Non-indigenous ascidians in V. O. Chidambaram port, Thoothukudi, India

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V. O. Chidambaram port, formerly Tuticorin port, is one among the 12 major ports in India with regular services to United States, Europe, China, Red sea port and Mediterranean countries and so serving as gateway for bioinvasion. Ascidians belonging to the Class Ascidiacea of sub-Phylum Tunicata are abundant in many ports around the world and most of them are non-indigenous. They are one of the key ecological groups because of their invasive potential and ability to thrive in eutrophic environments. During a survey of the port area over the past three years (2011-2013), sixteen species of ascidians were identified belonging to diverse families: Ascidiidae (4), Styelidae (2), Pyuridae (4), Polycitoridae (2), and Didemnidae (4). Among the identified species, Phallusia nigra, P. arabica, Ascidia gemmata, Microcosmus exasperates, Herdmania pallida, Eudistoma viride, E. laysani, Didemnum psammatode and Diplosoma similis are classified as cryptogenic species while M. squamiger, M. stoloniferus, Styela canopus, A. sydneiensis Didemnum candidum, Lissoclinum fragile and Eusynstyela tincta are invasive species. Presence of large number of cryptogenic and invasive ascidians in this harbour reinforces the need for continued and periodic monitoring of population expansion of these species and also for establishment of control programme. Utilization of non–indigenous ascidians for the betterment of humankind may be considered as one of management strategies of non–indigenous species.

[Key words: Ascidians, invasive species, V. O. Chidambaram port, Thoothukudi coast, cryptogenic species]

Introduction

Bioinvasion is one of the growing environmental issues in this century arising due to increasing globalization of markets, travel, global trade etc., which in turn impact significantly on all types of ecosystems. In the marine environment, ships and ballast waters are considered as the major vectors for introduction which represents a serious global threat to marine biodiversity, survival of native species and the economy13. Usually non indigenous species (NIS) are abundant in harbours and this higher propagule delivery rate of NIS is due to anchoring of a number of ships and mechanized boats which facilitate the introduction and also the available substrata favour the establishment of NIS. The Indian coast, being dotted with 12 major ports and a number of minor ports is susceptible for bioinvasions and hence warrants a close watch.

Aiscidians represent one of the main biofouling taxons, particularly on ship hulls4. They colonise all types of hard substrata, both natural and artificial 5, mainly in environments characterized by low diversity fauna (e.g. estuaries, lagoons, harbors, shellfish farms, eutrophic habitats etc.). By virtue of its sedentary nature and brief planktonic larval period they can easily be transported through hulls of ships and ballast water. Being strong spatial competitors, once they become established, they undergo population explosion and develop into dense stands or mats that over grow and cover available surfaces. Once non-indigenous ascidians become established, they provide large local sources of larvae for further possible invasions into other locales of harbours and nearby natural marine communities. Given these economic and ecological concerns, intense interest has developed to focus on the distribution of invasive ascidians in Thoothukudi coast.

Ascidian diversity is greater in Thoothukudi coast, given that the waters are tropical and that more than 70 species have been reported by many authors6,10 in several locations along the Thoothukudi coastline. However, little is known from marine ecosystems of India regarding the presence and distribution of invasive alien ascidians11,12. Some species have been reported only from specific habitat types rather than in the port area proper.

V.O. Chidambaram port, formerly called as Tuticorin port, located along the Gulf of Mannar region, is important regionally and internationally with intense services to United
States, Europe, China, Red Sea and Mediterranean countries and may serve as a gateway for bioinvasion. This port is provided with artificial substrates such as extensive pilings, floating docks, super barges and large cargo ships which act as suitable substrata for fouling organisms. Hence, the present study was aimed to understand the occurrence of alien ascidians in this harbour.

Materials and methods

Area description

V.O.Chidambaranar port is within the Gulf of Mannar (8° 44' 53.82" N-Latitude, 78° 12’15.77" E longitude) in the south east coast of India. This port is one of the important and major ports in south India, with year round heavy traffic by a number of ships and other mechanised boats. In addition to the port, large marinas are also permanently found in this region. The study area (Fig 1) (8°48’N and 78°11’E) is situated opposite to the Central Electrochemical Research Institute and finger jetty, 1200 meters away from the South Break Water (SBW) with very limited wave action. Granite rocks have been laid down to prevent soil erosion on the slopes of the Harbor basin. Few large barges are anchored in the harbor area regularly.

Method of collection

Specimens were collected seasonally during May 2011 to January 2013. Search for species at each habitat lasted for approximately two hours. Intertidal sites were visited at low tides and a variety of collection methods were employed to obtain the organisms. Hand tools were used to remove animals from solid surfaces such as bumper tires, docks, small rocks, stones and marina floats. SCUBA divers were engaged to sample at marinas to remove ascidians from the undersides of floating docks and barges.

Method of identification

All organisms collected were narcotized using menthol crystals for up to three hours (for colonial ascidians) and five or more hours (for solitary ascidians). After narcotization, the specimens were preserved in 10% buffered formalin in seawater. The specimens were sorted and identified to species or the lowest practicable taxon, with dissection and/or compound microscope using various taxonomic references.

The identified species were classified into cryptogenic or introduced by adopting possible criteria such as, appearance in local regions where not found previously, prevalence or restriction to new or artificial environments and also based on published geographic records.

After an extensive review of literature on global invasive species, their spread based on history, species origin, species behavior and field observations at Indian coastal areas, a list of invasive alien ascidians was prepared. The websites were also examined extensively for background information (http://www.marinespecies.org, http://www.catalogueoflife.org and http://www.sealifebase.org). The specimens were deposited in the Ascidian collection at the Museum of Islamiah College, Newtown Vaniyambadi – 635752, Tamil Nadu, India.

Results

Sixteen species of ascidians were identified belonging to diverse families: Ascidiidae (4), Styelidae (2), Pyuridae (4), Polycitoridae (2), and Didemnidae (4). Most of these species are known from Thoothukudi coast rather than in the port area proper.

Most taxa were found in harbor installations rather than in the natural substrata. Some species such as *Eudistoma viride*, *Didemnum psammatodes* and *Lissoclinum fragile* were common to both natural and artificial substrata. In contrast, 12 species were found only in port installations (Table 1). Among these, *Phallusia nigra*, *P. arabica*, *Ascidia gemmata*, *Microcosmus exasperatus*, *Herdmania pallida*, *Eudistoma viride*, *E. laysani*, *Didemnum psammatodes* and *Diplosoma similis* are classified as cryptogenic species while *M. squamiger*, *M. stoloniferus*, *Styela canopus*, *A. sydneiensis Didemnum candidum*, *Lissoclinum fragile* and *Eusynstyela tincta* as invasive species.
Table 1. List of ascidians collected from different habitats of the study area

<table>
<thead>
<tr>
<th>Ascidians</th>
<th>Status</th>
<th>Large cement blocks</th>
<th>Granite stones</th>
<th>Small embedded rocks</th>
<th>Pearl oyster cages</th>
<th>Hull of ship</th>
<th>Old barges</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
</tr>
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<tr>
<td>Microcosmus exasperatus</td>
<td>C</td>
<td>-</td>
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<td>x</td>
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<td>x</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<tr>
<td>M. squamiger</td>
<td>I</td>
<td>x</td>
<td>-</td>
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<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<td>x</td>
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<tr>
<td>M. stoloniferus</td>
<td>I</td>
<td>x</td>
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<td>x</td>
<td>x</td>
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<tr>
<td>Herdmania pallida</td>
<td>C</td>
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<td>x</td>
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<td>x</td>
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<tr>
<td>E. laysani</td>
<td>C</td>
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<tr>
<td>Lissoclinum fragile</td>
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<td>C</td>
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<td>x</td>
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<tr>
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<td>-</td>
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<td>x</td>
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<tr>
<td>Didemnum candidum</td>
<td>I</td>
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Note:  C – cryptogenic;  I – invasive;

Fig. 1 - Sampling sites in V.O.Chidambaramar Port, Thoothukudi

Fig. 2 - *Microcosmus exasperatus* Heller, 1878
Fig. 3 - *M. squamiger* Michaelsen, 1927

Fig. 4 - *M. stoloniferus* Kott, 1952

Fig. 5 - *Herdmania pallida* (Heller, 1878)

Fig. 6 - *Phallusia nigra* Savigny, 1816

Fig. 7 - *P. arabica* Savigny, 1816

Fig. 8 - *Ascidia gemmata* Sluiter, 1895
Fig. 9 - *Ascidia sydneiensis* Stimpson, 1855

Fig. 10 - *Styela canopus* (Savigny, 1816)

Fig. 11 - *Eusynstelya tincta* Van Name, 1902

Fig. 12 - *Eudistoma viride* Tokioka, 1955

Fig. 13 - *E. laysani* (Sluiter, 1900)

Fig. 14 - white colony - *Lissoclinum fragile* (Van Name, 1902) and Dirty colony - *Didemnum psammatodes* (Sluiter, 1895)

Fig. 15 - *Diplosoma simile* (Sluiter, 1909)

Fig. 16 - *Didemnum candidum* Savigny 1816
Non indigenous ascidians:  
Family Pyuridae  
**Microcosmus exasperatus** Heller, 1878 (Fig 2)  
*Material examined:* Collected from large cement blocks, pearl oyster cages and old barges at 3-4 m depth (ICBT Asc.S18)  
*External appearance:* Solitary species. The globular body is enclosed within a leathery and wrinkled tunic. The tunic is orange or purple, maintaining colour in formalin, and contains some sand and encrusting organisms on the surface. Gonads are partly enclosed. The long siphon and leathery reddish orange coloured test are the identification marks in the field. Characterized by eight branchial folds lining with small rounded scales and pointed unique siphonal spines.  
*World Distribution:* Indo-West Pacific and the Mediterranean: Sub-Antarctic region

**Microcosmus squamiger** Michaelsen, 1927 (Fig 3)  
*Material examined:* Collected from large cement blocks, pearl oyster cages and barges at 3-4 m depth (ICBT Asc. S19)  
*External appearance:* Solitary species with long siphon and leathery orange coloured test and characterized by unique small rounded scales in siphon. It has the gonads divided into three lobes and has been confused with *M. exasperatus*, but the siphonal spines are very different, with roof-tile shape and spiny rims. The species light brown or light yellowish brown in live condition.  
*World Distribution:* Indo-Pacific, Southwest Atlantic and the Mediterranean Sea, Sub-Antarctic region.

**Microcosmus stoloniferus** Kott, 1952 (Fig 4)  
*Material examined:* Collected from large cement blocks and barges at 3-4 m depth. Few specimens were collected from cages installed for aquaculture (ICBT Asc. S20)  
*External appearance:* Solitary species with short curved siphon and leathery dirty brown coloured test with few encrustations around the base. Presence of both siphonal spines and scales is the unique character of this species.  
*World Distribution:* Australian waters and Gulf of Mannar, India  

**Herdmania pallida** (Heller, 1878) (Fig 5)  
*Material examined:* Collected from large cement blocks, barges at 3-4 m depth and also from cages installed for aquaculture at a depth of 4-5 meters (ICBT Asc. S21)  
*External appearance:* Large sized flesh coloured solitary species with cylindrical siphon turned away from each other. Numerous barbed spines in both test and mantle bodies are the characteristic features of this species.  
*World Distribution:* Indo-West Pacific and the Mediterranean: Sub-Antarctic Region

Family Ascididae  
**Phallusia nigra** Savigny, 1816 (Fig 6)  
*Material examined:* Collected from large barges at 1-3 m depth and also from cages installed for aquaculture at a depth of 4-5 meters (ICBT Asc. S22).  
*External appearance:* This is a common large solitary ascidian. The unique feature is the typical velvety-black or dark-brown colour. It is free of epibionts throughout its life. It is characterized by the presence of accessory openings in the neural gland duct.  
*World Distribution:* Widely distributed throughout the warm water of the Western Atlantic ranging from Bermuda to Brazil. It is also found in Red Sea, the Gulf of Aden, other parts of Arabian Sea, Mediterranean, Hawaiian Island and Micronesia. Off late, it is recorded from the both east and west coast of India especially in harbours and embayments.

**P. arabica** Savigny, 1816 (Fig 7)  
*Material examined:* Collected from large barges at 1-3 m depth and also from cages installed for aquaculture at a depth of 4-5 meters (ICBT Asc. S23).  
*External appearance:* This is also a common large solitary ascidian free of epibionts throughout its life. It is characterized by the presence of accessory openings in the neural gland duct. It differs from *P. nigra* by its colouration (dark grey) and rudimentary siphons.  
*World Distribution:* North Atlantic Ocean, European waters, Red Sea, South Pacific Ocean and Indian waters.

**Ascidia gemmata** Sluiter, 1895 (Fig 8)  
*Material examined:* Collected from large barges at 1-3 m depth and also from cages installed for aquaculture at a depth of 4-5 meters (ICBT Asc. S24).  
*External appearance:* Simple ascidian. Dirty white colour, translucent, wrinkled test with epibionts. Posterior position of atrial siphon is the peculiar feature. Four stigmata per mesh are present. Intermediate papillae on the longitudinal vessels are absent.  
*World Distribution:* Central Indo-Pacific and south Pacific and Indian Ocean.
**Ascidia sydneiensis** Stimpson, 1855 (Fig 9)  
*Materials Examined:* Collected from barges at 4-5 m depth and also from pearl oyster cages (ICBT Asc. S25).  
*External appearance:* Simple ascidian with translucent, wrinkled and leathery test. Have prominent branchial and atrial siphons. No accessory openings from the neural duct. The peculiar feature is the bilobed nature of anal border.  
*World Distribution:* Hawaii Islands and Indian Ocean.

**Family Styelidae**  
**Styela canopus** (Savigny, 1816) (Fig 10)  
*Materials Examined:* Collected from large cement blocks, barges at 3-4 m depth and also from cages installed for aquaculture at a depth of 4-5 meters. Few specimens were collected from shallow water regions also (ICBT Asc. S26)  
*External appearance:* The species is readily identified by its deeply curved gut loop, long stomach with fine longitudinal pleats, long anal lobes, and leaf-like endocarps on and in the gut loop. Presence of brown coloured wrinkles around the branchial siphon is the peculiar feature. The species is light red, dark rose or white in living condition.  
*World Distribution:* Japan, France, Indonesia, Hong Kong, New South Wales (Central E coast), Queensland (Central E coast, NE coast), Western Australia (Lower W coast, NW coast); Torres Strait, Coral Sea, west Pacific Ocean, Korea, tropical and temperate Atlantic Ocean, Persian Gulf, Adriatic, Mediterranean, Ascension Is., Channel Is., west coast of France.

**Eusynstelya tincta** Van Name, 1902 (Fig 11)  
*Materials examined:* Collected from various harbour installations such as cement blocks, calcareous stones, ropes etc at varying depths from 1-4 m and also from cages installed for aquaculture at a depth of 4-5 meters, encrusting on bivalve shells (ICBT Asc. C12).  
*External appearance:* Colonial ascidian with bright red colour in live condition and with brown colour in formalin. Zooids are embedded in a common test. Atrial and oral siphons have four lobes. Two different sizes of tentacles can be seen around the branchial siphon. Ten rows of stigmata with 4 folds per side. Smooth dorsal lamina. Two elongated testis on gonad. The stomach is also extended.  
*World Distribution:* Western Atlantic, Mozambique, Red Sea, Gulf of Suez, African Coast, South Indian Coast.

**Family Polycitoride**  
**Eudistoma viride** Tokioka, 1955. (Fig 12)  
*Materials examined:* Collected from small embedded rocks and calcareous stones at a depth of 1 meter and also from cages installed for aquaculture at a depth of 4-5 meters (ICBT Asc. C14).  
*External appearance:* Colonial ascidian. Colonies are Greenish yellow in colour. Lobes of the colonies are closely packed. Colonies are free of epibionts. Black spots on either side of the oral siphon’s basal region are the characteristic mark. No distinct constriction between thorax and abdomen.  
*World Distribution:* Western Central Pacific and Indian Ocean.

**E. laysani** (Sluiter, 1900) (Fig 13)  
*Material Examined:* Collected from small embedded rocks and calcareous stones at a depth of 1 meter and also from cages installed for aquaculture at a depth of 4-5 meters (ICBT Asc. C14).  
*External appearance:* Colonial ascidian. Colonies are lobed. The lobes have rounded head which is covered by delicate transparent test. No pigment spot in the siphon. The branchial sac has three rows of rectangle stigmata. The atrial siphon originates opposite to the first row of stigmata. The abdomen is longer than the thorax. Gonads are in the gut loop. The unique features of this species are the existence of lobed colonies with a common test devoid of sand and the terminal part of the lobe with bluish white iridescence.  
*World Distribution:* Pacific Ocean and Indian Ocean.

**Family Didemnidae**  
**Lissoclinum fragile** (Van Name, 1902) (Fig14)  
*Materials Examined:* The ascidians collected from small embedded rocks and calcareous stones at a depth of 1 meter and also from cages installed for aquaculture at a depth of 4-5 meters (ICBT Asc. C15).  
*External appearance:* The colony is soft, thin, encrusting with extensive cloacal cavity. The thorax is almost free, and the abdomen is included in pillars in the tunic. Larvae are located in the basal lamina. The branchial sac shows a brown pigment, light or dark. The body wall is almost missing, visible at the level of a short atrial languet and posteriorly where it holds thoracic lateral organs at the basis of the fourth stigmata row. There is no fixative appendage. The testis has a straight sperm duct and ovary is present above the testis. The tadpole has 3 suckers and 4
small papillae. The colony is in varying shades of white colour.  

**World Distribution:** East Pacific Ocean off South America to California, West Indies, North Atlantic Ocean to Arctic Ocean and Northwest of Iceland, West Pacific Ocean.

*Didemnum psammatodes* (Sluiter, 1895) (Fig 14)  
**Materials Examined:** Collected from all kinds of substrates available in the study area (ICBT Asc. C16).  
**External appearance:** Colony forms thin encrusting sheets, although sometimes they are produced into fleshy lobes or have irregular twig–like branches. Faecal pellets embedded throughout the colony are a peculiar feature. No green cells are found. All have characteristically restricted thoracic common cloacal centre. Spicules never crowded and numerous in surface test. Zooids are minute and the atrial aperture is wide and exposes a large part of the branchial sac to the common cloacal. The colony is brown or light yellowish-brown in live condition.  

**World Distribution:** Japan, Malaysia, Indonesia, New Zealand, New South Wales (Central E coast), Queensland (Central E coast, Great Barrier Reef, NE coast), Victoria (Bass Strait); west Indian Ocean, Red Sea and India

*Didemnum simile* (Sluiter, 1909) (Fig 15)  
**Materials Examined:** Collected from calcareous stones at a depth of 1 meter and also from cages installed for aquaculture at a depth of 4-5 meters (ICBT Asc. C18)  
**External appearance:** This genus is characterized by the absence of spicules in the test and no coiled vas deferens. Test is gelatinous not firm. The colony is translucent. Prochloron present in common cloacal cavities. Zooids are evenly spread. Six branchial lobes are triangular. Symbiotic cells are present. Retractor muscle is from the base of the oesophagus. The colony is very transparent and gelatinous with black dots.  

**World Distribution:** Gulf of Mannar in India, Japan, Singapore, Indonesia, QLD (Great Barrier Reef); West Pacific Ocean.

*Didemnum candidum* Savigny 1816 (Fig 16)  
**Materials examined:** Collected from calcareous stones at a depth of 1 meter and also from cages installed for aquaculture at a depth of 4-5 meters (ICBT Asc. C19).  
**External appearance:** Colonial ascidian with thin, smooth, irregular sheets encrusting on available substrates. Flesh colour in live condition but white in formalin. Sometimes shade of red or orange colour can be noticed. Spicules have 7-9 rays. Vas deferens always spirals 7 times around the outer surface of the testis.  

**World Distribution:** Canada, Madagascar, North Atlantic Ocean, Red Sea, South Pacific Ocean and Indian Ocean

**Discussion**  
As expected due to marine context of port, large numbers of introduced ascidian species which include several cryptogenic species were found at V.O.Chidambaranar port, Thoothukudi coast. It is well-known that a number of ships and mechanized boats would have introduced these sedentary tunicates through ballast water or from hulls of ships. Besides the availability of man-made substrata, other artificial structures such as boulders, prolonged barges, concrete submerged blocks etc, provide suitable substratum for attachment and help the dispersal of species. It is stated that *Pseudopolydora paucibranchiata* might have been introduced to northeastern Pacific through ballast water or from ship fouling or with Japanese oysters. Many researchers have reported that shipping has introduced exotic species into many ports worldwide.

There is ample evidence that the artificial substrata in and around ports often have large number of introduced ascidians. In the present investigation, the presence of large number of cryptogenic ascidians could be explained by the availability of artificial substrata. Similar results are known in Southern California, Mediterranean Sea and Brazil. The Selection Regime Modification (SRM) states that highly disturbed environments will have a greater abundance of exotic species and that the impact of invading species on native species will be greater in other coastal environments. In this study the occurrence of four species such as *Microcosmus squamiger*, *M. exasperatus*, *M. stoloniferus* and *Styela canopus* are new records in this port area.

Due to uncertainty in the origin of some species, their status as non–indigenous species remains unclear. Various level of evidences suggest that *Phallusia nigra*, *P. arabica*, *Ascidia gemmata*, *Microcosmus exasperates*, *Herdmania pallida*, *Eudistoma viride*, *E. laysani*, *Didemnum psammatode* and *Diplosoma similis* are classified as cryptogenic species. *Phallusia nigra* was first described from the Red Sea but most records come from the West Atlantic. The ambiguity of native region of *P. nigra* has also been reported. Records of *P. nigra* and *P. arabica* in Indian waters revealed a very restricted distribution of these species in Gulf of Mannar suggesting that they may also be considered as
lessepsian migrant. There is a high possibility that these species have been introduced through Red Sea and /or Mediterranean since they are recorded from these areas. *Microcosmus squamiger* which seems to have an invasive potential is actually present in southern California. In the Mediterranean Sea, specimens of *M. squamiger* have been recorded in several harbours and coastal bays from Bizerte (Tunisia), to Savona, Taranto, Imperia (Italy).

Taxonomy and identification have been the major limiting factors in the ability to detect non – indigenous species of ascidians in the area. This is compounded by the lack of updated local fauna inventories and the lack of taxonomic expertise and knowledge in Indian waters. Furthermore, some of the earlier records of alien ascidians in the Thoothukudi coast remain uncertain. The effect of non-indigenous species on natural communities and habitats occurring in this region is unknown and research programmes to examine these issues should be promoted.

In fact not all non-indigenous species are harmful, some are highly useful in fisheries. The Government of Chile recognizes the threats posed by alien invasive species, but also recognizes the significant societal benefits associated with aquaculture. Non indigenous ascidians can sustainably be utilized for the benefit of mankind since ascidians are prolific producers of novel bioactive metabolites exhibiting antimicrobial, antineoplastic, antitumor, anti-inflammatory, antifouling, antioxidant, deterrent and insect control activities. Tyrosine derived antimicrobial compounds have been isolated from the non – indigenous ascidian *Phallusia nigra* from Thoothukudi coast of India. A value added product, ascidian pickle, was prepared from alien ascidian *Herdmania pallida*. Besides, recent study reveals pellet feed prepared using *H. pallida* showed better performance on growth, survival and reproduction during culturing periods of black molly (personal communication). Utilization of non – indigenous ascidians for the betterment of humankind may be considered as an alternative way of managing non – indigenous species.

Acknowledgement

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