000; Tsuda et al. 2003), hydrodynamics (Holloway & Connell 2002), competition (Lambert 2000; Lambert 2001) and predation (Castilla et al. 2002) are the most important environmental factors determining the distribution of ascidians.

Cluster Analysis is useful to find natural groupings of samples. It shows that samples within a group are more similar to each other than samples in different groups (Clarke & Warwick 2001). In the present study, the dendrogram and the MDS plot show close similarity (60–80%) in species composition between the sites of Andaman and Nicobar Islands. It appears from the dendrogram that the ascidian assemblage pattern is similar during the pre-monsoon and monsoon seasons. The stress value of the MDS plot (stress = 0.19) shows excellent ordination of the samples collected in all seasons at all sites excluding the Haddo Wharf harbor. Furthermore, this harbor is located within two parallel arms and the maritime port area is prone to pollutants such as domestic sewage, industrial effluents, oil spillage, grease from ships, boat yards and surface runoff. These factors directly or indirectly slow down the settlement of larvae by creating an unfavorable environment for the survival and growth of organisms. Kott (2002) reported that fertilizers, insecticides, organic pollutants and suspended sediments in the terrestrial run-off could significantly affect filter-feeding organisms. In the dominance plot, the curve that lies on the lower side extends further and rises slowly due to the presence of a larger number of species demonstrating the rich diversity of ascidians at the beaches of Burmanella, Bquarry and Wandoor during all seasons.

Seasonal fluctuations in the abundance and the number of ascidian species reported in the present study at different sites may be due to different long-term patterns of organisms as well as different reproductive cycles. Rinkevich et al. (1993) reported that life history traits, such as longevity and reproduction, were subjected to changes in environmental and biotic conditions, so that the species may show phenotypic flexibility. There are many examples among ascidians that show variation in life history traits in different seasons between populations of *Ciona intestinalis, Dendrodoa grossularia, Podoclavella mollucensis, Aplidium glabrum* and *Botrylloides* sp. (Rinkevich et al. 1993; Durante & Sebens 1994).

## Conclusions

It is extremely important to study the distribution, diversity and abundance of ascidians, because the results obtained could be used as baseline data for sustainable management of the marine ecosystem. Therefore, the presented research on the diversity of ascidians provides basic information on tunicates and knowledge of this group of organisms (which has not yet been fully research) will help to preserve the marine environment. Extensive research on ascidian diversity of Andaman and Nicobar Islands is required, which will provide sufficient information necessary to maintain the environment free from invasive species and protected for sustainable use to preserve its biological heritage.

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