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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.

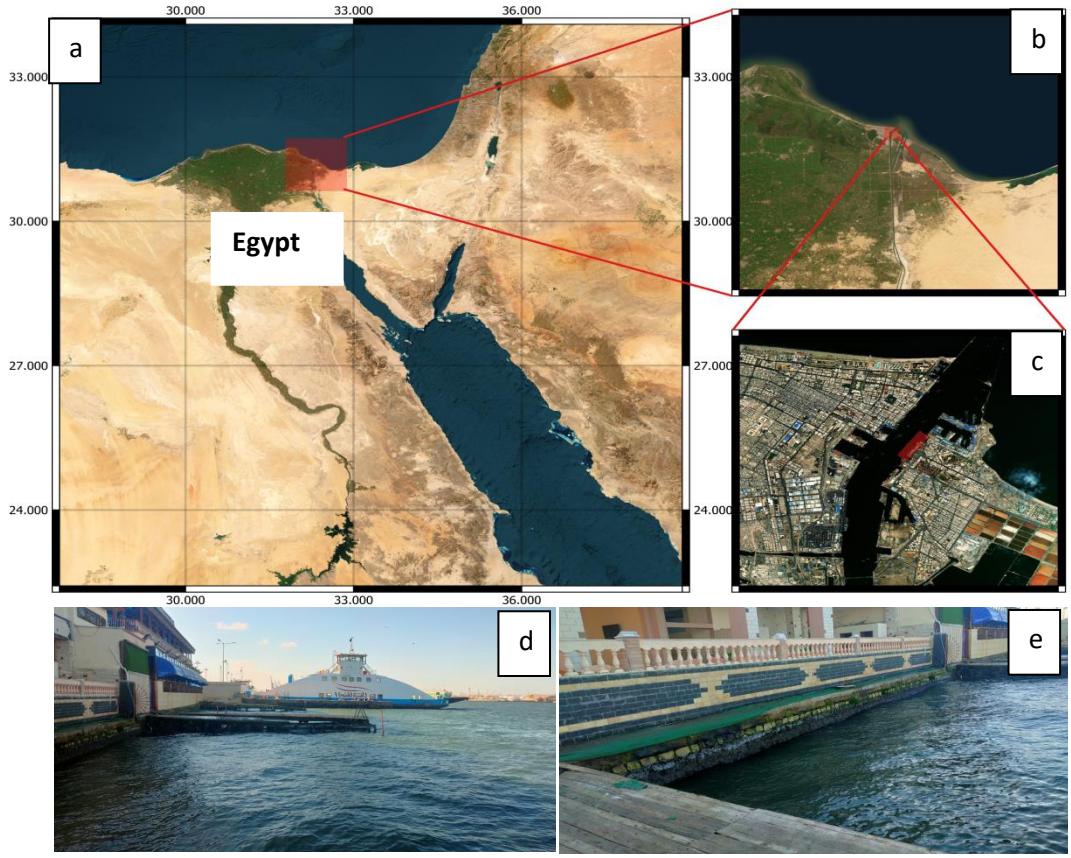

Figure (1): (a) The study area, **(b)** location of Port Said, **(c)** magnified position of the study area in Port Fuad, and **(d and e)** the sampling site.

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).

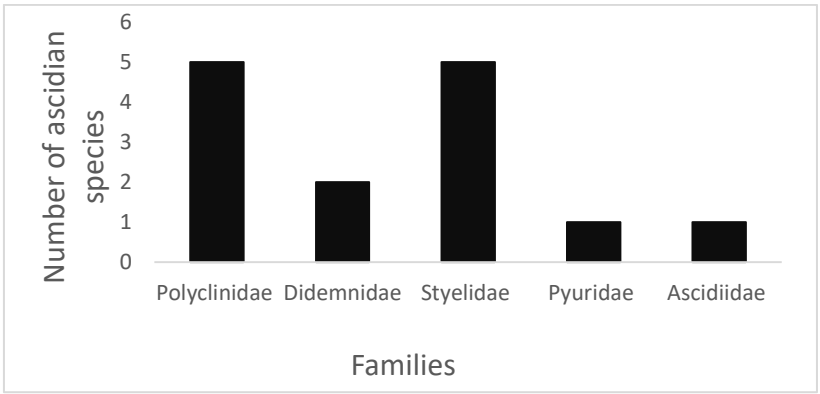

Figure 2. Count distribution of the ascidians' families in the period of study (2023–2024).

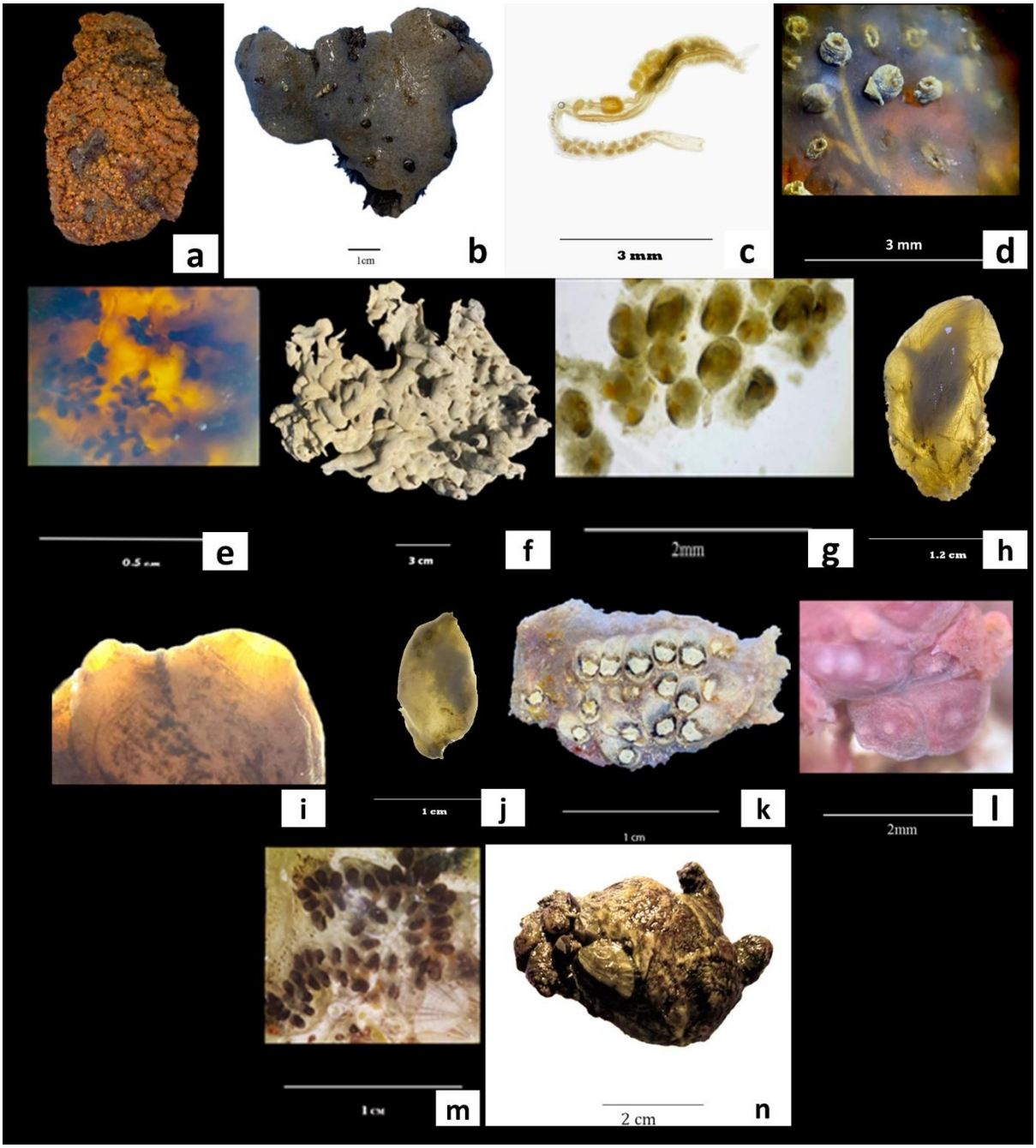

Figure 3. The identified 14 ascidian species in the study area: **(a)** *A. grisiatum*, **(b)** *Aplidium* sp, **(c)** *A. accarense* (zooid form), **(d)** *P. constellatum*, **(e)** *P. indicum*, **(f)** *D. perlucidum*, , **(g)** *D. listerianum*, **(h)** *A. mentula*, **(i)** *S. plicata*, **(j)** *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).

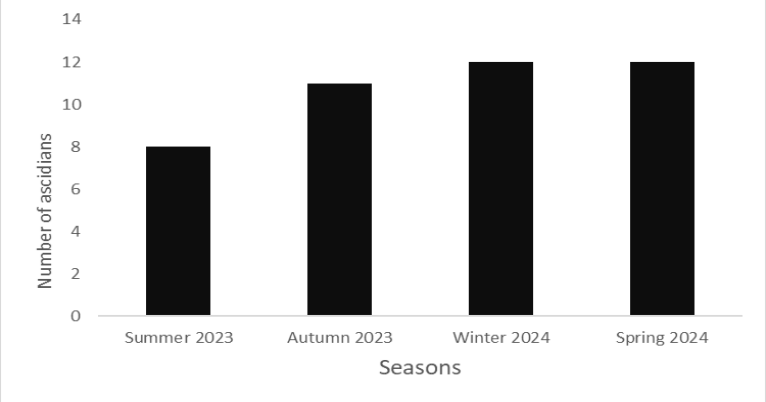

Fig. 4: Seasonal variations in ascidian numbers during the period of study.

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				
1- Family: Polyclinidae				
P. constellatum	+	+	+	+
P. indicum	+	-	-	-
A. grisiatum	-	+	+	+
A. accarense	-	-	-	+
Aplidium sp.	-	-	+	-
2- Family: Didemnidae				
D. perlucidum	+	+	+	+
D. listerianum	-	+	+	+
Order: Phlebobranchia				
3- Family: Ascidiidae				
A. mentula	-	+	+	+
Order: Stolidobranchia				
4- Family: Styelidae (Subfamily: Styelinae)				
S. plicata	+	+	+	+
S. partita	+	+	+	+
4- Family: Styelidae (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]. Seven species out of 14 followed Aplousobranchia which present 50 % 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 Messeih 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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