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RESEARCH PAPER

Distribution of Ascidians with a New Record of the Non-Indigenous Species *Polyclinum constellatum* Savigny, 1816 from the Aegean Coast of Turkey

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Abstract

The current study attempts to investigate the distribution of ascidian species collected from eight stations located along the eastern coasts of Aegean Sea, Turkey. For this purpose, field studies were conducted in July 2016. This study revealed the occurrence of ascidian species belonging to 7 families and 12 genera. The invasive species *Polyclinum constellatum* Savigny, 1816 is reported for the first time from Turkish waters and for the second time in the Mediterranean Sea. Furthermore, *Symplegma brakenhielmi* (Michaelsen, 1904) is found as a new record for the Turkish Aegean Sea fauna. Knowledge about the ascidian diversity of the Turkish Seas is still incomplete and large-scale surveys, additional taxonomic and molecular studies are needed.

Keywords: Tunicates, non-indigenous ascidians, invasive species, Aegean Sea, *Polyclinum constellatum*.

Introduction

In marine ecosystems, ascidians (Phylum: Chordata, Subphylum: Tunicata, Class: Ascidiacea) are commonly known as sea squirts and they comprise an important group of marine fouling organisms around the world (Pennai & Rothbächer, 2016). Moreover, a number of ascidians are successful invaders in marine ecosystems (Shenkar & Swalla, 2011; Zhan, Briski, Bock, Ghabooli, & MacIsaac, 2015).

On a global scale, great attention has been given to ascidians due to a) their nutritive value (Lambert, Karney, Rhee, & Carman, 2016), b) their ability to produce bioactive compounds, specifically substances which serve as cytotoxic, antibacterial, antipyretic, analgesic, and histamine (Gopalakrishnan, Meenakshi, & Shanmuga, 2011), and compounds against various solid-type tumors (Gab-Alla, 2008), c) and also their use in studies of evolutionary developmental biology (Lemaire, 2011). Furthermore, invasive ascidians have ability to a) change community structure (Tracy & Reyns, 2014), b) they can lead to the loss of native genotypes (Katsanevakis *et al*., 2014), c) they can modify habitats, d) they can affect ecosystem processes and trophic relationships in the food chain, e) they can economically affect fishing and aquaculture activities and also f) they can introduce new diseases and pathogens or toxic substances (Marques *et al*., 2013).

According to Shenkar *et al*. (2017), about 3,000 ascidian species have been described worldwide. In addition, knowledge on ascidian diversity across the Turkish coasts is relatively limited. Çınar (2014) published a comprehensive regional tunicate species checklist. According to the latter review the faunistic studies on the ascidians, thaliaceans, and appendicularians (or larvaceans) in the Turkish Seas began with Colombo (1885), who reported six species from the entrance of the Çanakkale Strait to the Aegean Sea. After then, Ostroumoff (1894) conducted a marine survey in the Sea of Marmara and reported two ascidian species. Moreover, Ostroumoff (1896) investigated plankton and benthos during the Selanik Expedition to the Sea of Marmara after the invitation of the Ottoman Sultan Abdulhamit II and he determined 17 tunicate species belonging to class Ascidiacea and one species belonging to class Thaliacea. Afterwards, ascidians have been documented in different studies from the Turkish coasts (Demir, 1952; Demir, 1954a, 1954b; Nalbantoğlu, 1955; Băcescu, 1961; Kiseleva, 1961; Ergen, 1967; Kiseleva, 1969; Geldiay & Kocataş, 1972; Gökalp, 1972; Pınar, 1974; Uysal; 1976, Kocataş; 1978; Mavili, 1987; Ergen, Kocataş, Katağan, & Cınar, 1994; Koçak, Ergen, & Çinar, 1999; Koçak & Küçüksezgin, 2000; Tarkan, 2000; Dinçaslan & Öber, 2004; Öztürk *et al*., 2004; Aslan, 2006; Çinar, Bilecenoglu, Öztürk, & Can, 2006a; Çinar *et al*., 2006b; Lakkis & Toklu, 2007; Okuş *et al*., 2007; Çinar *et al*., 2008; Mutlu & Ergev, 2008; Çınar *et al*., 2011; Aslan-Cihangir, Izquierdo-Muñoz, Pancucci-Papadopoulou, & Can-Yılmaz, 2011; Gözcelioğlu, 2011; Ramos-Espla, Izquierdo, & Çınar, 2013; Artüz, Sönmez, & Kubanç, 2014).

In total 61 species belonging to the three tunicate classes Ascidiacea (50 species), Thaliacea (3 species), and Appendicularia (8 species) were reported from the Turkish coasts by Çınar (2014). Four of these species, namely *Symplegma brakenhielmi* (Michaelsen, 1904), *Microcosmus exasperatus* Heller, 1878, *Herdmania momus* (Savigny, 1816), and *Phallusia nigra* Savigny, 1816 are non-indigenous (NIS) (Çinar, Bilecenoglu, Öztürk, & Can, 2006a; Ramos-Espla, Izquierdo, & Çınar, 2013). According to this annotated checklist, the Aegean records include 29 species of Ascidiacea, 3 species of Thaliacea, and 7 species of Appendicularia. Two NIS, namely *M. exasperatus* and *P. nigra* were reported from this region (Çınar, 2014). After this publication, one more NIS, *Styela clava* Herdman, 1881 was recorded in one locality (Karamürsel, İzmit Bay) in the Sea of Marmara on rocks at 10 m depth (Çınar, 2016). Up to now, the addition of this new alien species brings the total number of identified tunicate species in Turkish coasts to 62.

The aim of the present work was to study ascidian diversity and to detect the occurence of alien and invasive ascidian species through field surveys in selected stations situated in the eastern Aegean Sea coasts.

Materials and Methods

Study Area

The study area is located along the coastline of the eastern Aegean Sea, Turkey (Figure 1). In this study, eight sampling stations were selected from potential places that are susceptible to introductions or the dispersal of ascidian species. Sampling was carried out in areas which have high numbers of local fishing boats, commercial fishing vessels and recreational boats (Figure 1). Station S1 is located in Aliağa Bay and Station S2 is placed in Yeni Foça located on the Aegean Coast. These stations host seasonal fishing and recreational vessels. Station S3 is situated in Güzelbahçe in İzmir Bay and it is subject to intense anthropogenic activities and urban stress. Station S4 is found in Özbek Bay (Urla, İzmir) and Station S5 is located on the coast of Özdere (İzmir). The number of fishing and recreational boats is high at these two stations, especially during summer months. Station S6 is placed in Kuşadası Bay (Aydın), which is a highly developed area with maritime traffic by fishing and recreational boats, including cruise vessels. Stations 7 and 8 are placed in Taşburun (Didim, Aydın) located on the Aegean

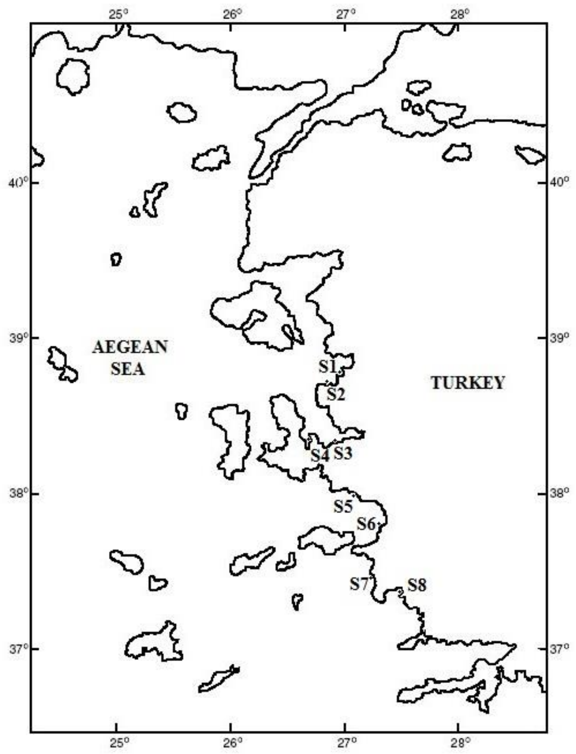

Figure 1. Map of the study area showing locations of sampling stations. S1: Aliağa, S2: Yeni Foça, S3: Güzelbahçe, S4: Özbek, S5: Özdere, S6: Kuşadası, S7: Taşburun, and S8: Akbük.

Coast and Akbük Bay (Didim, Aydın), respectively. These stations are used as sheltering places for commercial fishing vessels, recreational fishing boats, and yatchs (Figure 1).

Station numbers sites, geographical coordinates, and sampling dates for each study site are given in Table 1.

Methods

Ascidians were sampled in July 2016. At each station, ascidians were collected by examining associated subtidal structures such as ropes, wheels, pontoons, buoys, tires, chains, walls, plastic structures and other floating materials. Specimens were taken by hand from artificial and natural substrates using hand tools to scrape the ascidians from surfaces.

Specimens were photographed in situ and were collected from a depth zone of 0-1 m. In addition to photographs, observation notes (i.e. color, shape, substrate type, and depth) were recorded, and then the samples were narcotized with menthol crystals for relaxion and preserved in fixatives (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. Moreover, few ascidians were preserved in ethanol 95% for future molecular studies.

Ascidians were separated, identified and solitary species were counted. Specimens were examined and then identified under an Olympus-SZX16 stereomicroscope. Dissected ascidians were stained with haemalum. Images were taken by using a digital camera. Authoritative and valid taxonomic keys and publications (Van Name 1921, 1931, 1945; Kott 1985, 1990, 1992, 2001; Monniot, Monniot, & Laboute, 1991; Monniot & Monniot, 2001; Rocha, Zanata, & Moren, 2012) were used for species identification.

Three replicate surface water samples were collected in each station with sampling bottles (precleaned and rinsed twice with seawater) and then physicochemical variables such as temperature, salinity, pH, and dissolved oxygen (DO) were measured by using a portable instrument (HACH-HQ40D, Hach Lange, Düsseldorf, Germany).

Statistical Analysis

Community variables such as number of species, number of individuals were calculated for each sampling station. Frequency Index (F) was calculated according to Soyer (1970) in order to determine the frequency value [(constant ($F \geq 49\%$), common ($49\% > F \geq 25\%$) and rare ($F < 25\%$)] of each ascidian species. The frequency index formula is the following: $f = m/M * 100$, where m= the number of stations where the species was detected and M= the numbers of all stations. Data analysis was performed using STATISTICA v.7.1 (STATSOFT) software package. Spearman’s rank order correlation test was used to examine relationships among the physicochemical variables, number of individuals, and the number of species.

Results

Taxonomic Composition

A total of 396 solitary ascidians were examined corresponding to 7 species which belonged to 5 families namely Ascidiidae (2), Cionidae (1), Molgulidae (1), Pyuridae (1), and Styelidae (2) (Table 2). However, three taxa could be determined only to genus level (*Didemnum* sp., *Aplidium* sp., and *Microcosmus* sp.) (Table 2). The species *Didemnum* sp. and *M. exasperatus* were found in only one station, while *Styela plicata* (Lesueur, 1823) and *Styela canopus* (Savigny, 1816) were observed at all sampling stations (Figure 2).

Three taxa, namely *Microcosmus* sp., *Botrylloides leachii* (Savigny, 1816), and *S. canopus* were found on both natural and artificial substrata (Table 2). The orders Aplousobranchia and Phlebobranchia were represented with three colonial forms and three solitary species, respectively. Furthermore, the order of Stolidobranchia had the greatest number of species (8), three of which were colonial and others were solitary.

Table 1. Stations numbers, sampling sites, geographical coordinates, and sampling dates of each site surveyed in Aegean coasts of Turkey

Station Numbers	Sampling Sites	Geographical Coordinates		Sampling Dates
		Latitude (N)	Longitude (E)	
S1	Aliağa	38°48'23.63"	26°58'28.86"	02.07.2016
S2	Yeni Foça	38°44'30.24"	26°50'19.47"	02.07.2016
S3	Güzelbahçe	38°22'39.56"	26°53'02.06"	02.07.2016
S4	Özbek	38°22'28.66"	26°40'56.10"	02.07.2016
S5	Özdere	38° 0'37.55"	27° 6'18.02"	03.07.2016
S6	Kuşadası	37°51'41.15"	27°15'20.10"	04.07.2016
S7	Taşburun	37°27'11.44"	27°13'07.87"	06.07.2016
S8	Akbük	37°23'11.70"	27°25'54.48"	06.07.2016

Table 2. Number of ascidian species and abundance of solitary ascidians recorded in the sampling stations. Substrate and substrate type, A: Artificial, N: Natural, Colonial C

	STATIONS								Substrate, Substrate Type
	S1	S2	S3	S4	S5	S6	S7	S8	
Phylum: Tunicata									
Classis: Ascidiacea									
Order: Aplousobranchia Lahille, 1886									
Family: Didemnidae Giard, 1872									
Didemnum sp.		C							on Lamellibranch, N
Family: Polyclinidae Milne Edwards, 1841									
Aplidium sp.			C			C			Wheels, A
**Polyclinum constellatum* Savigny, 1816						C		C	Ropes, A
Order: Phlebobranchia Lahille, 1886									
Family: Ascidiidae Herdman, 1882									
Ascidia mentula Müller, 1776	2			1	1		1		Ropes, wheels, buoys, A
Ascidiella aspersa (Müller, 1776)	4				2				Ropes, wheels, buoys, A
Family: Cionidae Lahille, 1887									
Ciona robusta Hoshino & Tokioka, 1967	4		15		2	1			Ropes, A
Order: Stolidobranchia Lahille, 1886									
Family: Molgulidae Lacaze-Duthiers, 1877									
Molgula manhattensis (De Kay, 1843)				4			3	1	Ropes, A
Family: Pyuridae Hartmeyer, 1908									
Microcosmus exasperatus Heller, 1878								3	Ropes, A
Microcosmus sp.	4	61			62	8	1	1	Ropes, wheels, buoys, on *S. plicata*, A, N
Family: Styelidae Sluiter, 1895									
Botrylloides leachii (Savigny, 1816)	C		C	C	C	C		C	Ropes, wheels, buoys, on bivalve shell and *S. plicata*, A, N
Botryllus schlosseri (Pallas, 1766)	C					C			Wheels, A
Styela canopus (Savigny, 1816)	5	6	3	12	21	5	7	25	on *S. plicata*, *M. squamiger*, N
Styela plicata (Lesueur, 1823)	13	8	10	9	30	7	29	25	Ropes, wheels, buoys, A
***Symplegma brakenhielmi* (Michaelsen, 1904)								C	on Lamellibranch, N
Colonial	+	+	+	+	+	+		+	
Total species number	8	4	5	5	7	8	6	8	
Total number of soliter ascidians at stations	32	75	28	26	118	21	45	55	

*First record for the Turkish Sea fauna. **First record for the Aegean Sea fauna.

The ascidian species identified in the study area along with their systematic classification and ecological information (substrate type) are presented in Table 2. The highest ascidian species richness (8) occurred at stations S1, S6, and S8, while the lowest species richness (2) was found at Station S2. Of these recorded species, S3 and S4 stations were represented by five species followed by S7 station six species and S5 station seven species (Table 2).

A high abundance of solitary ascidians was recorded at Station S5 situated in Özdere (118 individuals) with *S. plicata*, *S. canopus*, *Microcosmus* sp., and *B. leachii* being the most frequent species. However, the lowest number of individuals was encountered at station S6 situated in Kuşadası (21) (Table 2).

The species *Microcosmus* sp. accounted for 34.6%, *S. plicata* 33.1%, *S. canopus* 21.2%, *C. robusta* 5.6%, *M. manhattensis* 2.0%, *A. aspersa* 1.5%, *A. mentula* 1.3%, and *M. exasperatus* 0.8% of the solitary ascidian community at all sampling stations.

According to frequency index values, *S. plicata* was constant at S1, S8, and S7 stations, *S. canopus* was constant at S4 and S8 stations, *Microcosmus* sp. was constant at S2 and S5 stations, and also *C. robusta* was constant at S3 (Table 3).

Non-Indigenous Ascidians

Species were classified by origin status as native, cryptogenic or established non-indigenous (Zenetos *et al.*, 2017). The species, *Ascidia mentula* Müller, 1776 was classified as native (Figure 2), while *B. leachii*, *Botryllus schlosseri*, *Molgula manhattensis*, and *S. canopus* labeled as cryptogenic (Figure 3) in the Mediterranean Sea. Seven species, namely such as *P. constellatum*, *Ascidiella aspersa*, *C. robusta*, *M. exasperatus*, *S. plicata*, and *S. brakenhielmi* were assigned as established non-indigenous species in the Mediterranean Sea (Figure 2, 3). However, three taxa, which were identified only to the genus level, could not be assigned to any of the above categories.

Taxonomic Remarks

Order APLOUSOBRANCHIA Lahille, F. (1886)
Family POLYCLINIDAE Milne Edwards, 1841
Genus *Polyclinum* Savigny, 1816
Polyclinum constellatum Savigny, 1816
Sites (Figure 1): Station S6, S8

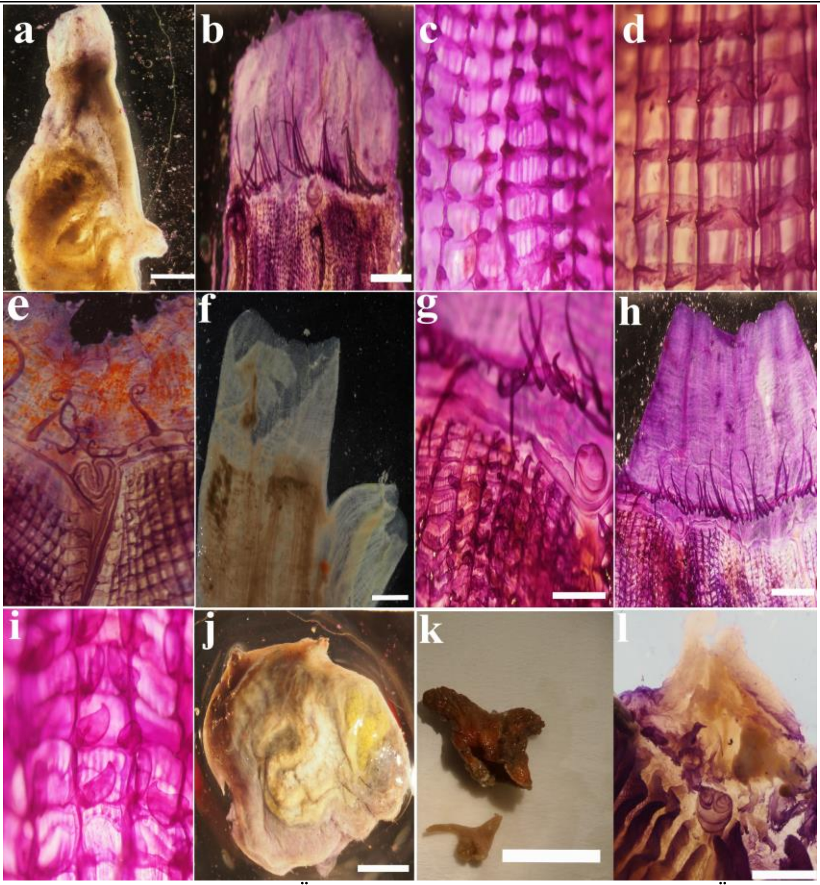

Figure 2. a) *Ascidia mentula* Müller, 1776, S5-Özdere, 03.07.2016; b) *A. mentula*, S5-Özdere, 03.07.2016, dissected; c) *A. mentula*, S5-Özdere, 03.07.2016, stigmata and papillae; d) *Ascidiella aspersa* (Müller, 1776), S5-Özdere, 03.07.2016, stigmata and papillae; e) *A. aspersa*, S5-Özdere, 03.07.2016; f) *Ciona robusta* Hoshino & Tokioka, 1967, S6-Kuşadası, 04.07.2016, without tunic; g) *C. robusta*, S6-Kuşadası, 04.07.2016, dorsal tubercule; h) *C. robusta*, S6-Kuşadası, 04.07.2016, dissected; i) *C. robusta*, S6-Kuşadası, 04.07.2016, stigmata and papillae; j) *Molgula manhattensis* (De Kay, 1843), S5-Özdere, 03.07.2016; k) *Microcosmus exasperatus* Heller, 1878, S8-Akbük, 06.07.2016; l) *M. exasperatus*, S8-Akbük, 06.07.2016. Scale bar a, f, h, j: 3 mm; b, e, k, l: 2 mm; g: 1 mm.

Table 3. Frequency index values of solitary taxa (F<25: Rare, 25≤F≤49: Common, F>49: Constant)

Species	S1	S2	S3	S4	S5	S6	S7	S8
Ascidia mentula	6.25			3.85	0.85		2.44	
Ascidiella aspersa	12.50				1.69			
Ciona robusta	12.50		53.57		1.69	4.76		
Molgula manhattensis				15.38			7.32	1.82
Microcosmus exasperatus								5.45
Microcosmus sp.	12.50	81.33			52.54	38.10	2.44	1.82
Styela canopus	15.63	8.00	10.71	46.15	17.80	23.81	17.07	45.45
Styela plicata	40.63	10.67	35.71	34.62	25.42	33.33	70.73	45.45

Examined material: 19 colonies, S6, Kuşadası, 37°51'41.15" (N) 27°15'20.10" (E), 1 m, 04 July 2016
Examined material: 4 colonies, S8, Akbük (Didim), 37°23'11.70" (N) 27°25'54.48" (E), 1 m, 06 July 2016

The non-indigenous *P. constellatum* Savigny, 1816, which is reported for the first time in Turkish waters, was especially abundant at stations S6 and S8. The biggest colony of *P. constellatum*, which collected from station S6, was measured 6.9 cm in diameter by 5.3 cm in height. It formed ball-shaped colonies filled with a gelatinous tunic, with the zooids located only around the outer edge (Goodbody, 2003) (Figure 4a-d). It had similar characteristics as reported by Rocha and Costa (2005). The studied colonies of *P. constellatum* were encrusting and on the colony surface, they had dark green with white or beige stellate systems (Figure 4d). There were from 10 to 20 rows of stigmata and transverse muscle fibers

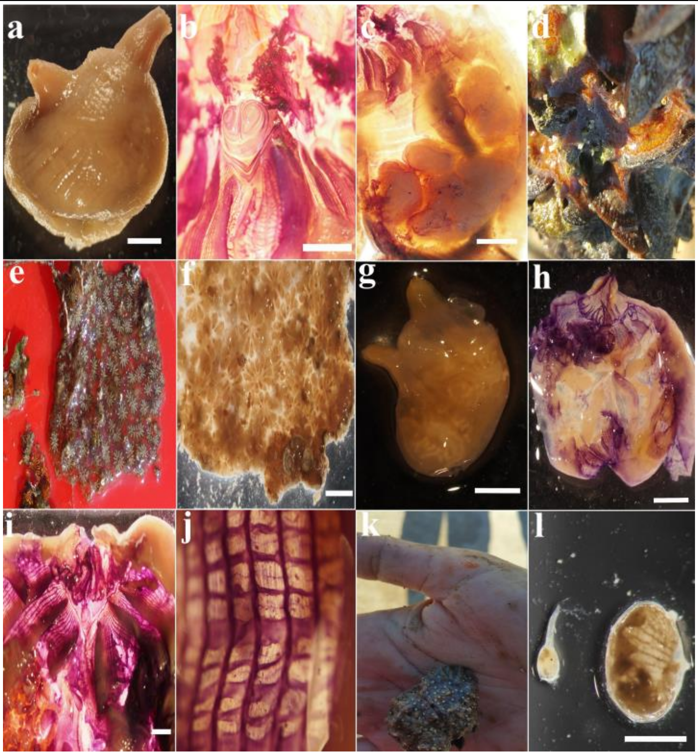

Figure 3. a) *Microcosmus* sp.S6-Kuşadası, 04.07.2016, without tunic; b) *Microcosmus* sp., S6-Kuşadası, 04.07.2016, dorsal tubercule; c) *Microcosmus* sp., S6-Kuşadası, 04.07.2016, intestine and gonads; d) *Botrylloides leachii* (Savigny, 1816), S8-Akbük, 06.07.2016; e) *Botryllus schlosseri* (Pallas, 1766), S6-Kuşadası, 04.07.2016; f) *B. schlosseri*, S6-Kuşadası, 04.07.2016, zooids; g) *Styela canopus* (Savigny, 1816) S7-Taşburun, 06.07.2016; without tunic; h) *S. canopus*, S7-Taşburun, 06.07.2016; dissected; i) *Styela plicata* (Lesueur, 1823), S2-Yeni Foça, 02.07.2016, dissected; j) *S. plicata*, S2-Yeni Foça, 02.07.2016, stigmata and papillae; k) *Symplegma brakenhielmi* (Michaelsen, 1904), S8-Akbük, 06.07.2016; l) *S. brakenhielmi*, S8-Akbük, 06.07.2016, zooid; Scale bar a, b, f, g, h, i: 3 mm; c: 2 mm; l: 1 mm.

between them (Figure 4e). The abdomen was short with the distal end of the esophagus bending at a right angle, leaving the stomach horizontal to the thorax. It brooded inside the atrial cavity or in a small pouch projecting from the thorax at the anus level (Figure 4f).

The shape of oral siphon was tubular, fringed by six long and triangular lobes (Figure 4g). And also it had a large circular atrial siphon and its dorsal languet had diminute lobes. Its margin of pharyngeal transverse vessels was papillated (Rocha, Zanata, & Moren, 2012) (Figure 4h). The stomach was globular with a smooth wall and the mid-intestine was short (Figure 4g-i). The intestine was twisted and the bilobed anus ended between the seventh and the ninth rows of stigmata. Anus was located at the 8th transverse vessel in zooids (Figure 4g). Its abdomen was more or less 90° position in relation to the thorax. The post-abdomen was sac-like and the ovary of this species surrounded by male follicles (Rocha & Costa, 2005) (Figure 4e-i).

The established NIS *S. brakenhielmi* formed flattened, encrusting colonies of gray-green color. It had gonads in both of sides of the body wall, and the slightly lobed testis. The maximum colony diameter was 3.3 cm. The colony was observed at station S8 and it is reported for the first time in the Aegean ecoregion (Antoniadou *et al*., 2016). Due to having, tubercular prominences as small raised regions on the surface of the tunic of the siphons *C. intestinalis* type A individuals in this study were accepted as *C. robusta* (Brunetti *et al*., 2015). The measured minimum and maximum lengths of the solitary species are presented at Table 4.

Distribution Patterns of the Species

A literature search was done to investigate the identified species distribution in various regions of the world and in Turkish Seas. Worldwide and Turkish Seas distribution of the recorded ascidian species were presented in Table 5.

Physicochemical Variables

The mean±standard errors (SE) of physicochemical variables of the seawater samples at all stations are given in Table 6. The mean water temperature levels ranged from 25.70±0.04 ºC at Station S8 to 28.67±0.02 ºC at Station S3. As for salinity, the present data showed that sampling stations revealed narrow variations, the mean salinity values changed from 31.73±0.21 at Station S6 to 40.43±0.02 ppt at Station S7 (Table 6).

A great variability was not observed in recorded pH levels. Moreover, the water pH is slightly basic and the mean pH values which ranged from 7.77±0.01 at Station S6 to 8.09±0.01at Station S7. In addition, the water conductivity was slightly basic and the mean conductivity levels varied from 48.77±0.74 at Station S6 to 60.70±0.01 mS/cm at Station S7. The average DO values changed between 5.18±0.01 at Station S6 and 7.01±0.05 mg/l at Station S3 (Table 6).

Correlation with Abiotic Variables

According to Spearman's rank correlation analysis there was a negative correlation between temperature (°C) and the number of species (r= -0.7120, P<0.05). Moreover, there was a positive relationship between *S. plicata* and pH (r= 0.7143, P<0.05).

Discussion

The current study aimed to characterize the ascidian assemblages of the eastern Aegean coasts and investigate the presence of non-indigenous species. According to the findings of this study, *S. plicata* and *S. canopus* were frequently occurring species at all sampling stations. Skinner, Barboza and Rocha (2016) mentioned that *S. plicata*, *S. canopus*, *S. brackenhielmi*, *P. constellatum*, and *M. exasperatus* have a wide geographic distribution and are generally found in marinas and harbors. *Ascidiella aspersa* is also another widespread species that is introduced in many parts of the world. Moreover, it is assigned as an established alien species in the Mediterranean Sea (López-Legentil *et al*., 2015; Zenetos *et al*., 2010).

Stations with the highest species richness (S1, S6, and S8) hosted a high number of alien species. The increasing maritime traffic (e.g. fishing vessels and international cruise ships) and anthropogenic impacts (e.g. domestic sewage discharge) at these A

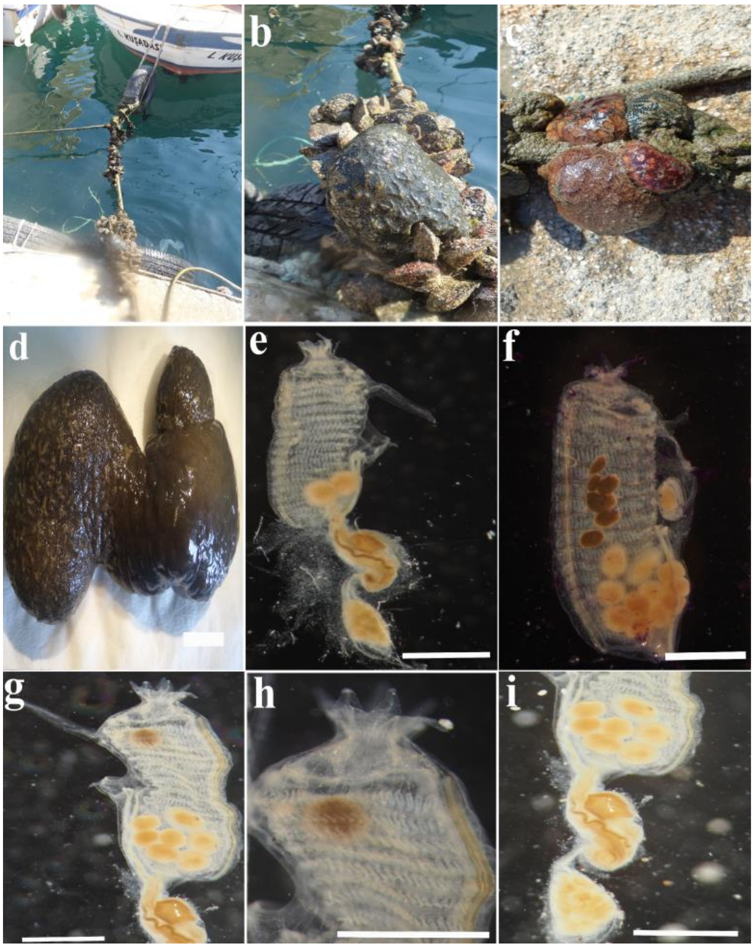

Figure 4. (a-d) *Polyclinum constellatum* Savigny 1816-colony, S6-Kuşadası, 04.07.2016, (e-i) zooid of *P. constellatum*, S6-Kuşadası, 04.07.2016. Scale bar d: 2 cm; e-i: 1 mm.

Table 4. The measured minimum and maximum lengths of solitary species at the sampling stations

Species	Minimum Length (cm)	Stations	Maximum Length (cm)	Stations
Ascidia mentula	2.8	S7	4.6	S7
Ascidiella aspersa	1.9	S1	4.4	S1
Ciona robusta	1.9	S1	11.3	S3
Microcosmus exasperatus	1.9	S7	2.8	S7
Microcosmus sp.	4.9	S2	4.9	S2
Styela canopus	0.2	S3	2.1	S4
Styela plicata	0.8	S5	7.5	S3

Table 5. Worldwide and Turkish Seas distribution of the recorded ascidian species in this study

	Worldwide Distribution	Turkish Seas Distribution
Polyclinum constellatum Savigny, 1816	Guam, Brazil, Pacific Mexico, Atlantic Canada, Brazil, Trinidad, France, North Pacific Ocean, Panama, Bermuda, USA (Florida, Gulf of Mexico), South Africa, West Indian Ocean, West Pacific, Hawaii, South California, Suez Canal, Mediterranean Sea.	Kuşadası Bay, Akbük Bay
Ascidia mentula Müller, 1776	Mediterranean, Adriatic to the Straits of Gibraltar, the Atlantic coast of France, Spain into the English Channel, Greece, Algericas Bay (Southern Spain), Baléares, Tunisia Lagoons.	Dardanelles, Kepez Harbor, Aegean Sea, Black Sea, Levantine Sea
Ascidiella aspersa (Müller, 1776)	Argentina, New England, New Zealand, South Africa, South Australia, Tasmania	Black Sea, İzmir Bay,
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 South Africa.	Aegean Sea, Black Sea, Sea of Marmara, Levantine Sea
Molgula manhattensis (De Kay, 1843)	California Harbors, China/Korea, Europe, NE Pacific, Netherlands, South Australia, Tasmania, Western Atlantic, Sweden, Norway, UK, Portugal, Spain, Morocco, Canada, USA, Argentina.	Aegean Sea, Sea of Marmara, Mersin Bay
Microcosmus exasperatus Heller, 1878	Atlantic Panama, Guam, Hawaii, India, Mediterranean Sea, Trinidad, Bermuda, Caribbean, SE USA, Brazil, South Africa, West Africa, Australia, Tunisia, Lebanon, Israel, North-Western Cyprus.	Alsancak Harbor (Izmir Bay), Levantine Sea
Botrylloides leachii (Savigny, 1816)	South Australia, Tasmania, Northeast Atlantic, Indo West Pacific, Mediterranean, the Black Sea, Australia, Europe.	Mersin Harbor
Botryllus schlosseri (Pallas, 1766)	Atlantic Canada, California Harbors, India, New England, San Francisco Bay, South Africa, South Australia, Tasmania, US West coast.	Marmara Sea, Black Sea, Aegean Sea, Mersin Harbor
Styela canopus (Savigny, 1816)	Atlantic Panama, Atlantic Canada, Adriatic Sea, Brazil, Mexico, California Harbors, Mediterranean, Guam, Indian Ocean, Australia, West Pacific, Hawaii, Easter Island.	Marmara Sea, Mersin Harbor, İzmir Bay
Styela plicata (Lesueur, 1823)	Atlantic Canada, Brazil, California Harbors, China/Korea, Gulf of Mexico, South Africa, South Australia, Tasmania, the Mediterranean Sea (Iberian Peninsula), the Northeastern Atlantic Ocean (Iberian Peninsula, Canary Islands), the Northwestern Atlantic Ocean (US east coast), the Southwestern Atlantic ocean (Brazil), the Northwestern Pacific Ocean (Japan and China), the Southwestern Pacific Ocean (Australia), the Northeastern Pacific Ocean (US West coast), the Southwestern Indian Ocean (South Africa), Hong Kong, China, East Asian Seas.	Mersin Harbor, Aegean Sea
Symplegma brakenhielmi (Michaelsen, 1904)	California Harbors, Guam, Hawaii, Mediterranean Sea, Trinidad, Caribbean, Brazil, Atlantic Oceans, Indian Ocean, West Pacific, Lebanon, Israel.	Levantine Sea, Fethiye Bays, Finike Bays

number of invasive ascidian species introductions have been linked to hull fouling stations could facilitate the introduction and spread of non-native species from recreational boating (Lambert & Lambert, 1998; Smiard *et al*., 2017).

In this study, *S. plicata* and *S. canopus* were observed at all sampling stations (Table 2). *Styela plicata* is a solitary ascidian native to NW Pacific Ocean (Carlton, 2009; Pineda, López-Legentil, & Turon, 2011) and *S. canopus* is originated from the Indo-West Pacific (Kott, 1985). They were commonly found in warm and temperate oceans, usually colonizing widely fluctuating environments, particularly marinas, harbours, and places used for

Table 6. Physicochemical variables (mean±SE) of the seawater at each sampling station

Stations	Temperature (°C)	Salinity (ppt)	pH	Conductivity (mS/cm)	DO (mg/l)
S1	26.03±0.27	33.53±0.13	7.80±0.02	51.23±0.38	6.03±0.01
S2	27.33±0.05	35.03±0.11	7.84±0.01	52.97±0.42	6.09±0.03
S3	28.67±0.02	39.50±0.00	8.04±0.00	58.87±0.06	7.01±0.05
S4	28.13±0.05	39.53±0.05	7.92±0.01	59.20±0.10	6.52±0.04
S5	28.30±0.04	40.03±0.02	8.05±0.01	59.63±0.12	6.82±0.02
S6	26.10±0.04	31.73±0.21	7.77±0.01	48.77±0.74	5.18±0.01
S7	26.63±0.11	40.43±0.02	8.09±0.01	60.70±0.01	6.67±0.01
S8	25.70±0.04	38.20±0.09	7.91±0.01	57.07±0.38	5.56±0.07

recreational and commercial fishing and boating. These species are able to survive in disturbed habitats and may dominate the sessile communities and reduce local diversity (Stachowicz, Whitlatch, & Osman, 1999). In addition, *S. plicata* (Lesueur, 1823) has been categorized as a harmful invasive species in several regions around the world (Pineda, López-Legentil, & Turon, 2011). The alien species *S. plicata, S. canopus, P. constellatum*, and *S. brackenhielmi* have been reported from both polluted and non-polluted environments (Marins, Novaes, Rocha, & Junqueira, 2010; Skinner, Barboza, & Rocha, 2016).

The results of this study showed that collected *S. plicata* individuals were fouled by *S. canopus* and *Microcosmus* sp. specimens. According to Yakovis and Artemieva (2017) most ascidians generally prefer to reside on barnacles, empty barnacle tests, and other ascidians.

In the present study, a negative relation was found between temperature (°C) and the number of species. Thiyagarajan and Qian (2003); Tracy and Reyns (2014) mentioned that temperature is an important factor which can affect the community composition, distribution and diversity of ascidians.

The circumtropical ascidian *S. brakenhielmi* is globally distributed in the Atlantic (Van Name, 1921; Monniot, 1983, Goodbody, 2003, Rocha *et al*. 2005), Indian (Millar, 1954; Monniot & Monniot, 1997) and Pacific Oceans (Monniot & Monniot, 2001; Lambert 2003). It occurs in the Red Sea and the Suez Canal (Michaelsen, 1918) and according to Abdel Messeih (1994) it can be a Lessepsian migrant. It has not previously reported from the Aegean Sea (Çınar, 2014; Antoniadou *et al*., 2016). In the Mediterranean, it was reported from Lebanon (Bitar & Kouli-Bitar, 2001; Bitar, Ocana, & Ramos-Esplá, 2007), Israel (Shenkar, 2008) and the Levantine coast of Turkey (Çinar *et al*., 2006). Moreover, it was also reported from Fethiye and Finike Bays (the Turkish Levantine Sea/Mediterranean coast) (Ulman *et al*., 2017).

The species *M. exasperatus* has probably reached the Mediterranean via the Suez Canal; it has an extra-tropical Indo-Pacific distribution and its distribution in the eastern Mediterranean is restricted (Izquierdo-Muñoz, Diaz-Valdéz, & Ramos-Esplá, 2009; Shenkar & Loya, 2009). Alien species of tropical/subtropical Indo-Pacific origin, which are present in Egypt, Israel, and Turkey were probably introduced via the Suez Canal, shipping traffic, ballast water, fouling of ship hulls, aquaculture, and aquarium trade (Zenetos *et al*., 2010; Galil *et al*., 2016). Transportation, shipping activities, ballast water, and hull fouling of recreational yachts, boats, and fishing vessels can be important vectors for the spread of ascidian species (Ashton, Boos, Shucksmith, & Cook, 2006).

The invasive tunicate *P. constellatum* is recorded for the first time from Turkish coastal waters (Table 5). Being a fouling organism, it often develops on artificial substrata in harbours (Monniot & Monniot, 1997). In the Mediterranean Sea, it was first recorded from Port Said in Egypt by Halim and Abdel Messeih (2016).This species has an invasive potential (Locke, 2009) so that it should be investigated.

Invasive alien species and NIS are a focus of good environmental status in the Marine Strategy Framework Directive (European Commission, 2008) and Aichi Biodiversity Target 9 (CBD, 2010), respectively due to their significant ecological and economic impacts on marine communities, aquaculture, and humans. As a result, invasive and alien ascidians should monitored regularly to avoid possible economic and/or environmental impact. Additional taxonomic and molecular studies are needed to enhance knowledge about the ascidian diversity and NIS of the Turkish Seas.

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