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Research Article

Settlement of non-native *Watersipora subtorquata* (d'Orbigny, 1852) in artificial collectors deployed in Colombo Port, Sri Lanka

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Abstract

Sri Lanka has been a key node in the historical maritime "silk route" since the 14th century. Colombo Port, with its strategic location in the Indian Ocean, handles most of the country's foreign cargo. With increased foreign trade and ship traffic, Colombo Port environs are highly susceptible to invasions by non-indigenous species (NIS), which may threaten native biota. There is a dearth of information in Sri Lanka on the present status of the biofouling community and the nature and level of threats posed to native biota. The present study was conducted to investigate the biofouling assemblage within the Colombo Port. Artificial settlement collectors were deployed in order to study the colonization of fouling organisms. The encrusting foliaceous colonial bryozoan, *Watersipora subtorquata* was recorded for the first time in Sri Lanka. It was found on settlement collectors in two of the eight sampling locations one in New Pilot Station (12.50% cover) at 2 m depth and the other in Bandaranayake Quay (2.19% cover) at 1 m depth. Random and patchy distribution within the port environment and the absence of *W. subtorquata* on permanently located collectors indicate that their arrival is relatively recent. However, invasive behavior of *W. subtorquata* is significant since they can facilitate the settlement of other biofoulers providing a non-toxic substrate for them to attach. Furthermore, they pose deleterious impacts on ecology through interspecific competition with native organisms modifying community structure and dynamics. Therefore, continuous monitoring is vital for early detection and management of a possible invasion by globally known invasive species.

Key words: biofouling, non-indigenous species, encrusting bryozoan, biological invasions, settlement collectors

Introduction

Globalization of world trade has resulted in increased levels of long-distance travel and commerce where shipping plays a major role, transporting nearly 90% of the commodities across the globe (International Maritime Organization 2012). Ships not only carry goods but are also known for dispersal of organisms to areas they never existed; ballast water and ship hull fouling act as major vectors for the introduction of non-indigenous species (NIS) (Hewitt et al. 2009). After the introduction, NIS can become invasive in indigenous habitats altering native biodiversity, ecosystems and community structure (Kelso and Jackson 2012). With increasing population size, they may transform entire ecosystems causing deleterious impacts on native biota and biological diversity (Simberloff and Holle 1999; Lonhart 2012; Chan et al. 2015). Invasive species may adversely impact shipping, port operations, aquaculture, or even commercial fishing (Johnson et al. 2007). Thus, successful invasions may endanger both ecology and economy of a country.

Sri Lanka has been a key node in the historical maritime "silk route", and since the 14th century Colombo Port has played a major role in handling most of the country's foreign trade due to its strategic location in the Indian Ocean (SAGT 2014). With increased foreign trade and ship traffic, Colombo Port environs are highly susceptible to invasions by NIS from around the globe threatening native fauna, flora and pristine ecosystems. Although ship hull fouling is considered one of the major vectors of transporting non-indigenous species around the world, there is a dearth of information in Sri Lanka. Therefore, the present status of NIS in Sri Lanka and the threats they pose to native biota are virtually unexplored.

The outer sea of the Colombo Port is mainly a sandy seabed without any protected water bodies, and the near-shore area receives a large amount of sediments and freshwater from nearby Kelani river (SLPA 2006). Previous study by Ranatunga et al. (2015), recorded 38 species of biofouling organisms out of which five species were globally known invasive species. Another study by Priyadarshani and Ranatunga (2013) on biofouling assemblage of ship hulls in Colombo Port have recorded nine globally known invasive biofouling organisms. These include: *Musculista senhousia* (Benson, 1842) (Asian date mussel), *Balanus amphitrite* (Darwin, 1854) (striped barnacle), *Elminius modestus* (Darwin, 1854) (acorn barnacle), *Mytilus galloprovincialis* (Lamarck, 1819) (Mediterranean mussel), *Perna viridis* (Linnaeus, 1758) (Asian green mussel), *Crassostrea gigas* (Thunberg, 1793) (Pacific or Japanese Oyster), *Carcinus maenas* (Linnaeus, 1758) (European shore crab), *Ostrea edulis* (Linnaeus, 1758) (European flat oyster) and *Pomatoceros triqueter* (Linnaeus, 1758) (keelworm), all of which have the potential to cause deleterious impacts. Therefore, further investigations and monitoring are imperative for early detection and warning of potential invasions and to recognize any impacts that they would pose. However, no detailed investigations have been carried out regarding the present existence of NIS in Colombo Port. Thus, the present study is to augment the knowledge gap on NIS in Colombo Port environs.

Bryozoans (moss animals) are colonial invertebrates, the majority of them inhabiting marine waters and a major component of the biofouling communities settling on artificial substrates (Abdelsalam 2016). Among the species recorded on hard substrates, bryozoans play a major role in structure and dynamics of the fouling community and the presence of *Watersipora subtorquata* d'Orbigny, 1852 in such communities is significant due to its global concern as one of the widely spread invasive species fouling on ship hulls and other floating objects. *Watersipora subtorquata* is widely distributed in both tropical and subtropical regions around the world (Kuhlenkamp and Kind 2013). However, their native range is uncertain (Mackie et al. 2012). They are mostly found in lower intertidal and shallow subtidal areas and some areas confined to harbors (Vieira et al. 2014).

Table 1. Sampling locations within the Colombo Port.

Location	Abbreviation	Lat, N	Long, E
New Pilot Station	NPS	6.960261	79.83994
Passenger Jetty	PJ	6.940703	79.84441
Bandaranayake Quay	BQ	6.938734	79.84525
Dockyard Berth	DP	6.956168	79.857
Dolphin Pier	DOC	6.955625	79.85576
Unity Container Terminal	UCT	6.95914	79.85502
Old Pilot Station	OPS	6.955226	79.84468
Colombo International Container Terminal	CICT	6.945795	79.82765

One of the key features of *W. subtorquata* is that they are tolerant to copper-based biocides (antifouling paints) so they can facilitate the spread of other invasive species by providing a non-toxic surface for them to settle (GISD 2008). Despite the widespread global distribution of *W. subtorquata*, its presence in Sri Lankan waters has not been reported previously.

Methods

The present study was conducted in eight sampling locations within Colombo Port (Table 1, Figure 1). Two settlement collectors were deployed in each location (one permanently placed and another replaced monthly). Monthly sampling was conducted from October 2014 to July 2015.

Artificial settlement collectors that were derived from Hewitt and Martin (2001) and Marshall and Cribb (2003) were deployed to study the fouling aggregations, and modified to suit the local conditions. The structure consists of a rope supporting horizontal PVC pipes where 10 × 10 cm aluminum plates were attached as settlement surfaces. The structures were suspended from a mooring buoy with weights so that the first level is 1 m below the water surface; and second, third and fourth levels at depths of 2, 3 and 4 m, respectively (Figure 2).

Samples were collected by pulling the entire collector out of the water and replacing one vertically oriented plate and one horizontally oriented plate from one side of a collector with clean plates at each depth. Plates that were replaced monthly in this way were called "replacing plates". Left and right sides of the settlement collectors were treated separately. During monthly sampling events, either left or right-side plates were removed from the collectors. Therefore, although the sampling frequency is monthly, at the time of collecting one side of the replacing plates, they were two-months old. The removed plates were chilled immediately and transported to the laboratory for further analysis. "Permanent collectors"

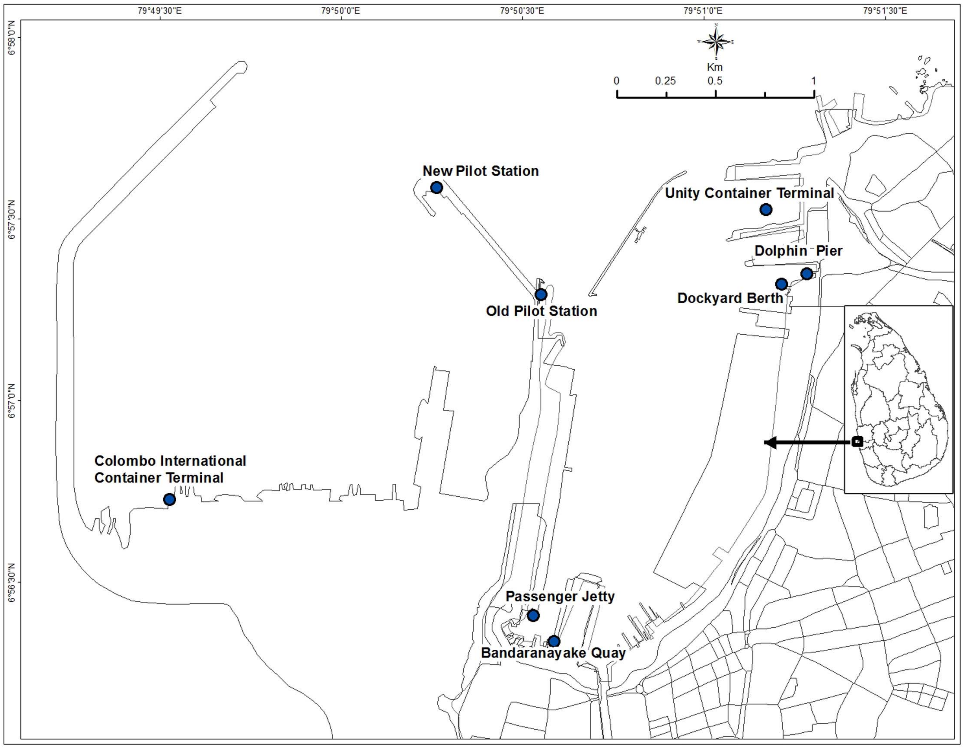

Figure 1. Sampling locations within the Colombo Port, and location of Colombo Port in Sri Lanka (Inset).

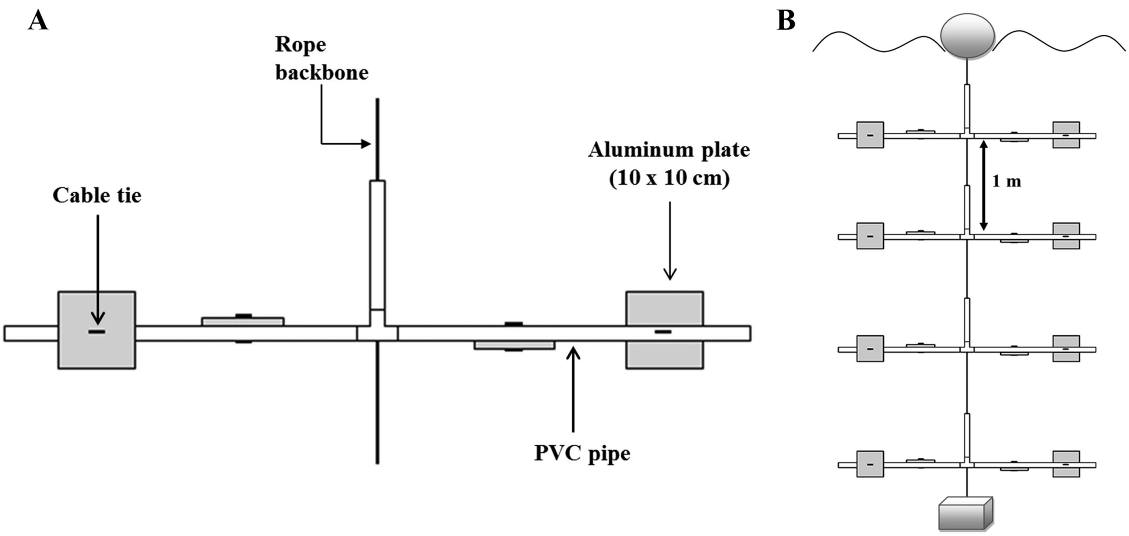

Figure 2. Schematic diagram of settlement collectors; A – a single level, B – all four levels once set up.

were left throughout the study (i.e., for nine months) in order to understand the community succession. Each permanent collector was photographed during the monthly sampling cycle before placing the collectors back in the water.

Percentage cover for each species in a panel was determined using 400 quadrats (5 mm × 5 mm). All the specimens encountered were preserved as type specimens for further studies and future references. Species were identified to their lowest taxonomic

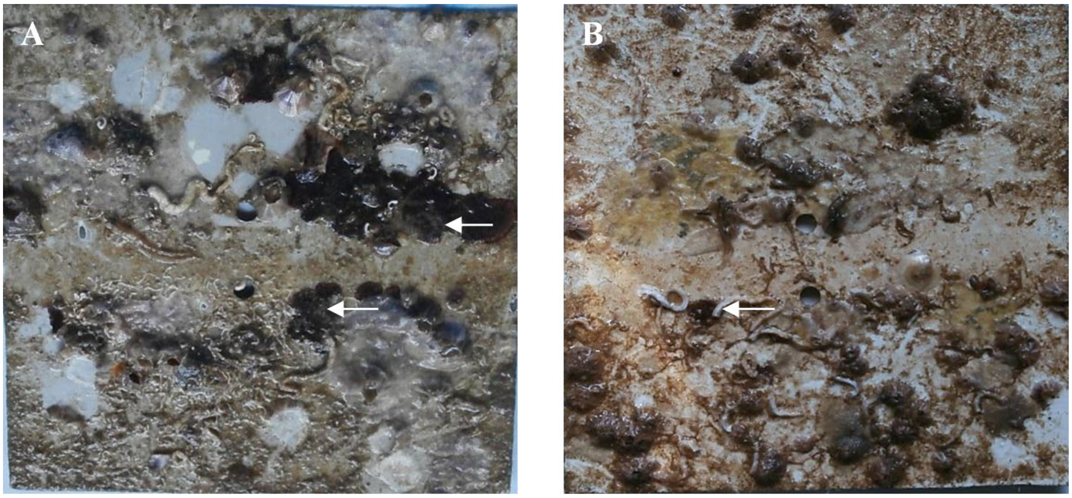

Figure 3. Photographs of the settlement plates where *W. subtorquata* were recorded: (a) 2 m depth at NPS; (b) 1 m depth at BQ. Photograph by MMKI Marasinghe.

level observing fine morphological features using stereomicroscope and Scanning Electron Microscope (SEM). Specimens were examined under the light microscope as described in Kuhlenkamp and Kind (2013) to certain important morphological characters which were not distinguishable in SEM images (i.e., pigmented operculum, distinct spots, etc.). Taxonomic confirmation of *W. subtorquata* was based on the description given by, Fofonoff et al. (2003), GISD (2008), Ryland et al. (2009), Cohen and Andrew (2011), Kuhlenkamp and Kind (2013), Vieira et al. (2014) and Abdelsalam (2016). Microscopic fine morphological features that are identical to the genus *Watersipora,* such as orifice and operculum were considered in detail as described by Kuhlenkamp and Kind (2013). Apart from their microscopic details, morphometric measurements were also carried out to confirm the taxonomic identity. Length and width of zooids, orifice, and sinus were measured using UTHSCSA Image Tool 3.0 software.

Results and discussion

Previous studies by the authors (Marasinghe et al. 2015a; Marasinghe et al. 2015b; Marasinghe et al. 2016a; Marasinghe et al. 2016b; Marasinghe et al. 2017) have recorded eight species of encrusting bryozoans (*Hippoprina indica* Pillai, 1978; *Celleporaria volsella* Tilbrook, 2006; *Parasmittina parsevalii* Audouin, 1826; *Schizoporella errata* Waters, 1878; *Electra bengalensis* Stoliczka, 1869; *Sinoflustra annae* Osburn, 1953; *Hippopodina iririkiensis* Tilbrook, 1999) from the Colombo Port. The most significant finding of the present study is the detection of the globally known highly invasive encrusting bryozoan *W. subtorquata.* This is the first record of *W. subtorquata* in Colombo Port. However, Abdel-Salam and Ramadan (2008) and Cohen (2011) have mentioned the existence of *W. subtorquata* in Sri Lanka based on geographic distribution; however, there are no previous empirical studies to confirm the presence of *W. subtorquata* in Sri Lanka. Therefore, the present study provides the first evidence for the existence of this species in artificial settlement collectors deployed in Colombo Port and Sri Lanka as well.

Among the replacing and permanently settled collectors, *W. subtorquata* was recorded only in two of the plates in replacing collectors in New Pilot Station (NPS) and Bandaranayake Quay (BQ). In NPS, they were recorded at 2 m depth covering 12.50% of the plate area in January 2015 and in BQ at 1 m depth covering 2.19% of the plate area in November 2014 (Figure 3). Their presence in short-term test panels and absence in long-term test panels indicates that they are early successional species settling in artificial structures (Abdel-Salam and Ramadan 2008). Furthermore, their random and patchy distribution within the port environment suggests its arrival is relatively recent. Therefore, continuous monitoring in port and adjacent coastal waters is necessary to determine their level of invasion.

Furthermore, present study records *W. subtorquata* colonies only on the underside of the plate confirming their requirement for low light conditions. Other studies have also shown their preference for shaded surfaces (i.e., existence in shaded areas of floating macroalgae *Himanthalia* in the North Sea (Kuhlenkamp and Kind

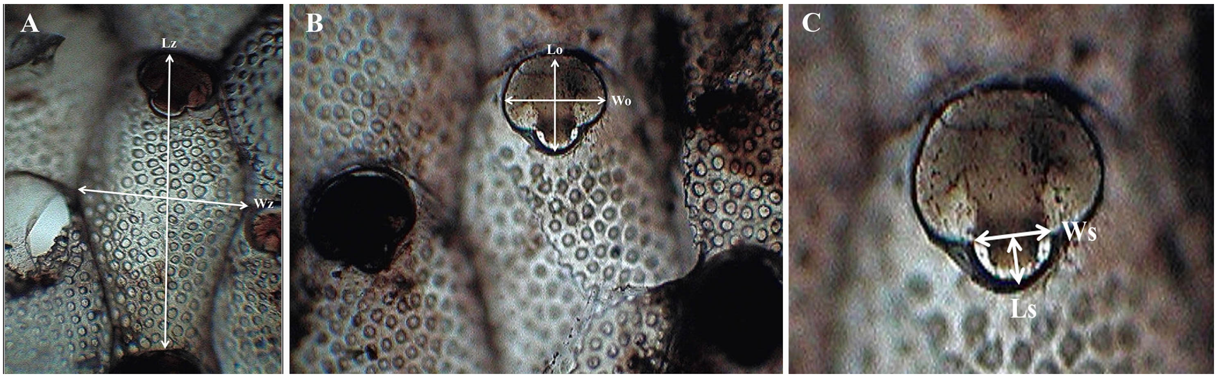

Figure 4. Measurements used for *W. subtorquata* morphometric analysis: (A) Zooid Length (Lz: 817–1220 µm) and width (Wz: 370–437 µm); (B) Orifice Length (Lo: 190–240 µm) and width (Wo: 190–280 µm); (C) Sinus Length (Ls: 30–50 µm) and width (Ws: 50–60 µm), also note the two distinct white spots. Photograph by MMKI Marasinghe.

2013); shaded side of pontoons in Ireland (Kelso and Jackson 2012); and underside of rock overhang in Brittany (Ryland et al. 2009). Since Colombo Port environment provides favorable habitats such as harbor walls, pilings, shipwrecks and long-term anchored ships, etc., *W. subtoruqata* has a high potential to be an early colonizer, expand their distribution within the port environment, and become an artificial reservoir for spreading to the other areas.

Watersipora subtorquata is an encrusting colonial bryozoan (family: Watersiporidae, order Cheilostomatida and suborder Ascophora). The colonies were dark in color with a bright orange outer growing edge, a ~ 5 ± 0.127 cm maximum length and ~ 2.5 ± 0.117 cm maximum width (Figure 4). The identification of *W. subtorquata* was challenging since it lacks many characters such as avicularia, ovicells, spines, etc., which are typical characters of bryozoan taxonomy. Therefore, morphological characters and dimensions described in the literature (Abdel-Salam and Ramadan 2008; Ryland et al. 2009; Kuhlenkamp and Kind 2013; Vieira et al. 2014; Abdelsalam 2016) were used for detailed identification. Further, *W. subtorquata* is a species which potentially may be misidentified since it shares closely similar morphological features with the other species of genus *Watersipora*. However, the present specimen was morphologically much closer to taxonomic details given in Ryland et al. (2009) and Kuhlenkamp and Kind (2013) and different from given reference for *W. subovoidea*, Ceylon in Ryland et al. (2009).

The colony was unilaminar and consists of comparatively large and distinct zooecia with numerous pores. Aperture and operculum consist of distinct mushroom shaped outline with the broad upper circular area and rounded extension at the bottom (Figures 5 and 6). Operculum was dark, strongly pigmented with a broad dark central band, paler peripheral regions and two distinct shining white spots located proximally (Figure 4C). The mean zooid measurements were, Lz = 817–1220 µm, Wz = 370–437 µm; orifice, Lo = 190–240 µm, Wo = 190–280 µm and sinus, Ls = 30–50 µm, Ws = 50–60 µm and these measurements were much similar to *W. subtorquata* materials examined by the Ryland et al. (2009).

The native region of *W. subtorquata* is still a question. However, as per Inglis et al. (2006), their native range is wider Caribbean and South Atlantic, and their introduced range includes United States of America, Australia, New Zealand, Ireland and United Kingdom (GISD 2008; Kelso and Jackson 2012). They also occur in the north-western Pacific and Torres Strait (between Australia and Papua-New Guinea) (Inglis et al. 2006). They are known as highly invasive in the coastal region of both temperate and tropical waters throughout the world (Ryland et al. 2009) displacing native bryozoan species (Kelso and Jackson 2012) and thus indicating their possibility to invade the Sri Lankan coastal region (Ryland et al. 2009). However, detailed studies are required to determine their invasive status in Sri Lanka.

Watersipora subtorquata is known to be one of the abundant organisms in port environments of many parts of the world fouling on ship hulls, pilings, pontoons and other man-made structures. They can also be found attached to rocks and seaweeds (Inglis et al. 2006; Kuhlenkamp and Kind 2013). They are well recognized as a highly invasive species around the world (Inglis et al. 2006; GISD 2008). The ability to tolerate a range of antifouling toxins (Inglis et al. 2006), especially, copper-based antifouling biocides is one of the major reasons for their wider distribution throughout the world. In addition to self-dispersion,

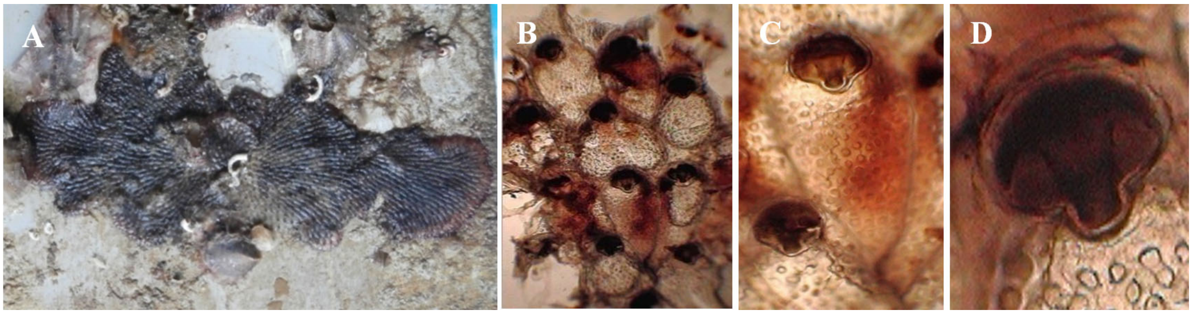

Figure 5. (A) *W. subtorquata* colony; (B) Light photomicrograph showing close-up view of zooids (x 40); (C) Zooid showing distinct operculum and pores (x 100); (D) mushroom shaped operculum strongly pigmented with a broad dark central band with paler peripheral regions. Photograph by MMKI Marasinghe.

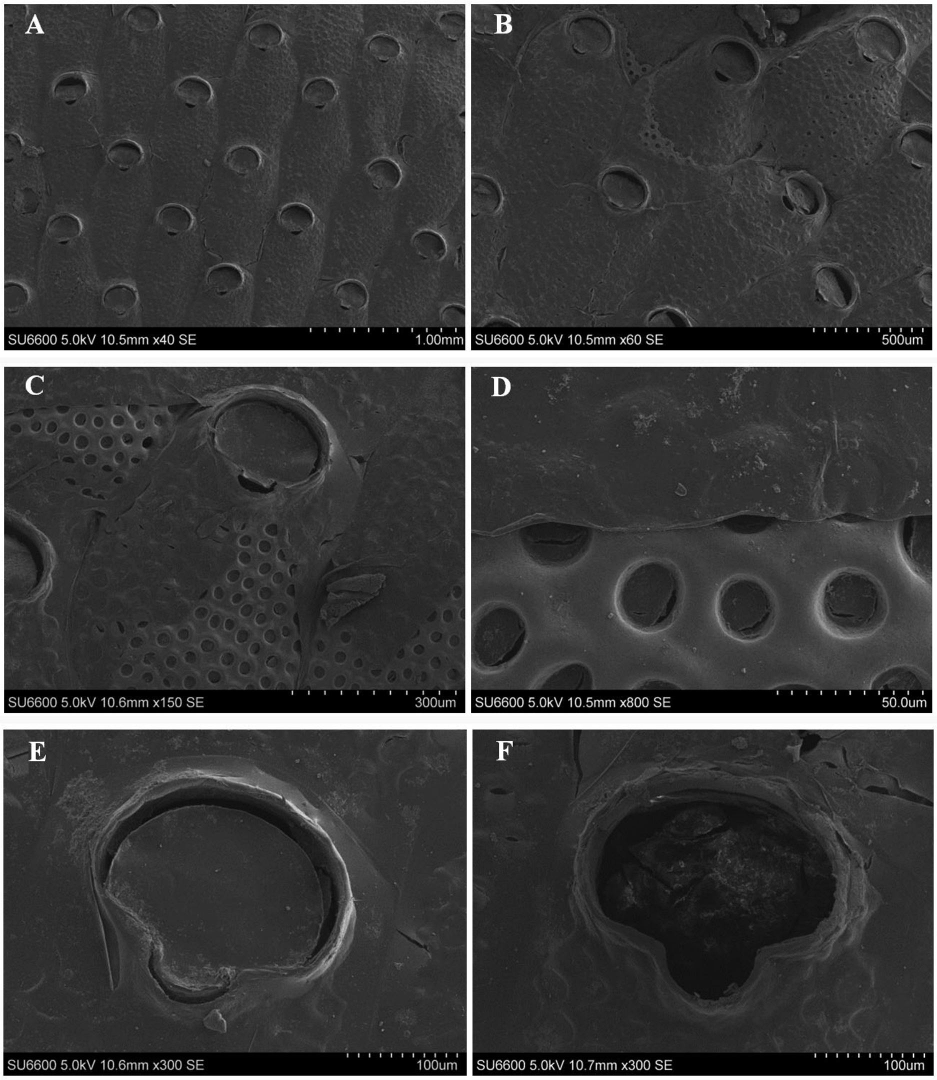

Figure 6. SEM photomicrograph of *W. subtorquata*: (A) colony; (B) Close-up view of the colony (1000 μm); (C) Close up showing zooids and surface covered with numerous pores (300 μm); (D) Close up view of pores; (E) Mushroom shaped orifice covered with operculum; (F) Mushroom shaped orifice without operculum. Photograph by Rashmi Kumarasinghe, Sri Lanka Institute of Nanotechnology (SLINTEC).

they also play a role spreading other invasive organisms providing non-toxic substrates to settle (GISD 2008). Further, they pose deleterious impacts on ecology through competing with native bryozoans as well as other fouling organisms altering native fouling community structure and dynamics (GISD 2008). They also settle on ship hulls increasing the vessel maintenance cost and fuel consumption, vessel speed by increasing the friction between the hull and the seawater (Johnson et al. 2007).

W. subtorquata was recorded only in two of the sampling locations with a very low covering percentage compared to other encrusting bryozoans (i.e., *Celleporaria volsella*, *Schizoporella errata*, *Electra bengalensis* and *Hipporina indica*) within Colombo Port (Marasinghe et al. 2015a; Marasinghe et al. 2015b; Marasinghe et al. 2016a; Marasinghe et al. 2016b). Further, the magnitude of their occurrence and distribution is extremely low compared to other NIS recorded (i.e., *Schizoporella errata*). This may be due to their low population size, seasonal occurrences or relatively recent arrival. Although the history of Colombo Port dating back to 14th century (SAGT 2014), the present study records only a few colonies with low abundance and narrow distribution suggesting their arrival is relatively recent. However, due to their potential for enormous impacts on the ecology and economy, the early detection of *W. subtorquata* is vital in preventing, and mitigating further introduction and spread of the species (Lehtiniemi et al. 2015).

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