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Faunistic Study of Ascidians (Chordata: Tunicata) From The northern Limit of Suez Canal, Port Said, Egypt

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ABSTRACT

Subphylum: Tunicata, Class Ascidiacea comprises ascidians either colonial or solitary species which have received little research attention in Port Said coasts, Egypt, despite their high biodiversity in the study area. Fouling samples were collected and some environmental parameters were also measured seasonally during the period from (August) 2023 to (June) 2024. Moreover, morphological and taxonomical characterizations of different ascidian specimens were carefully examined to be identified. The study revealed 14 ascidians species**,** six of them were confirmed as first records and one probably to be first record if identified to species level during the current study period. These species belong to five families: Polyclinidae, Didemnidae, Styelidae, Pyuridae and Ascidiidae, which were collected from different anchors: piers, nets, marinas, and wooden poles attached with the other fouling. Winter and spring 2024 exhibited the highest number of ascidian species (12 species in both), while summer 2023 exhibited the lowest number of species (only8 species). Additionally *Aplidium grisiatum* Kott, 1998 and *Polyclinum constellatum* Savigny, 1816 were the most dominant species. During the study period, the physico-chemical parameters of the water and the associated fauna played significant roles in the variations of number of the species among the ascidian's community.

Keywords: Ascidians, Taxonomy, Suez Canal, physico-chemical parameters.

1. INTRODUCTION

Ascidians follow Phylum: Chordata and Class: Ascidiacea, called sea squirts and considered as the largest and most diverse class of the subphylum Tunicata, known also as Urochordata [1].

Ascidians have been recognized as a fascinating group in zoology, with their distinctive characteristics first described by Aristotle (around 350 B.C.), who considered them exceptional due to their protected body enclosed within a leathery shield tied to rocks and featuring two distant siphons [1]. Members of order Aplousobranchia are exclusively colonial, whereas the Phlebobranchia and Stolidobranchia orders contain both colonial and solitary species [2].

Traditional taxonomic studies on ascidians have spanned many years [3-6]. On the other hand, the most recent phylogeny and taxonomy of tunicate samples using 18S rRNA give raise to three clades for the Tunicata [7]: (1) Appendicularia, (2) Stolidobranch, and (3) Aplousobranch. There is a realistic thought

that colonial ascidians can overgrow rapidly on corals and outcompete them for space [8-11]. Ascidians filter any minute particulate matter [12, 13]. All three groups of the last clade show similarity in association of the gonads to the gut in harmony with the Enterogona classification [14]. Despite the valid position of the Appendicularia and the Thaliacea remains unresolved, the Appendicularia has been considered to be at the base of the Tunicata [15, 16], but molecular phylogenies recently put them as sister group to Stolidobranchia [7, 17]. Furthermore, molecular work based on ribosomal phylogenies suggests that Thaliacea, as general group, is more closely related to Phlebobranchia than to Aplousobranchia [7, 17].

Colonial and solitary ascidian species foul various artificial substrates successfully such as man-made substrata and jetties adjacent to the natural coral reef [9, 18, 19].

Some adverse factors may affect the life of these creatures, for example period of exposure to hydrodynamic alterations, solar radiation, temperature, rainfall, sediment, and depth as well as, some biological parameters, for example, presence of conspecifics, reproductive patterns and period, predation, and larval phototaxis and other possible factors may also influence ascidians, either separately or collectively, from the stage of emancipation of larvae reaching to the attachment stage then metamorphosis, and finally the growth on hard substratum [4-6, 20-24]

In spots of harsh conditions changing along seasonal variation, temperature of the water is the most important factor affecting markedly on the control of the reproductive period of ascidians. Except for species of the boreal region which reproduces in short time while the most of ascidians reproduces in short or moderately long periods in the summer [20]. Ascidians in subtropical waters show continuous reproductive periods, though gonadal activity are affected badly during winter [21, 23].

The Ascidian fauna of the Suez Canal has been the subject of detailed investigations at two distinct time intervals. The first study was conducted by Harant [25], who documented the samples collected during the Cambridge Expedition of 1924-1926 [26]. However, Por and Ferber [27] conducted a less comprehensive study on a limited number of samples from the middle segment of the Canal. Abdel Messeih [28] undertook the second investigation, focusing on the Suez Canal and the Egyptian Mediterranean waters. The findings of Abdel Messeih [28] were subsequently summarized by Halim, et al. [29] . Recent studies included molecular identification, bioactive, antimicrobial and cytotoxic activities of ascidians were also done [30, 31]. Worthily mentioning that the Suez Canal is an important cause for transportation of fauna including the non-indigenous species.

Aim of the current work is to identify the collected species of ascidians from the area of study (Port Fuad on the Suez Canal), using the traditional taxonomical characterizations, as well as to study the seasonal variations in number of species of the ascidian community and the related affecting factors, either biological or non-biological.

2. MATERIALS AND METHODS

2.1. Study area: The area of study is located directly on the most northern limit of the Suez Canal at Port Fuad (Fig.1). The site of collection is situated on the same line of the pier where the ferry anchors from concrete walls, wooden marinas, wooden poles, and fishing nets. It lies at coordinates of 31°15'19.7"N and 32°18'51.0"E. The total surveyed area is approximately 220 m^2 (11x20 m) in a horizontal submerged panel.

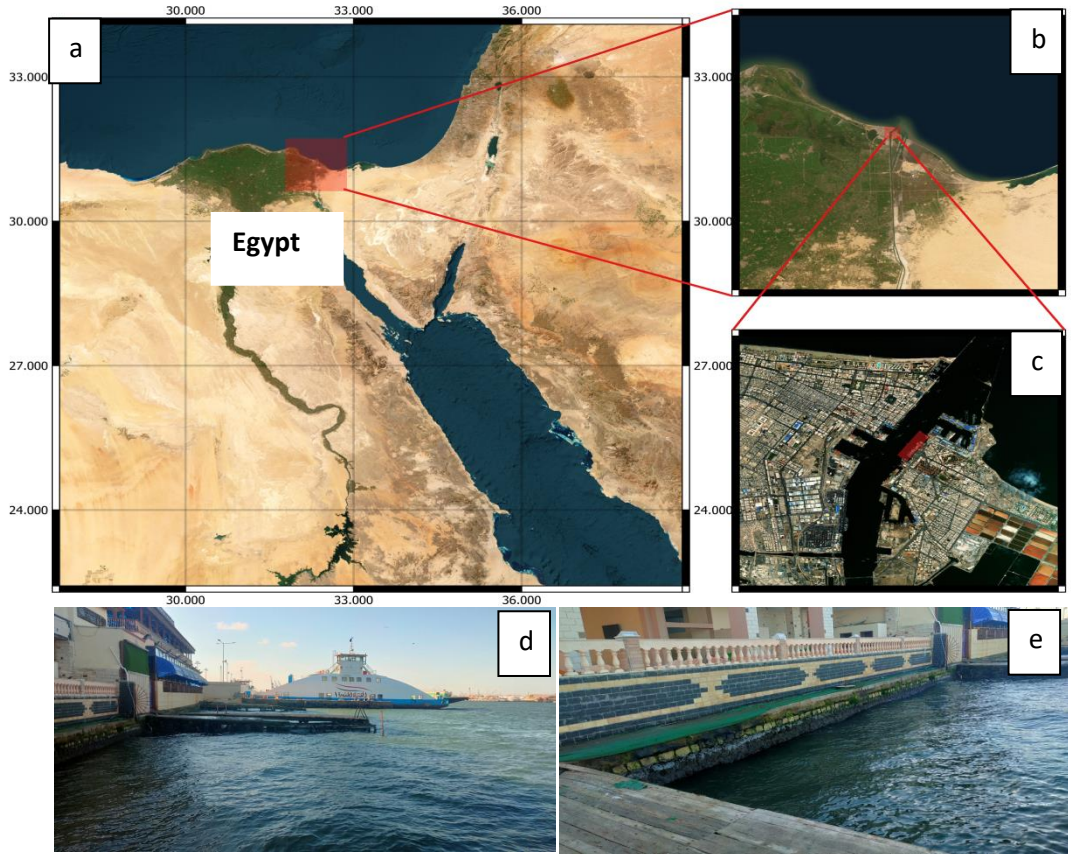

Figure (1): (a) The study area, **(b)** location of Port Said, **(c)** magnified position of the study area in Port Fuad, and **(d and e)** the sampling site.

2.2. Sampling: Sampling of ascidians and sea water physico-chemical parameters were seasonally done during the period from August 2023 to June 2024.

2.2.1. Water samples: Some physico-chemical parameters of sea water in the study area were measured comprised: depth (m), Temperature (°C), Dissolved oxygen (%) and Salinity (ppm).

2.2.2. Biological samples:

a. Collection: The fouling ascidian and its associated fauna were collected from <2m depth, using a hard, sharp knife to scrape off any underwater surfaces, e.g., man-made substrata, harbors, marinas, pillar, and fishing nets.

b. Narcotization and preparation**:** To narcotize the collected samples, $MgCl_2$ or crystal menthol were used in closed containers. To ensure full relaxation, test the response of the samples by examining their reaction by a pin or a rod. If the siphons do not shrink, this indicates that the organism is fully narcotized.

c. Preservation: A gradually changing in seawater using 70% alcohol or formalin 4 % to 10% solution was applied for further morphological examination.

d. Shooting: Photos for the wholemounts samples either solitary or colonial, were captured in the same day of sampling.

e. Identification: A stereo zoom microscope was utilized to examine external features, separate zooids from colonies, identifying samples, and capture images.

f.1. The dissection: The dissection methodology for internal identification differs according to the ascidians form. The solitary species dissection was carried out by making a longitudinal cut in the lateral line of the ascidians body parallel to the branchial siphon (oral siphon) in the left side view of the sample then. The sample becomes open wide to examine the internal organs. The colonial samples were dissected by putting the colony in a merged petri plate with water and making a slight cut in the tunic and shaking the colony slowly to extract the zooids [28].

f.2.Taxonomic identification: The taxonomic identification was carried out using the basic traditional references, as well as the authoritative keys and the international references [28, 32-34].

3. RESULTS

3.1. Sea water Physico-chemical parameters: During the course of this study, the seasonal physico-chemical sea water parameters were measured; temperature ranged from 20.7 to 29.3 (°C) in Winter and Summer, Salinity ranged from 26.63 to 37.00 ppm in summer and autumn and the dissolved oxygen ranged from 68.9 to 98.1 % in summer and winter respectively (Table 1).

Table (1). Results of some measured sea water physicochemical parameters during the period of study.

Parameters	**2023**		**2024**	
	Summer	**Autumn**	**Winter**	**Spring**
Depth (m)	< 2			
Temperature (°C)	29.30	25.00	20.70	27.50
DO (%)	68.90	84.50	98.10	75.00
Salinity (ppt)	26.63	37.00	34.50	32.50

3.2. Ascidian fauna: The examination revealed a total of 14 different ascidian species. These comprises; 4 species of solitary type, ranging in size from 0.5 to 7 cm, among them one species recorded for the first time in the study area (*Microcosmus Exasperatus* Heller, 1878) and 10 species of the colonial type ranging in size from 2 mm to 15 cm, among which five species were newly recorded in this study (*Aplidium* sp. Savigny, 1816, *Aplidium grisiatum* Kott, 1998, *Amaroucium accarense* Millar, 1953, *Polyclinum indicum* Sebastian, 1954; and *Didemnum perlucidum* Monniot F, 1983). Additionally, *Symplegma* sp. might be a new record if identified to species level.

The identified ascidians in this study could be affiliated to five families: Polyclinidae, Didemnidae, Styelidae, Pyuridae and Ascidiidae. Polyclinidae and Styelidae exhibited the highest diversity in terms of their total number of species, while Pyuridae and Ascidiidae were the least representative families (Fig. 2).

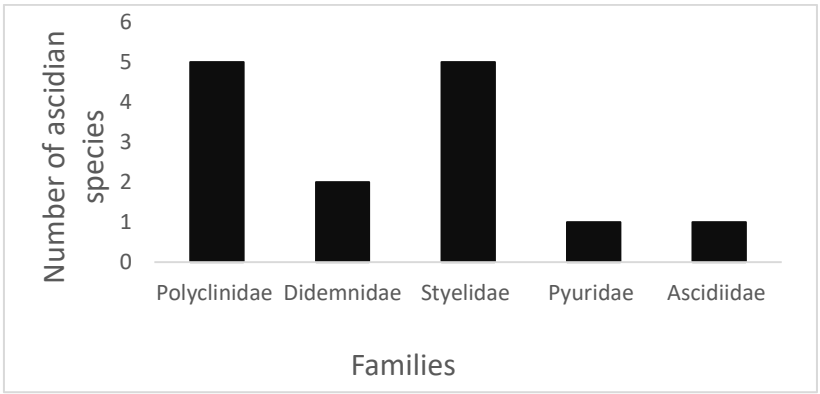

Figure 2. Count distribution of the ascidians' families in the period of study (2023–2024).

3.3 Community composition: Depending on the morphological taxonomy and the identification keys, the community of ascidian in this study consisted of: Aplidium sp. Savigny, 1816, Aplidium grisiatum Kott, 1998, Amaroucium accarense Millar, 1953, Polyclinum constellatum Savigny, 1816, Polyclinum indicum Sebastian, 1954; Didemnum perlucidum Monniot, 1983, Diplosoma listerianum (Milne Edwards, 1841), Ascidia mentula Müller, 1776, Styela plicata (Lesueur, 1823), Styela partita (Stimpson, 1852), Symplegma sp. Herdman, 1886, Symplegma viride Herdman, 1886, Botrylloides leachii (Savigny, 1816) and Microcosmus exasperatus Heller, 1878 (Fig. 3).

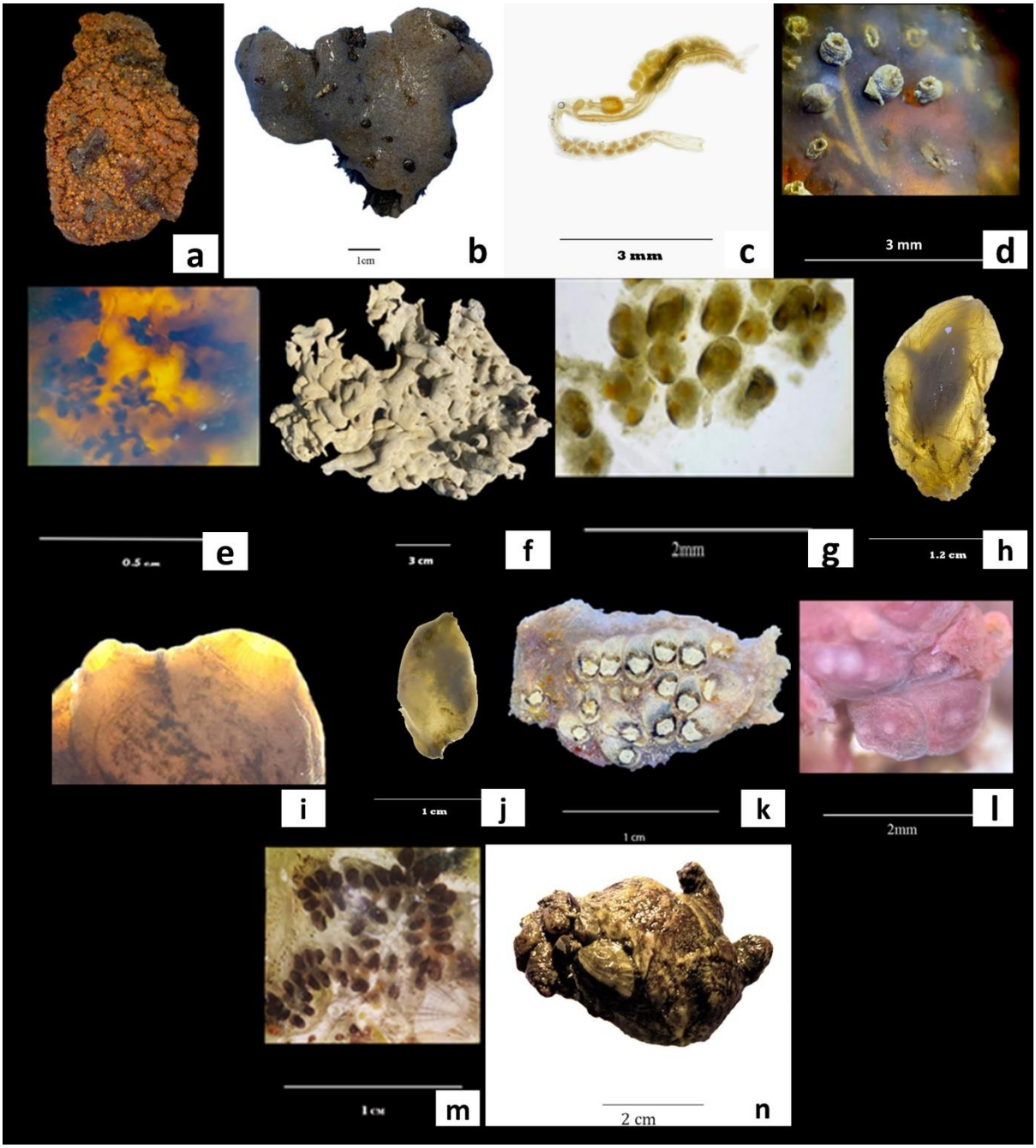

Figure 3. The identified 14 ascidian species in the study area: **(a)** *A. grisiatum*, **(b)** *Aplidium* sp, **(c)** *A. accarense* (zooid form), **(d)** *P. constellatum*, **(e)** *P. indicum*, **(f)** *D. perlucidum*, , **(g)** *D. listerianum*, **(h)** *A. mentula*, **(i)** *S. plicata*, **(j)** *S. partita*, **(k)** *Symplegma* sp, **(l)** *S. viride*, **(m)** *B. leachii*, and **(n)** *M. exasperatus*.

3.3. Seasonality: Seasonal variations in the number of ascidians were detected during the whole period of study (Fig. 4). It was noted that winter and spring 2024 exhibited the highest number of species (12 species for each), while the summer 2023 exhibited the lowest (8 species). Moreover, different species showed variable occurrences during the different seasons. Seasonal occurrences of the ascidians species during the period of study are represented in Table (2). *P. constellatum*, *D. perlucidum*, *S. plicata*; *S. partita*, *Symplegma* sp., *S, viride* and *M. Exasperatus* were observed throughout the entire period of the study. Another species, including *P. indicum*, *A. accarense*, and *Aplidium* sp., were recorded only during one season. *P. indicum* was observed in the summer, while *Aplidium* sp. was recorded in the winter, and *A. accarense* was observed in the spring (Table 2).

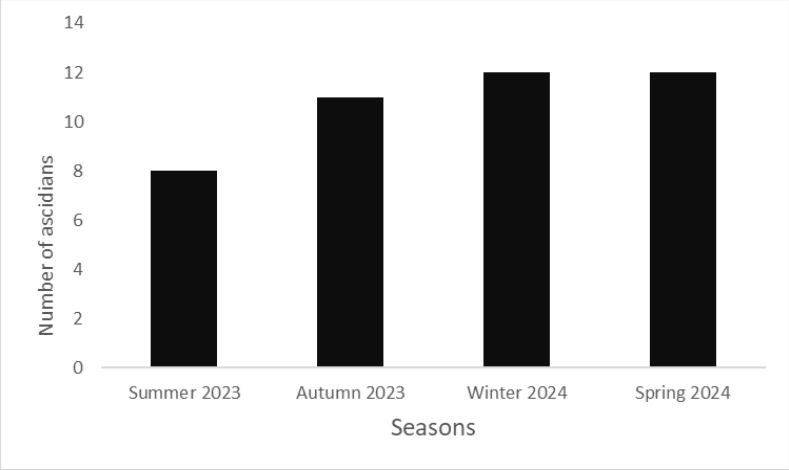

Fig. 4: Seasonal variations in ascidian numbers during the period of study.

Table (2): Seasonal fluctuations (presence + / absence -) of the studied ascidians during this study

Species / Seasons	2023		2024	
	Summer	**Autumn**	**Winter**	**Spring**
Phylum:				**Chordata**
Sub-phylum:				**Tunicata**
Class:				**Ascidiacea**
Order:				**Aplousobranchia**
Family: Polyclinidae				
Aplidium sp.	-	-	+	-
A. grisiatum	-	+	+	+
A. accarense	-	-	-	+
P. constellatum	+	+	+	+
P. indicum	+	-	-	-
Family: Didemnidae				
D. perlucidum	+	+	+	+
D. listerianum	-	+	+	+
Family: Ascidiidae				
A. mentula	-	+	+	+
Order:				**Stolidobranchia**
Family: Styelidae				
Subfamily: Styelinae				
S. plicata	+	+	+	+
S. partita	+	+	+	+
Subfamily Botrylliae				
Symplegma sp.	+	+	+	+
S. viride	+	+	+	+
B. leachii	-	+	+	+
Family: Pyuridae				
M. exasperatus	+	+	+	+
Total No. of species	**8**	**11**	**12**	**12**

4. DISCUSSION

In the sub-phylum Tunicata, class Ascidiacea represents the largest and most diversified groups. It comprises about three thousand known species that can be found in all marine environments, ranging from shallow water to the deep sea [35-37]. Order Aplousobranchia boasts the most abundant variety [38]. Eight species out of 14 followed Aplousobranchia which present more than 57% of the total number of species.

The colonial species represented more than 60% of the identified species [21]. The present study shows that the colonial species form about 70% of the total samples which are compatible with Rocha [21].

In the last century, a total of 26 ascidians species were recorded from 1924 to 2024 (the present study). Regarding the previous investigations, the first study on ascidian fauna of the Suez Canal was performed by Harant [25] during the Cambridge expedition in 1924. Compared with our findings, as well as the other previous studies, the surveyed locations included five sites in Port Said where 10 species of ascidians were recorded. After more than 65 years, Abdel Messeih [28] recorded 17 species in Port Said as well. Gab-Alla [39] recorded the colonial ascidian *Ecteinascidia thurstoni* as a new record in the Red Sea and the Suez Canal including Port Said location. Recently, Halim and Abdel Messeih [40] reported0 20 ascidians species from Port Said which considered the highest recorded number of species. In our study we recorded 14 ascidian species; six of which are newly recorded species, and one species is expected to be a new record in Port Said area. The occurrences: presence (+) / absence (-) of the ascidians species compared with the previous recorded are shown in (Table 3).

Table 3. Comparison between the present study and the previous ones depending on occurrence presence (+) / absence (-) of the recorded ascidian species in the study area.

Species	Previous studies			Present study 2023-2024[(4)]
	Harant 1927[(1)]	Abdel Messeih 1994[(2)]	Halim& Abdel esseih 2016[(3)]	
Amaroucium accarense Millar, 1953	-	-	-	+
Aplidium sp. Savigny, 1816	-	-	-	+
Aplidium grisiatum Kott, 1998	-	-	-	+
Ascidia cannelata Oken, 1820	-	+	+	-
Ascidia conchilega Müller, 1776	+	-	+	-
Ascidia mentula Müller, 1776	-	+	+	+
Ascidia obliqua Alder, 1863	-	+	+	-
Ascidiella aspersa (Müller, 1776)	+	-	+	-
Botrylloides leachii (Savigny, 1816)	-	-	+	+
Botryllus schlosseri (Pallas, 1766)	-	+	-	-
Ciona intestinals (Linnaeus, 1767)	-	+	-	-
Cnemidocarpa margaritifera Michaelsen, 1919	-	-	+	-

Didemnum amethystem (Van Name, 1902)	-	+	-	-
Didemnum candidum Savigny, 1816	+	+	+	-
Didemnum moseleyi (Herdman, 1886)	-	+	+	-
Didemnum perlucidum Monniot F., 1983	-	-	-	+
Diplosoma listerianum (Milne Edwards, 1841)	+	-	+	+
Distaplia magnilarva (Della Valle, 1881)	+	-	+	-
Distomus fuscus Delle Chiaje, 1841	+	-	-	-
Distomus variolosus Gaertner, 1774	-	-	+	-
Ecteinascidia thurstoni Herdman, 1890	-	-	+	-
Eusynstyela hartmeyeri Michaelsen, 1904	+	-	-	-
Herdmania momus (Savigny, 1816)	-	-	+	-
Macroclinum duboscqui Harant, 1927	-	+	-	-
Microcosmus sulcatus (Coquebert, 1797)	+	-	-	-
Microcosmus exasperatus Heller, 1878.	-	-	-	+
Molgula occidentalis Traustedt, 1883	-	+	-	-
Molgula siphonalis Kiaer, 1896	-	+	-	-
Perophora listeri var. senegalensis Pérès, 1951	+	+	+	-
Polyclinum aurantium Milne Edwards, 1841	-	+	+	-
Polyclinum constellatum Savigny, 1816	-	+	+	+
Polyclinum indicum Sebastian, 1954	-	-	-	+
Polysyncraton amethysteum Van Name, 1902	-	-	+	-
Styela partita (Stimpson, 1852)	+	+	-	+
Styela plicata (Lesueur, 1823)	-	+	-	+
Symplegma sp. Herdman, 1886	-	-	-	+
Symplegma brakenhielmi (Michaelsen, 1904)	-	-	+	-
Symplegma viride Herdman, 1886	-	+	-	+
Synoicum duboscqui (Harant, 1927)	-	-	+	-

[1] Samples collected during Cambridge expedition to Suez Canal in 1924 later in 1927, Tunicata group were identified by Harant [25].
[2] Abdel Messeih [28].
[3] Halim and Abdel Messeih [40].
[4] Present study 2023-2024.

Tropical areas exhibit the greatest species diversity, primarily composed of colonial species [38]. However, the Mediterranean Sea has significant temperature variations, both geographically from the Alborán to the Levantine Seas and temporally from tropical to moderate in the summer and winter which represents a suitable area for the introduction of species from different origins [41]. This is in coincidence with global climatic changes.

The highest number of ascidians (12 species) was recorded in winter and spring 2024, while the lowest (8 species) was recorded in summer 2023. This could be attributed to water temperature changes, Aydin-Onen [42] reported a negative relation between temperature (°C) and the number of ascidian species from the Aegean Coast of Turkey. In the current study, the maximum water temperature (29.3°C) is recorded in summer 2023 which is coupled with the minimum number of species of ascidian fauna (8 species). However, along the Suez Canal, the tropical *Ecteinascidia thurstoni* has been observed growing on metal pilings of jetties in habitats with salinities as high as 46 ppt, during autumn as indicated by Gab-Alla [39]. The present study exhibited the most elevated degree of salinity was 37 ppm in autumn (2023), the number of ascidian species was not affected severely with the high salinity and increased to 11 species.

In the current study, *Aplidium grisiatum* was the most dominant species among all the ascidians followed by *P. constellatum*, which was also a leading colonial encrusting species. The latter species was initially identified in Mauritius by Savignyii in 1816. While this finding points to an Indian Ocean origin, its native presence in the tropical Atlantic remains a possibility. This species was first recorded from Port Said, Egypt, by Abdel Messeih [28]. Few studies have been conducted on *A. grisiatum* and it exhibits no obvious invasive behavior; it remains mysterious. The only published information is that samples were recorded in the Northern Territory, Australia, according to the WoRMS site.

Based on the study of Aydin-Onen [42] in Turkey, within the Mediterranean Sea, six species have been identified as established non-indigenous: *P. constellatum*, *Ascidiella aspersa*, *Ciona robusta*, *M. exasperatus*, *S. plicata*, and *S. brakenhielmi*. Moreover, a number of ascidians are successful invaders in marine ecosystems [38, 43]. Additionally, *P. constellatum* is regarded as invasive species in the Turkish waters [42]. In this study *P. constellatum*, *S. plicata* and *M. exasperates* were recorded in all seasons.

Virgili *et al*. [44] studied the lake of Miseno (Central-Western Mediterranean Sea, Italy) as an overlooked reservoir of non-indigenous ascidians in a marine reserve. They indicated that the native communities are likewise thought to be seriously threatened by the renowned invader *Polyclinum* sp. [45, 46]. Moreover, some ascidian species, namely *A. accarense*, *P. constellatum*, *Botrylloides niger*, and *Polyandrocarpa zorritensis*, are well known to adapt to different environmental conditions [47-49]. This may explain the dominancy of *P. constellatum* in the present study.

According to Galil *et al*. [50] and Ashton *et al*. [51], the Suez Canal, shipping traffic, ballast water, fouling of ship hulls, aquaculture, and aquarium trade are likely the routes by which alien species of tropical/subtropical Indo-Pacific provenance that were found in Egypt, Palestine, and Turkey were introduced. Moreover, the movement of ascidian species can be facilitated by hull fouling, shipping operations, ballast water, and recreational yachts, boats, and fishing vessels [52].

In conclusion**,** during the current study, a total of 14 ascidian species were recorded in the northern limit of the Suez Canal at Fuad, Port Said, Egypt. These ascidians settled in this environment, influenced by both water physico-chemical and biological parameters. The ascidian community of Port Fuad shows variations in the number of species during the years 2023-2024 which might be attributed to water temperature variations. Meanwhile, the dominance of the colonial ascidian *P. constellatum* is primarily due to its invasiveness and well-known adaptation to different environmental conditions**.**

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6. REFERENCES

[1] E. Voultsiadou, M.-M. Pyrounaki, and C. Chintiroglou, "The habitat engineering tunicate Microcosmus sabatieri Roule, 1885 and its associated peracarid epifauna," *Estuarine, Coastal and Shelf Science,* vol. 74, no. 1-2, pp. 197-204, 2007.

[2] L. Zeng and B. J. Swalla, "Molecular phylogeny of the protochordates: chordate evolution," *Canadian Journal of Zoology,* vol. 83, no. 1, pp. 24-33, 2005.

[3] H. Harant and P. Vernières, "Faune de France 27. Tuniciers fascicule 1: Ascidies," ed: Paris: Paul Lechevalier, 1933.

[4] R. H. Millar, "The annual growth and reproductive cycle in four ascidians," *Journal of the Marine Biological Association of the United Kingdom,* vol. 31, no. 1, pp. 41-61, 1952.

[5] B. Rinkevich, R. Porat, and M. Goren, "Ecological and Life History Characteristics of Botryllus schlosseri Tunicata Populations Inhabiting Undersurface Shallow-Water Stones," *Marine Ecology,* vol. 19, no. 2, pp. 129-145, 1998.

[6] M. G. Hadfield and V. J. Paul, "Natural chemical cues for settlement and metamorphosis of marine invertebrate larvae," *Marine chemical ecology,* vol. 13, no. 431.461, 2001.

[7] G. Tsagkogeorga *et al.*, "An updated 18S rRNA phylogeny of tunicates based on mixture and secondary structure models," *BMC evolutionary biology,* vol. 9, pp. 1-16, 2009.

[8] R. Bak, D. Lambrechts, M. Joenje, G. Nieuwland, and M. Van Veghel, "Long-term changes on coral reefs in booming populations of a competitive colonial ascidian," *Marine Ecology Progress Series,* vol. 133, pp. 303-306, 1996.

[9] N. Shenkar, O. Bronstein, and Y. Loya, "Population dynamics of a coral reef ascidian in a deteriorating environment," *Marine Ecology Progress Series,* vol. 367, pp. 163-171, 2008.

[10] B. Vargas-Ángel, L. Godwin, J. Asher, and R. Brainard, "Invasive didemnid tunicate spreading across coral reefs at remote Swains Island, American Sāmoa," *Coral Reefs,* vol. 28, pp. 53-53, 2009.

[11] B. Sommer, P. L. Harrison, and S. Scheffers, "Aggressive colonial ascidian impacting deep coral reefs at Bonaire, Netherlands Antilles," *Coral Reefs,* vol. 29, pp. 245-245, 2010.

[12] R. Bak, M. Joenje, I. De Jong, D. Lambrechts, and G. Nieuwland, "Bacterial suspension feeding by coral reef benthic organisms," *Marine Ecology Progress Series,* vol. 175, pp. 285-288, 1998.

[13] Q. Bone, C. Carre, and P. Chang, "Tunicate feeding filters," *Journal of the Marine Biological Association of the United Kingdom,* vol. 83, no. 5, pp. 907-919, 2003.

[14] E. Perrier, "Note sur la classification des Tuniciers," *CR Acad Sci Paris,* vol. 124, pp. 1758-1762, 1898.

[15] H. Wada, H. Saiga, N. Satoh, and P. W. Holland, "Tripartite organization of the ancestral chordate brain and the antiquity of placodes: insights from ascidian Pax-2/5/8, Hox and Otx genes," *Development,* vol. 125, no. 6, pp. 1113-1122, 1998.

[16] B. J. Swalla, C. B. Cameron, L. S. Corley, and J. R. Garey, "Urochordates are monophyletic within the deuterostomes," *Systematic Biology,* vol. 49, no. 1, pp. 52-64, 2000.

[17] L. Zeng, M. W. Jacobs, and B. J. Swalla, "Coloniality has evolved once in stolidobranch ascidians," *Integrative and Comparative Biology,* vol. 46, no. 3, pp. 255-268, 2006.

[18] G. Paulay, L. Kirkendale, G. Lambert, and J. Starmer, "The marine invertebrate biodiversity of Apra Harbor: significant areas and introduced species, with focus on sponges, echinoderms and ascidians," *Draft Report Prepared for US Dept. of Defense, COMNAVMARIANAS,* 1997.

[19] U. Oren and Y. Benayahu, "Didemnid ascidians: rapid colonizers of artificial reefs in Eilat (Red Sea)," *Bulletin of Marine Science,* vol. 63, no. 1, pp. 199-206, 1998.

[20] R. Millar, "The breeding season of some littoral ascidians in Scottish waters," *Journal of the Marine Biological Association of the United Kingdom,* vol. 37, no. 3, pp. 649-652, 1958.

[21] R. M. d. Rocha, "Replacement of the compound ascidian species in a southeastern Brazilian fouling community," *Boletim do Instituto Oceanográfico,* vol. 39, pp. 141-153, 1991.

[22] R. W. Osman and R. B. Whitlatch, "The influence of resident adults on recruitment: a comparison to settlement," *Journal of Experimental Marine Biology and Ecology,* vol. 190, no. 2, pp. 169-198, 1995.

[23] R. M. d. Rocha, T. M. d. C. Lotufo, and S. d. A. Rodrigues, "The biology of Phallusia nigra Savigny, 1816 (Tunicata: Ascidiacea) in southern Brazil: spatial distribution and reproductive cycle," *Bulletin of Marine Science,* vol. 64, no. 1, pp. 77-88, 1999.

[24] R. B. Forward Jr, J. M. Welch, and C. M. Young, "Light induced larval release of a colonial ascidian," *Journal of experimental marine biology and ecology,* vol. 248, no. 2, pp. 225-238, 2000.

[25] H. Harant, "Rapport sur les Tuniciers. Par," *The Transactions of the Zoological Society of London,* vol. 22, no. 3, pp. 365-373, 1927.

[26] H. M. Fox, "Cambridge expedition to the Suez Canal, 1924," *The Transactions of the Zoological Society of London,* vol. 22, no. 1, pp. 1-64, 1926.

[27] F. Por and I. Ferber, "THE HEBREW UNIVERSITY—SMITHSONIAN INSTITUTION COLLECTIONS FROM THE SUEZ CANAL (1967–1972)," *Israel Journal of Zoology,* vol. 21, no. 3-4, pp. 149-166, 1972.

[28] M. Abdel Messeih, "Taxonomical studies on Ascidians in the Egyptian Mediterranean waters and the Suez Canal," *Unpublished Ph. D. thesis) Faculty of Science, University of Alexandria, Alexandria, Egypt,* 1994.

[29] Y. Halim, M. Abdel Messeih, M. Mikhail, and M. Shabana, "Ascidian fauna of the Suez Canal " *Rapp. Comm. int. Mer Médit.,* vol. 34, 1995.

[30] A. Metwally, S. A. Elgendy, T. A. Temraz, and M. I. Ahmed, "Antimicrobial and Cytotoxic activities of Ascidian Species Collected from Egyptian Coasts," *Bulletin of Faculty of Pharmacy Cairo University,* vol. 59, no. 1, pp. 64-75, 2021.

[31] A. Metwally, S. A. Elgendy, T. A. Temraz, and M. I. Ahmed, "Molecular Identification of Selected Ascidians from Egypt using COI," *Egyptian Journal of Aquatic Biology and Fisheries,* vol. 26, no. 1, pp. 313-326, 2022.

[32] R. H. Millar, "British ascidians, Tunicata: Ascidiacea; keys and notes for the identification of the species," *(No Title),* 1970.

[33] M. Abdel Messeih, "Studies of ascidians in Alexandria waters," MSc thesis. University of Alexandria, 1982.

[34] F. Monniot and C. Monniot, *Ascidians from the tropical western Pacific*. Publications Scientifique du Museum, Paris, 2001.

[35] C. Monniot, F. Monniot, and P. Laboute, *Coral reef ascidians of New Caledonia* (no. 30). IRD Editions, 1991.

[36] C. B. Cameron, J. R. Garey, and B. J. Swalla, "Evolution of the chordate body plan: new insights from phylogenetic analyses of deuterostome phyla," *Proceedings of the National Academy of Sciences*, vol. 97, no. 9, pp. 4469-4474, 2000.

[37] P. Kott, *Catalogue of Tunicata in Australian waters*. Australian Biological Resources Study Canberra, 2005.

[38] N. Shenkar and B. J. Swalla, "Global diversity of Ascidiacea," *Plos one*, vol. 6, no. 6, p. e20657, 2011.

[39] A.-F. Gab-Alla, "Distribution of the sea squirt Ecteinascidia thurstoni Herdman, 1890 (Ascidiacea: Perophoridae) along Suez Canal and Egyptian Red Sea Coasts," *Oceanologia*, vol. 50, no. 2, pp. 239-253, 2008.

[40] Y. Halim and M. Abdel Messeih, "Aliens in Egyptian waters. A checklist of ascidians of the Suez Canal and the adjacent Mediterranean waters," *The Egyptian Journal of Aquatic Research*, vol. 42, no. 4, pp. 449-457, 2016.

[41] A. Izquierdo Muñoz, M. Díaz Valdés, and A. A. Ramos-Esplá, "Recent non-indigenous ascidians in the Mediterranean Sea," *Aquatic Invasions*, vol. 4, no. 1, pp. 59-64, 2009.

[42] S. Aydin-Onen, "Distribution of ascidians with a new record of the non-indigenous species Polyclinum constellatum Savigny, 1816 from the Aegean coast of Turkey," *Turkish Journal of Fisheries and Aquatic Sciences*, vol. 18, no. 9, 2018.

[43] A. Zhan, E. Briski, D. G. Bock, S. Ghabooli, and H. J. MacIsaac, "Ascidians as models for studying invasion success," *Marine Biology*, vol. 162, pp. 2449-2470, 2015.

[44] R. Virgili *et al.*, "The Miseno Lake (central-western Mediterranean Sea): an overlooked reservoir of non-indigenous and cryptogenic ascidians in a marine reserve," *Frontiers in Marine Science*, vol. 9, p. 866906, 2022.

[45] M. A. Tovar-Hernández, E. Suarez-Morales, and B. Yáñez-Rivera, "The parasitic copepod Haplostomides hawaiiensis (Cyclopoida) from the invasive ascidian Polyclinum constellatum in the southern Gulf of California," *Bulletin of Marine Science*, vol. 86, no. 3, pp. 637-648, 2010.

[46] M. Govindharaj, N. S. Al Hashemi, S. S. Soman, and S. Vijayavenkataraman, "Bioprinting of bioactive tissue scaffolds from ecologically-destructive fouling tunicates," *Journal of Cleaner Production*, vol. 330, p. 129923, 2022.

[47] W. Van Name, "The north and south American ascidians," *Bulletin of the American Museum of Natural History*, vol. 84, pp. 1-476, 1945.

[48] F. Mastrototaro and R. Brunetti, "The non-indigenous ascidian Distaplia bermudensis in the Mediterranean: comparison with the native species Distaplia magnilarva and Distaplia lucillae sp. nov," *Journal of the Marine Biological Association of the United Kingdom*, vol. 86, no. 1, pp. 181-185, 2006.

[49] F. Mastrototaro, G. D'Onghia, and A. Tursi, "Spatial and seasonal distribution of ascidians in a semi-enclosed basin of the Mediterranean Sea," *Journal of the Marine Biological Association of the United Kingdom*, vol. 88, no. 5, pp. 1053-1061, 2008.

[50] A. Zenetos *et al.*, "Alien species in the Mediterranean Sea by 2010. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part I. Spatial distribution," 2010.

[51] B. S. Galil, A. Marchini, and A. Occhipinti-Ambrogi, "East is east and West is west? Management of marine bioinvasions in the Mediterranean Sea," *Estuarine, Coastal and Shelf Science,* vol. 201, pp. 7-16, 2018.

[52] G. Ashton, K. Boos, R. Shucksmith, and E. Cook, "Rapid assessment of the distribution of marine non-native species in marinas in Scotland," *Aquatic Invasions,* vol. 1, no. 4, pp. 209-213, 2006.