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First occurrence of fouling ascidian species *Microcosmus squamiger* Michaelsen, 1927 and *Didemnum ahu* Monniot C. & Monniot F., 1987 in İzmir Bay (Eastern Aegean Sea)

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ABSTRACT

The aim of the present study was to determine species composition of ascidians and to detect the occurrence of alien ascidian species in İzmir Bay (Eastern Aegean Sea). The study was conducted at seven stations situated in recreational marinas and frequently used places for boating, fishing, and shipping activities in İzmir Bay between 25 and 27 August 2015 and between 23 and 26 November 2015. According to the findings, the non-native ascidian species such as *Microcosmus squamiger* Michaelsen, 1927, *Didemnum ahu* Monniot C. & Monniot F., 1987, and *Polyclinum* sp. were recorded for the first time on natural and artificial substrates at the sampling stations. The results indicate an increase in the number of alien ascidian species in the region. Also, the findings of this study showed that global marine invasive species *M. squamiger* has spread from the western Mediterranean to the eastern Mediterranean.

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Non-indigenous ascidians; invasive species; *Microcosmus squamiger*; *Didemnum ahu*; Aegean Sea

Introduction

Ascidians (Chordata: Tunicata) can be found in all kinds of benthic communities and they are distributed in all marine habitats from the intertidal zones to abyssal depths (Monniot et al. 1991; Tatián and Lagger 2010).

As far as we know, about 3,000 ascidian species have been found worldwide (Shenkar et al. 2020). However, the number of studies that focus on ascidian diversity in Turkish coastal waters are few. Fifty ascidian species have been reported from Turkish coasts in a detailed study published by Çınar (2014). According to Çınar (2014), studies on the ascidians in Turkish Seas began with Colombo (1885), who reported six species from the Aegean Sea entrance of the Çanakkale Strait. Afterwards, ascidians have been documented in different studies from Turkish coasts (Aydin Onen 2018).

According to the literature, four of the recorded species, namely *Herdmania momus* (Savigny 1816), *Microcosmus exasperatus* Heller 1878 and *Phallusia nigra* Savigny, 1816, and *Symplegma brakenhielmi* (Michaelsen, 1904) were considered as alien species (Çinar et al. 2006a; Ramos-Esplá et al. 2013). In addition, a new alien species *Styela clava* Herdman, 1881 was found (Karamürsel, İzmit Bay) in the Sea of Marmara by Çınar

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(2016). Furthermore, *Polyclinum constellatum* Savigny, 1816 was reported from stations located in Kuşadası and Didim-Akbük (Aydin Onen 2018) and *Aplidium tabarquense* Ramos-Espla, 1991 was recorded from a station situated in İzmir [Sığacık (Kanyon)] (Çinar et al. 2019). Miroğlu and Yalçın (2020) conducted a study and they were collected ascidians from 28 sampling stations situated in the coastline of Ordu province. They reported ascidian species *Ascidiella aspersa, Ciona intestinalis, Molgula manhattensis, Botryllus schlosseri*, and *Botryllus* sp. as the first record for Middle Black Sea Region (Turkey). The addition of two newly recorded species in this study bringing the total number of ascidian species in Turkish coasts to 55.

Previous studies mentioned that invasive ascidian species should be monitored regularly because they have environmental, ecological, economic, societal, and cultural impacts (Bellard et al. 2016; Pergl et al. 2016). They have potential for biofouling (Vandepas et al. 2015) and also can out-compete and displace native species, lead to the loss of native genotypes, modify habitats, alter community structure, ecosystem processes, and trophic relationships in the food chain. Moreover, they economically affect fishing and aquaculture activities, introduce new diseases and pathogens or toxic substances that affect native organisms, biodiversity, and human populations (Shenkar and Swalla 2011; Rius and Shenkar 2012; Lucy et al. 2016).

It is known that artificial substrates in ports and marinas, such as buoys, boulders, ropes, tires, ship hulls, ballast water tanks, and also areas used for recreational boating, yachting, local fishing activities, and aquaculture facilities are frequently the first sites of colonisation for non-indigeneous species and they can also facilitate spread of these species. (Ashton 2006; Davidson et al. 2010). The primary objective of this study was, therefore, to identify the species composition of the ascidians and to record the presence of alien and invasive ascidian species collected from stations located at recreational marinas and frequently used places for boating and shipping activities in the eastern Aegean Sea.

Material and methods

In the present study, field surveys were conducted between 25–27 August 2015 and between 23–26 November 2015 at selected stations situated along the İzmir Bay (Eastern Aegean Sea) (Figure 1, Table 1). Stations S1 and S2 are located in inner İzmir Bay and five stations are located in the outer part of İzmir Bay. Station S1 is found at Pasaport and it is subject to intense anthropogenic activities and urban stress. Station S2 is placed in Levent Marina where leisure boats are intense. Station S3 is situated in Urla where fishing and recreational boats are in large number, especially in the summer months. Station 4 (Mordoğan) and Station 5 (Karaburun) are placed in outer İzmir Bay. Recreational and small fishing boats, ships, shipping activities are intense at Station 4. Station 5 is generally used for recreational and small fishing boat's launch site and activities. Station S6 is placed in Çeşme-Dalyan Marina and it is used for the mooring and servicing for pleasure boats and yachts. Station S7 is located in Seferihisar-Sığacık and the number of leisure boats and commercial fish boats increase in this area during the summer tourist season.

At each station, all specimens were collected by pulling up ropes and by examining walls and submerged structures such as tires, pontoons, buoys, chains, and plastic structures, etc., hand tools were used to remove the ascidians from surfaces.

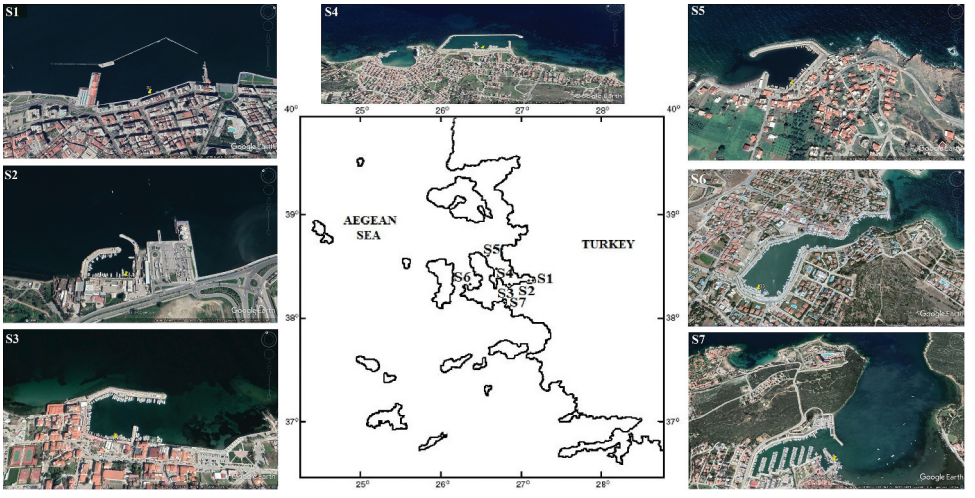


Figure 1. The study area and the locations of sampling stations. S1: Pasaport, S2: Levent Marina, S3: Urla, S4: Mordoğan, S5: Karaburun, S6: Çeşme-Dalyan Marina, and S7: Seferihisar-Sığacık. (Images of the sampling stations were taken from Google Earth).

Table 1. Station numbers, sampling sites, geographical coordinates, and sampling dates of each site surveyed in the Aegean Sea.

		Geographical Coordinates		
Station numbers	Sampling Sites	Latitude (N)	Longitude (E)	Sampling Dates
S1	Pasaport	38°25 31.26"	27°07 53.99"	27.08.2015
S2	Levent Marina	38°24 20.99"	27°04 06.01"	27.08.2015
S3	Urla	38°21 50.94"	26°46 15.79"	26.08.2015
S4	Mordoğan	38°30 45.83"	26°37 52.99"	25.08.2015
S5	Karaburun	38°40 17.14"	26°26 22.31"	25.08.2015
S6	Çeşme-Dalyan Marina	38°21 09.23"	26°18 44.67"	26.08.2015
S7	Seferihisar-Sığacık	38°11 41.74"	26°47 01.20"	27.08.2015
S1	Pasaport	38°25 31.26"	27°07 53.99"	26.11.2015
S2	Levent Marina	38°24 20.99"	27°04 06.01"	24.11.2015
S3	Urla	38°21 50.94"	26°46 15.79"	25.11.2015
S4	Mordoğan	38°30 45.83"	26°37 52.99"	23.11.2015
S5	Karaburun	38°40 17.14"	26°26 22.31"	23.11.2015
S6	Çeşme-Dalyan Marina	38°21 09.23"	26°18 44.67"	25.11.2015
S7	Seferihisar-Sığacık	38°11 41.74"	26°47 01.20"	26.11.2015

The ascidian samples were collected from the depth of 0–1 m. Moreover, observation notes (colour, substrate type etc.) were taken, and then the samples were narcotised with menthol crystals for relaxion and put into fixatives (the mixture of 850 ml of seawater, 50 ml of distilled water, 100 ml of full-strength formaldehyde, and 1 gram of sodium borate) for identification. Specimens were examined, solitary species were counted and identified under an Olympus-SZX16 stereomicroscope. Images were collected by using a digital camera and Scanning Elektron Microscope-IFE Quanta FEG 250. Identification of ascidians was carried out using authoritative and valid taxonomic keys and publications (e.g. Van Name 1945; Kott 1985, 1990, 1992, 2001; Monniot et al. 1991; Monniot and Monniot 2001; Rocha et al. 2012).

Statistical analysis

The data analysis was performed using the STATISTICA v.7.1 (STATSOFT) software package, and statistical significance was defined at the $p < 0.05$ level. Normality and homogeneity of variance tests were conducted by using Cochran-C and Levene tests, and data were transformed when necessary. If data were found to be non-normally distributed, nonparametric tests were preferred for the comparisons of changes in the number of individuals and the number of species. One-way analysis of variance (ANOVA; parametric data) and the Kruskal–Wallis test (nonparametric data) were applied to find any significant differences in the number of individuals and the number of species among different stations and between August and November. Significant differences were discriminate by using the Tukey (parametric data) and the Mann–Whitney U tests (non-parametric data).

Results

In the current study, 13 ascidians were identified belonging to diverse families Clavelinidae (1), Didemnidae (1), Ascidiidae (1), Cionidae (1), Pyuridae (2), Polyclinidae (3), and Styelidae (4). Of the recorded species, two species are considered as native status, three species are established, and five species are assigned as cryptogenic status in the Mediterranean Sea, as well as three taxa could be identified only to genus level [*Aplidium* sp. (1), *Aplidium* sp. (2), and *Polyclinum* sp.] (Table 2).

In the present study, *Polyclinum* sp. (Figure 2a), *Didemnum ahu* Monniot C. & Monniot F., 1987 (Figure 3a-e), and also a strong spatial competitor and global marine invader *Microcosmus squamiger* Michaelsen, 1927 (Figure 5a-c) were especially abundant at S4, S5, S7, and S5 and also S2 stations, respectively (Table 2) and they were recorded for the first time in the eastern Aegean Sea.

A total of 1564 solitary ascidians were observed, corresponding to 6 species and 4 families. *Didemnum ahu* and *Polyclinum* sp. were found in only one station, while *Styela plicata* (Lesueur 1823) specimens were found at all sampling stations (Table 2). In this study, numbers of each species found at each station colour coded by substrate type are given in Figure 4. According to Figure 4, it can be seen that, species generally collected from tires, ropes, and on *Styela plicata*. Moreover, *Microcosmus polymorphus* Heller, 1877, *M. squamiger, B. leachii*, and *Styela canopus* (Savigny, 1816) were found both on natural and artificial substrata (Table 2).

According to the results, the number of recorded species in November was generally more than the number of noted species in August sampling period. In spite of this, however, our findings revealed that spatial and temporal patterns were not statistically significant for the number of species. In August, Stations S5 (Karaburun) and S6 (Çeşme-Dalyan Marina) had the lowest number of ascidian species (2), while Station S4 had the highest number of species (7). However, in November, Stations S1 (Pasaport) and S5 (Karaburun) had the lowest number of ascidian species (4), while Station S7 (Sığacık) had the greatest number of ascidian species (9) (Table 2).

A high abundance of solitary ascidians was recorded at Station S3 (501 individuals) in November. In addition, the lowest number of individuals (2) was encountered at Station S6 in August. In terms of occurrence, both *S. plicata* and *M. squamiger* were found to be the most common species in both seasons. However, no statistical spatial and temporal

Table 2. The number of ascidians, their substrates, and their status recorded at 7 stations during surveys in 25–27 August 2015 and 23–26 November 2015, August: A, November: N; Colonial: C; Cryptogenic: Cr; Established: E; Native: N.

	STATIONS															
	S1		S2		S3		S4		S5		S6		S7			
	A	N	A	N	A	N	A	N	A	N	A	N	A	N	SUBSTRATE	STATUS
Phylum: Tunicata																
Classis: Ascidiacea																
Order: Aplousobranchia Lahille, 1886																
Family: Clavelinidae Forbes & Hanley, 1848																
Clavelina lepadiformis (Müller, 1776)												C		C	Tires	Cr
Family: Didemnidae Giard, 1872																
**Didemnum ahu* Monniot C. & Monniot F., 1987										C					On bivalve shell	Cr
Family: Polyclinidae Milne Edwards, 1841																
Aplidium sp. (1)	C						C					C		C	Tires	
Aplidium sp. (2)		C		C		C									Tires	
**Polyclinum* sp.				C											Ropes	
Order: Phlebobranchia Lahille, 1886																
Family: Ascidiidae Herdman, 1882																
Ascidia mentula Müller, 1776	1	1	20	104	4	3						2	9	17	Ropes, tires, and buoys	N
Family: Cionidae Lahille, 1887																
Ciona robusta Hoshino & Tokioka (1967)							1							5	Ropes	E
Family: Pyuridae Hartmeyer, 1908																
Microcosmus polymorphus Heller, 1877						3	2	1							Ropes, tires, on *M. squamiger*, and on *S. plicata*	N
**Microcosmus squamiger* Michaelsen, 1927							50	34	95	40			7	1	Ropes, tires, and on *S. plicata*	E
Family: Styelidae Sluiter, 1895																
Botrylloides leachii (Savigny, 1816)				C	C		C	C			C	C	C	C	Ropes, tires, buoys, on *S. plicata*, and on bivalve shell	Cr
Botryllus schlosseri (Pallas, 1766)										C		C		C	Tires, buoys, on *S. plicata*, and on bivalve shell	Cr
Styela canopus (Savigny, 1816)	1	10	5	99	12	450	64	43					1	19	Tires, buoys, ropes, on *S. plicata* and on *M. squamiger*	Cr
Styela plicata (Lesueur, 1823)	9	45	55	53	73	45	37	22	10	14	2	34	22	39	Ropes, tires, buoys, and on *S. plicata*	E
Total species number	4	4	3	6	4	5	7	5	2	4	2	6	5	9		
Total number of solitary ascidians	11	56	80	256	89	501	154	100	105	54	2	36	39	81		

*The first record for the Turkish Sea fauna.

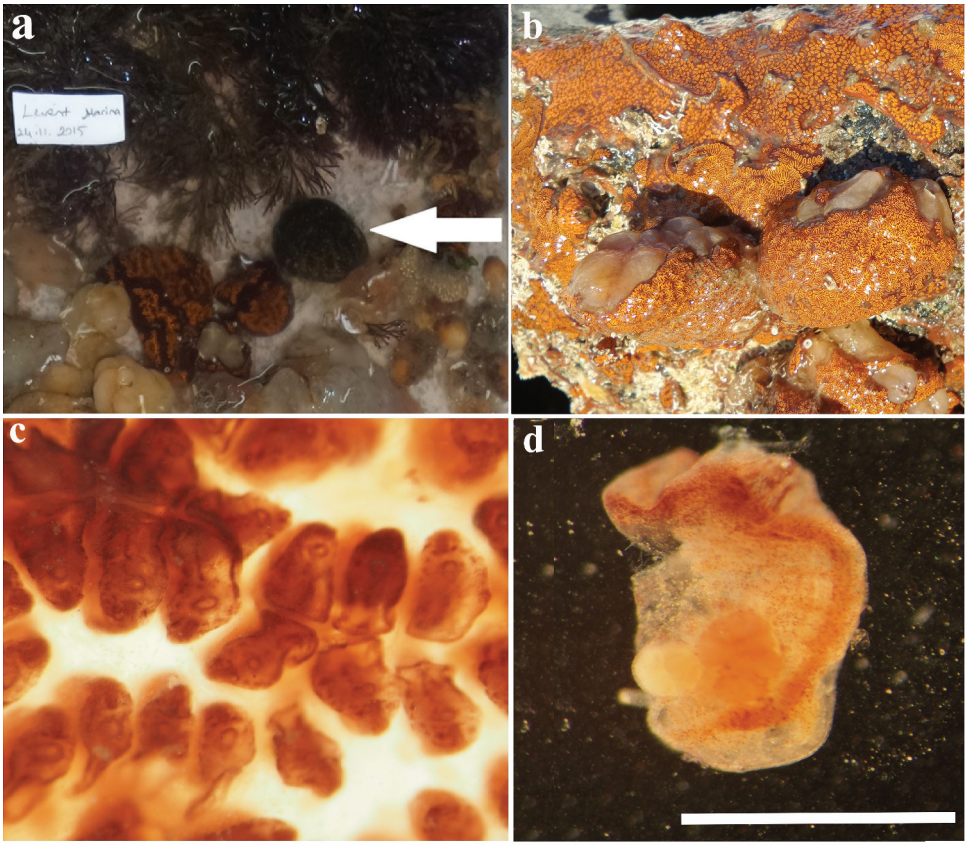


Figure 2. (a) *Polyclinum* sp. (Levent Marina, 24.11.2015), (b-d) *Botrylloides leachii* colony and detail of zooids (Urla, 26.08.2015), Scale bars: D: 1 mm.

changes were detected for the number of individuals. With regard to the total number of solitary ascidians in August, *S. plicata* accounted for 43.3% of the ascidian community, followed by *M. squamiger* 31.7%, *S. canopus* 17.3%, *Ascidia mentula* Müller, 1776 7.1%, *M. polymorphus* 0.4%, and *Ciona robusta* Hoshino & Tokioka, 1967 0.2%. However, in November sampling period, *S. canopus* (57.3%) was the most common species and *S. plicata* accounted for 23.2%, *A. mentula* 11.7%, *M. squamiger* 6.9%, *C. robusta* 0.5%, and *M. polymorphus* 0.4% of the individuals.

In the present study, the minimum length of *M. squamiger* (0.3 cm) was recorded at station S5 in November, while its maximum length (4.5 cm) was measured at S5 station in the same period. It had a leathery tunic, usually encrusted by other invertebrates and algae. Siphons were red and elongate showing white or yellow stripes and they separated approximately one-third of the total length. The pharynx had 6–10 folds on each side and it had straight stigmata and ramified tentacles. Its dorsal lamina consisted of a continuous membrane. It had endocarps on body wall, gonads and intestine. Its stomach had a large digestive gland. It had one gonad on each side of the body and they divided into up to five lobes (Figure 5). *M. squamiger* spines are very short and about 12.74 μm long and they

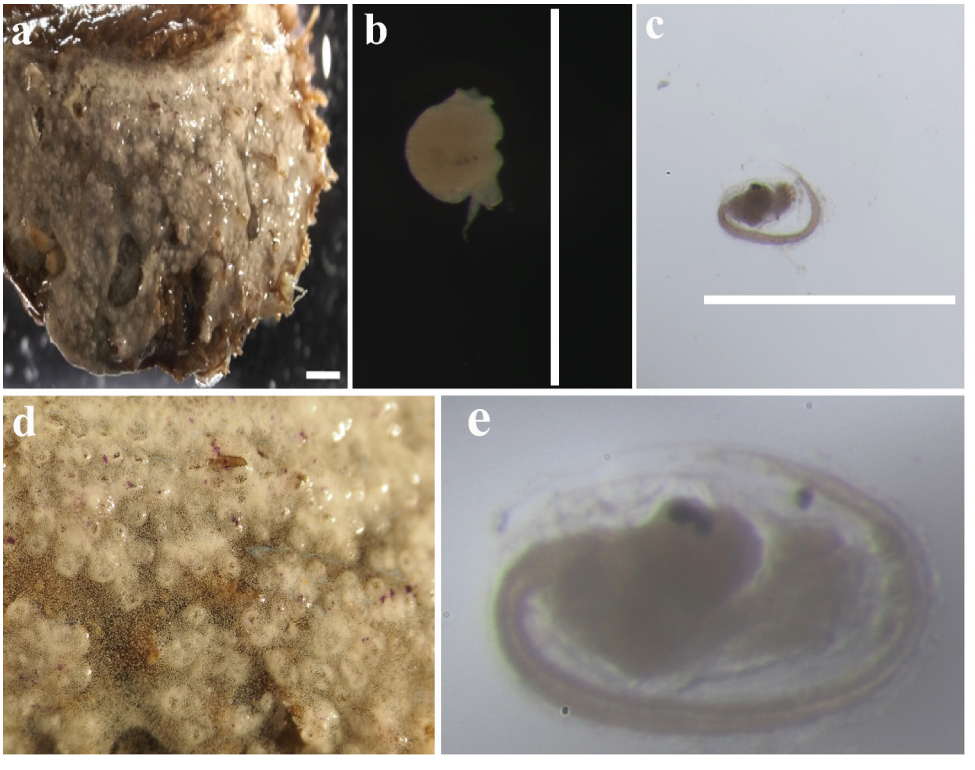


Figure 3. (a) *Didemnum ahu* colony, (b) thorax of a zooid, (c) larva, (d) spicules, (e) larva (Karaburun, 23.11.2015), Scale bars: A, B, C: 1 mm.

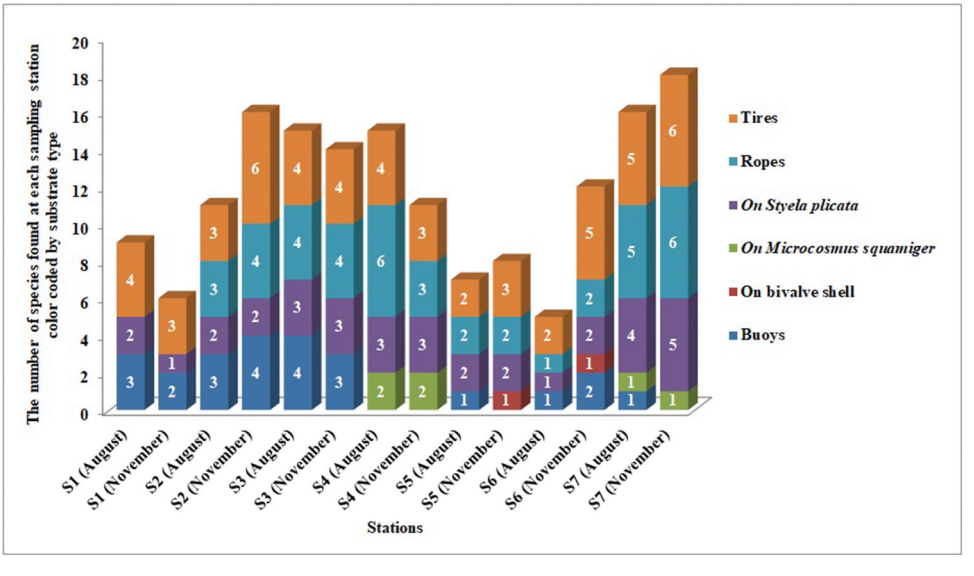


Figure 4. The number of species found at each sampling station colour coded by substrate type.

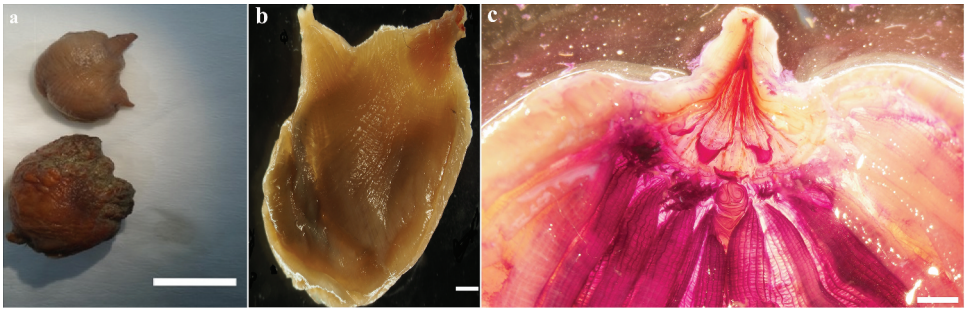


Figure 5. (a) *Microcosmus squamiger* (Mordoğan, 25.08.2015), (b) *M. squamiger* without tunic, (c) detail of the pericoroneal area (Karaburun, 25.08.2015), Scale bars: A-C: 2 mm.

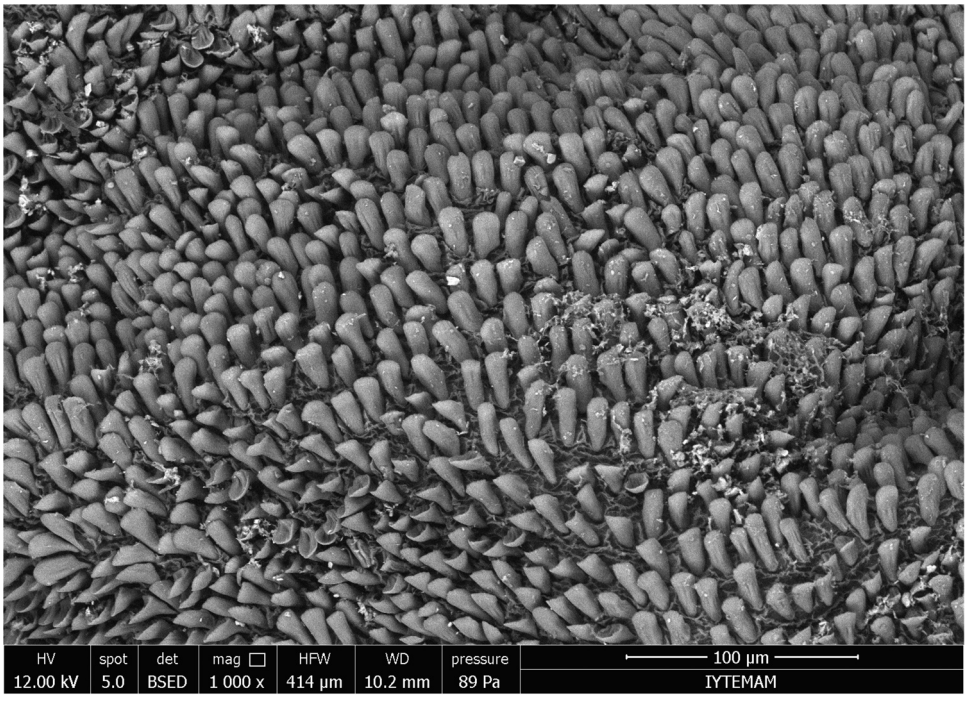


Figure 6. Siphonal spines of *Microcosmus squamiger* (Mordoğan, 25.08.2015). Scale bar: 100 µm.

look like a fingernail shape (Figures 6 and 7) (Kott 1985; Monniot et al. 2001; Mastrototaro and Dappiano 2008).

Didemnum ahu was recorded on a bivalve shell at Station S5 in November. The colony was white with small zooids and small larvae of 0.3 mm for the trunk. The colonies were very thin and the cloacal canals were visible on the surface of the colonies. The shape and size of spicules were irregular (Figures 8 and 9). The branchial siphon had six lobes. The thoracic lateral organs were small, placed over the third transversal vessel or over the fourth row of stigmata. The shape of the single testis was spherical or conical. It was surrounded by the six to eight coils of the sperm duct. The larva had three adhesive papillae and four pairs of fingerlike lateral ampullae (Figure 3c, e).

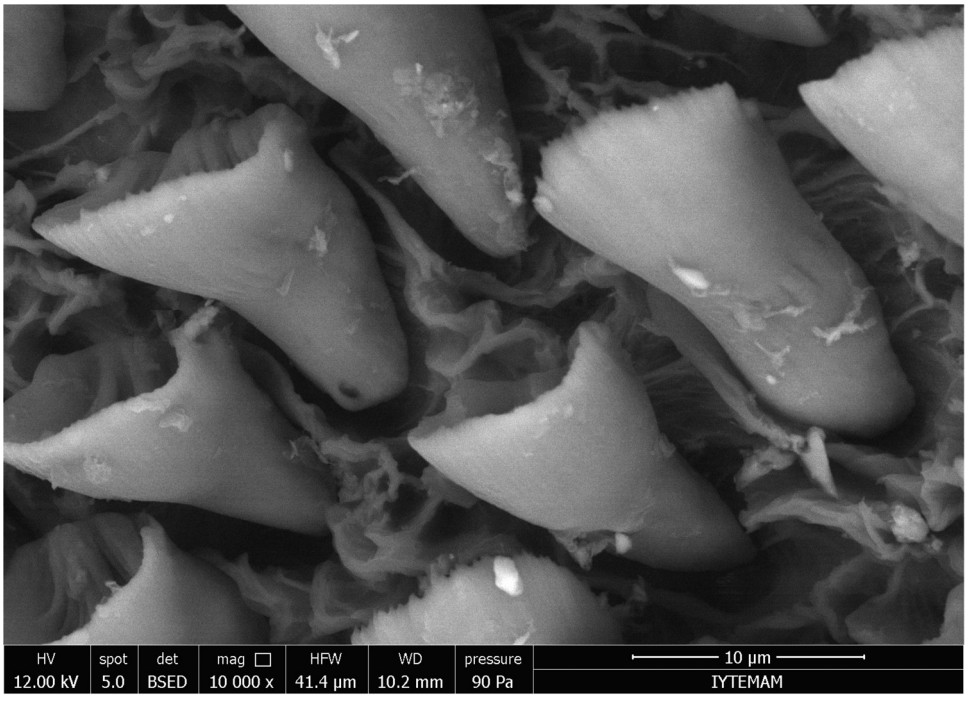


Figure 7. Siphonal spines of *Microcosmus squamiger* (Mordoğan, 25.08.2015). Scale bar: 10 µm.

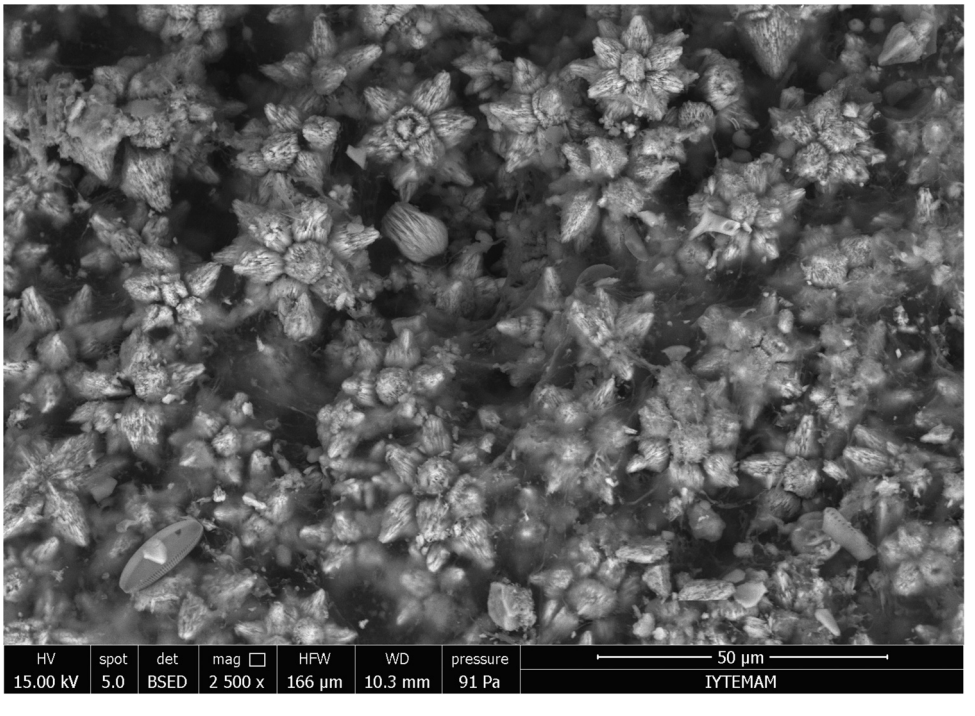


Figure 8. Spicules of *Didemnum ahu* (Karaburun, 23.11.2015). Scale bar: 30 µm.

The colony of *Polyclinum* sp. which was collected from Station S2 in November had an attachment area 2.7 cm in length and approximately 2.5 cm in height (Figure 2a). Zooids completely embedded in the tunic and zooids arranged in systems with common cloaca. The oral siphon of *Polyclinum* sp. had six lobes. It had small papillae on pharyngeal transverse vessels. Its post abdomen sack like and joined to abdomen with a neck. Its gut loop was small. External surface of stomach wall was smooth. This species could not be clearly identified at species level due to the lack of mature gonads and larvae that are essential for identification.

The global and Turkish Seas distributions of the identified ascidian species in this study are given in Table 3.

Discussion

In the present study, non-indigeneous species and species which have invasive potential were generally observed at stations (S2, S4, S6, and S7) selected from marinas and places where the number of fishing vessels are high. According to Mastrototaro et al. (2004) sessile suspension-feeders like ascidians particularly prefer to live in the marina and the harbour ecosystems characterised by protected and eutrophic waters.

In this study, *Styela plicata, Styela canopus, Ascidia mentula, Botrylloides leachii*, and *Microcosmus squamiger* were the most dominant species and they occurred frequently at the studied stations. Of the seven species recorded in Aydin Onen (2018), the colonial tunicate *B. leachii* and the solitary ascidian *S. canopus* were generally common at the stations and it was also common five of the seven sampling stations in this study. Furthermore, *S. plicata* was the only ascidian species found at all sampling stations in both studies undertaken by Aydin Onen (2018) and in this study. According too the findings it can be seen that these species fouled on both natural and artificial substrata. Rocha and Kremer (2005); Pineda et al. (2011); Pérez-Portela et al. (2013) reported that *S. plicata, S. canopus*, and *M. squamiger* have globally widespread distribution. They are generally known as common port fauna cosmopolitan species present around the world and also they found in marinas and harbours located in tropical and sub-tropical regions. In addition, five (5) named species such as *Polyclinum constellatum, Ascidiella aspersa, Molgula manhattensis, Microcosmus exasperatus*, and *Symplegma brakenhielmi* which were recorded at the sampling stations by Aydin Onen (2018) were not reported in the list of species given in this study.

A large number of *S. canopus* were found on the tunic of *S. plicata* individuals collected from Station S3 in November. Furthermore, sampled *S. canopus* individuals were also observed on the tunic of *M. squamiger*. The ascidians provide a favourable substratum for sessile organisms such as algae, sponges, hydrozoans, bryozoans, and *Microcosmus* spp. (Monniot 1965; Voultsiadou et al. 2007, 2010). *Styela plicata* usually fouled by ascidians and provide suitable substrate for non-ascidian epibionts (Lambert and Lambert 1998). Moreover, Naranjo et al. (1996) reported that *M. squamiger* individuals live together with barnacles and mussels and form a surface for the settlement of other fouling organisms.

Microcosmus squamiger is a solitary ascidian, probably southeast Australian in origin (Monniot et al. 2001; Rius et al. 2012). Furthermore, it has now spread to temperate waters worldwide. It has ability to foul hard substrata, especially in harbours, bays, and shallow littoral habitats, and to outcompete with native species for food and space, and also to form dense aggregations (Turon et al. 2007; Mastrototaro and Dappiano 2008; Otero et al. 2013).

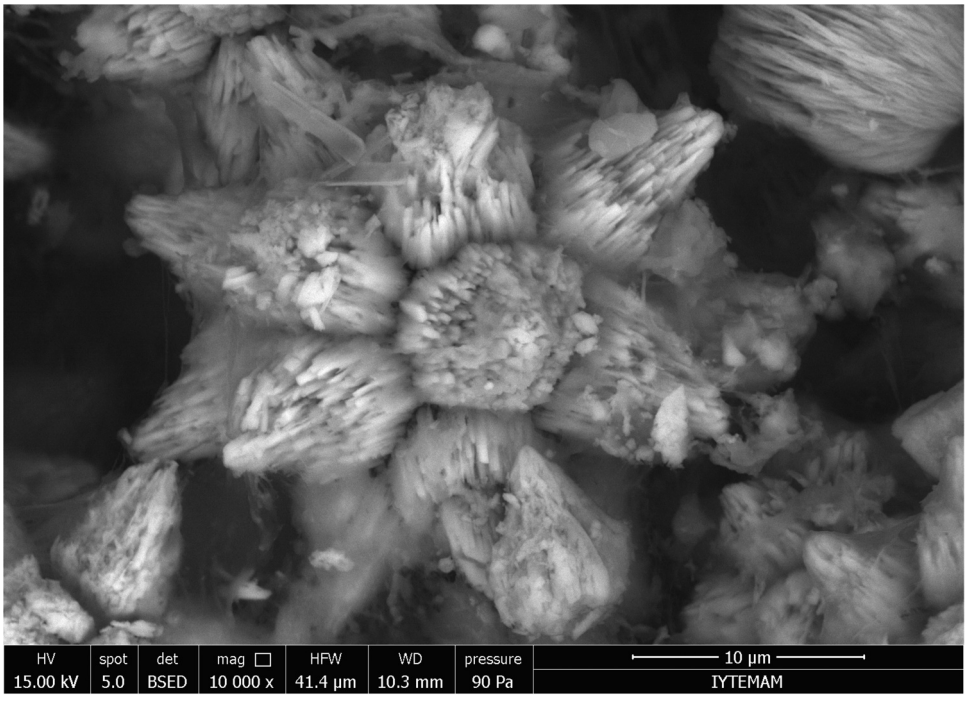


Figure 9. Spicules of *Didemnum ahu* (Karaburun, 23.11.2015). Scale bar: 50 µm.

According to Izquierdo-Muñoz et al. (2009) both invasive species of *Microcosmus*, the Indo-Pacific *M. exasperatus* spread from the Gulf of Gabes in the central Mediterranean Sea to the eastern Mediterranean Sea and the circumtropical *M. squamiger* spread from the western Mediterranean Sea to the central Mediterranean Sea (Malta) (Izquierdo-Muñoz et al. 2009; Ramos-Esplá et al. 2013). Turon et al. (2007) re-examined most of the *Microcosmus* specimens from previous studies and that they reported that *M. squamiger* has probably entered the Mediterranean through the Gibraltar Strait. It can be introduced as fouling on ships and recreational boats, in the ballast waters of shipping vessels, and through aquaculture. *Didemnum ahu* was first recorded in French Polynesia (Pernambuco, Bahia, São Paulo, Santa Catarina). (Monniot and Monniot 1987), and it is reported from New Caledonia and Papua (Monniot 1995, 2009; Monniot and Monniot 2008); and in the South-Eastern Brazil (Ponta do Baleeiro, Praia Grande, Praia do Cabelo Gordo de Dentro) (Rocha and Monniot 1995).

Brunetti et al. (2015) mentioned that, *Ciona intestinalis* type A specimens have been observed in the Mediterranean Sea, the Pacific Ocean, the Atlantic coasts of South Africa, English Channel, and French Atlantic coasts (i.e. in Plymouth, UK, and in Brest, France). According to these authors (Brunetti et al. 2015), the presence or absence of tubercular prominences in the tunic of *C. intestinalis* can be used to discriminate types A and B specimens and they reported that *C. intestinalis* type A corresponds to *C. robusta*. In the present study, observed *Ciona* specimens have tubercular prominences as small raised regions on the surface of the tunic of the siphons, so that our results confirmed that these species were *C. robusta*.

Table 3. The worldwide and Turkish Seas distributions of the recorded ascidian species in this study.

	Worldwide Distribution	Turkish Seas Distribution
Clavelina lepadiformis (Müller 1776)	Native to Norway, Northeast Atlantic (Ireland, Scotland, England, Channel Islands, France, Sweden, Portugal, Spain), Mediterranean Sea, Connecticut of Northwest Atlantic, South Africa, Japan, Korea.	İzmir Bay, Fethiye Bay
Didemnum ahu Monniot C. & Monniot F., 1987	Western Pacific (New Caledonia), French Polynesia (Pernambuco, Bahia, São Paulo, Santa Catarina), West Papua, South-Eastern Brazil (Ponta do Baleeiro, Praia Grande, Praia do Cabelo Gordo de Dentro).	
Ascidia mentula Müller, 1776	Norway to Canary Islands, Mediterranean and the Black Sea.	Dardanelles, Kepez Harbour, Aegean, Black, and Levantine Seas
Ciona robusta Hoshino & Tokioka, 1967	Mediterranean Sea, the Pacific Ocean (Australia, Japan, New Zealand, South Korea, West coast of North America), the Atlantic coasts of Africa.	Aegean Sea, Black Sea, Marmara Sea, Levantine Sea
Microcosmus polymorphus Heller, 1877	Mediterranean Sea to the Atlantic coast of Portugal.	İzmir Bay, Levantine Sea
Microcosmus squamiger Michaelsen, 1927	Native to Australia, California Harbours, USA, Atlantic Ocean, Indian Ocean, Mediterranean Sea, Spain, France, Italy, Portugal, Tyrrhenian Sea, Pacific Ocean, South and West coasts of Africa, Egypt, Mozambique, Tunisian Lagoons, the Suez Bay in the Red Sea.	
Botrylloides leachii (Savigny, 1816)	Northeast Atlantic, Mediterranean Sea, Black Sea, Australia, Tasmania, Indo West Pacific.	Mersin Harbour
Botryllus schlosseri (Pallas, 1766)	Northeast Atlantic, Mediterranean and Black Sea, Atlantic Canada, India, South Africa, South Australia, Tasmania, US West coast.	Marmara Sea, Black Sea, Aegean Sea, Mersin Harbour
Styela canopus (Savigny, 1816)	Eastern and Western Atlantic, California Harbours, Mediterranean, Guam.	Marmara Sea, Mersin Harbour, İzmir Bay,
Styela plicata (Lesueur, 1823)	Pacific Ocean, Atlantic Ocean, Atlantic Canada, Brazil, California Harbours, Gulf of Mexico, South Africa, South Australia, Indian Ocean Tasmania, Mediterranean Sea China/Korea, Hong Kong, China.	Mersin Harbour, Aegean Sea

Regarding *Polyclinum* sp., Galil et al. (2018) also reported this NIS, which has tropical/subtropical Indo-Pacific origin disperse to Turkish seas via Suez Canal.

It is known that some ascidian species such as *M. squamiger, S. plicata, B. leachii*, and *Botryllus schlosseri* (Pallas, 1766) can successfully introduce to new habitats due to their tolerance to high levels of pollution (Naranjo et al. 1996; Lambert and Lambert 2003; López-Legentil et al. 2015). In this study, *M. squamiger* and *S. plicata* formed dense populations at the studied stations. As reported by previous studies, both of them prefer to live in harbours and marinas of warm and temperate oceans (Pineda et al. 2011; Rius et al. 2012) and they coexist at high densities and they have tolerance to large variations in environmental conditions such as temperature and salinity changes, high levels of organic matter in the

water column and turbidity (Naranjo et al. 1996; Mastrototaro et al. 2008). Chabbi et al. (2010) studied ascidians collected from 12 stations situated in the port of Hammamet and in open sea along the Northern coast of Hammamet city. Moreover, they reported that *S. plicata* and an Atlanto-Mediterranean species *Clavelina lepadiformis* (Müller, 1776) were found in large numbers at sampling stations characterised by polluted waters.

Ascidia mentula and *M. polymorphus* are Mediterranean native species, they live on natural substrata, and are either found only in the Mediterranean Sea or have an Atlanto-Mediterranean distribution (Koukouras et al. 1995; Coll et al. 2010; Aslan et al. 2011). Furthermore, the golden star tunicate *B. schlosseri, B. leachii*, and *S. canopus* are labelled as cryptogenic in the Mediterranean Sea by López-Legentil et al. (2015). According to Zenetos et al. (2017), the status of *C. lepadiformis* is also considered as cryptogenic species in the Mediterranean Sea. Moreover, *C. robusta, M. squamiger*, and *S. plicata* are regarded as established species in the Mediterranean Sea.

The rate of Lessepsian migration has been increasing particularly in the last decade due to the continued enlargement of the Suez Canal (Zenetos et al. 2012), climate change (Belkin 2009) which affect ascidian spawning and recruitment patterns, and also 'tropicalisation' of the Mediterranean Sea (Bianchi and Morri 2003). The Aegean Sea, and in particular its southern part, is the area where the majority of the alien marine species is distributed, mainly due to its vicinity to the Levantine Basin (Zenetos et al. 2009). Moreover, Çinar et al. (2006b) conducted a study on zoobenthic organisms in the polluted inner region of the İzmir Bay, and they were reported that the region was significantly invaded by exotic species. According to the findings of this study, the most common species *S. plicata* and *M. squamiger* are considered a potential threat and further monitoring is needed to assess their impacts. So that to evaluate future introductions and to detect invasive non-indigenous ascidian species genetic monitoring and more detailed studies throughout Turkish seas are also needed.

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