Distribution of the sea squirt Ecteinascidia thurstoni Herdman, 1890 (Ascidiacea: Perophoridae) along Suez Canal and Egyptian Red Sea coasts*

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KEYWORDS Distribution Ascidian Tunicate Sea squirt *Ecteinascidia thurstoni* Red Sea Suez Canal

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Abstract

Ecteinascidia thurstoni is a colonial sea squirt. It has a seasonal rhythm and a tropical and subtropical distribution; it is usually present during the summer months. It synthesizes a group of molecules called ecteinascidins. One of these is ET-743, a compound that has a most original anti-tumoral activity and is today considered to be one of the most promising substances effective against various solid-type tumors (currently sold under the trade name of Yondelis for the treatment of sarcomas and related tumors; it is undergoing phase II/III clinical trails for other kinds of tumors). Worldwide, Ecteinascidia species represent the only available source of this bioactive compound, which was first discovered in E. turbinata. During the present study, the ecology of E. thurstoni along the Suez Canal and Red Sea was investigated. Its populations were observed to be highly gregarious due in part to their low larval dispersal, which is very localized; larvae therefore tend to settle close to their parent colonies. It is only recorded in shallow

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waters (0.5–1.5 m) as an epiphyte on the pneumatophores of mangroves by the Red Sea, on the pilings of jetties, and the metal or cement banks of the Suez Canal. The morphometric characteristics (zooid length, zooid weight, colony weight) of the Suez Canal population differ significantly from those of the Red Sea. Studying the distribution of this species and locating its different populations along the Suez Canal and Red Sea could help to characterize their genetics, chemistry and bacterial communities at different isolated locations. Ultimately, this will help to define the sources of ET-743 and hence promote its biosynthesis on a commercial scale.

1. Introduction

Many marine invertebrates such as sponges and ascidians (Rinehart 2000), not traditionally exploited as fisheries resources, have recently attracted increased attention because of their great economic potential as regards the production of compounds with pharmaceutical properties (Carballo et al. 2000, Rinehart 2000).

Ecteinascidia species, which are colonial tunicates from the family Perophoridae, are an important component of the benthic fauna of mangroves (Carballo 2000b, Hernández-Zanuy et al. 2007). Ecteinascidia turbinata has been the subject of various studies undertaken to examine its settlement, species succession, and larval behavior (Young & Bingham 1987, Bingham & Young 1991, 1995). In the 1960s, interest in this species was heightened when an extract of the animal was found to have potent cytotoxic properties due to the ecteinascidins it contained; they were identified and characterized by Rinehart et al. (1990). Little is known about the production of these secondary metabolites or what function, if any, they play in the animal (Moss et al. 2003). One of these metabolites – ecteinascidins – synthesized by E. turbinata is ET-743, a compound with a most original anti-tumoral activity. It is today considered to be one of the most promising substances effective against various solid-type tumors (Rinehart et al. 1990, Garcia-Rocha et al. 1996, Jimeno et al. 1996). It has recently come onto the market under the trade name of Yondelis for the treatment of sarcomas and related tumors; it is also undergoing clinical trails (phases II/III) for other kinds of tumors (Pharmamar 2008).

The supply of ecteinascidins for clinical development has been maintained over a period of several years by the sustainable harvesting of *E. turbinata*, an example of the production of metabolites while protecting natural populations (Carballo et al. 1999). Harvesting of this tunicate in large quantities was possible with the establishment of a rational extraction program in diverse habitats, as is the case with a lot of shellfish for human consumption. In order to find out the recovery capacity of *E. turbinata* populations, a research program involving harvesting experiments in the Caribbean and Mediterranean Sea was carried out (Carballo et al. 1999). PharmaMar, a Spanish pharmaceutical company, produced ecteinascidins for its clinical studies from an aquaculture in a natural environment in quantities of > 70 tonnes per year up to 2000 (Carballo et al. 2000, Naranjo et al. 2001). Now, however, production through collection or aquaculture is no longer necessary or expensive. The production of Yondelis is now completely synthetic: neither natural resources nor aquaculture activities are involved (Cuevas et al. 2000). The synthesis of this compound takes place in a multi-step process, and the cost of reagents is affordable on a small scale; large-scale synthesis for commercial purposes is, however, still too expensive.

A recent investigation of secondary metabolites in the tunicate *Ecteinascidia thurstoni*, pretreated with potassium cyanide, highlighted its pharmaceutical and medicinal importance: the ecteinascidins, ET-743, ET-770, ET-729 and ET-759 were present in samples collected from the Suez Canal and the Red Sea in 2005 (Youssef 2007, unpublished). A group of alkaloids – ecteinascidins (ET) including ET-770 and ET-786 – were isolated with very high yields from *E. thurstoni* (Phuket Province, on the Andaman Sea coast of southern Thailand) pretreated with potassium cyanide. These extracts exhibited potent cytotoxic activity against cancerous cells in breast, lung, colorectal and nasopharyngeal tissues (Suwanborirux et al. 2002). These findings imply that there are other types of ecteinascidins, not just ET-743, which could be of potential therapeutic value for the treatment of other diseases.

The aim of the present work was to study the ecology of the tunicate *E. thurstoni* along the Suez Canal and Red Sea: to describe its distribution pattern in different habitats, to locate different populations along the shore, and to measure the morphometric characteristics of these populations. This information will be helpful for studying the genetics, chemistry and bacterial communities of different *Ecteinascidia* populations along the Suez Canal and Red Sea. Ultimately, this will aid the further laboratory development of the compound and hence its biosynthesis on an economically viable scale.

2. Material and methods

The present study was carried out along the coasts of the Suez Canal and the Egyptian Red Sea. The coasts of the Suez Canal are sedimentary, whereas Red Sea shores are mainly rocky. A pilot survey was carried out in summer 2005, during which different habitats were surveyed by snorkeling. These included the pilings of jetties, metal and cement banks, and sea grasses along the Suez Canal, as well as the coral reefs, sea grasses, mangroves and rocky shores of the Red Sea. This pilot survey showed that the cement and metal banks, the pilings of jetties along the Suez Canal, and also the mangroves along Red Sea are suitable habitats for the tunicate *Ecteinascidia thurstoni*. Growth usually takes place during the summer months from late May to early October. The species then usually disappears during autumn and winter, since ecteinascidians are species characteristic of warm tropical waters, environmental conditions (especially temperature and salinity) that are suitable for its survival in natural shallow waters.

After the summer 2005 pilot survey, intensive surveys were carried out during the subsequent summers of 2006 and 2007 to study the ecology of this species by snorkeling in different habitats along the Suez Canal and Red Sea. Figure 1 and Tables 1 and 2 present the study sites and their GPS positions.



Figure 1. Sites surveyed along the Suez Canal (a) and Red Sea (b)

 $\ensuremath{\textbf{Table 1.}}$ Table 1. The sites surveyed along the Suez Canal and their GPS positions

No.	Site	Position		
1	Port Said	$31^{\circ}13'\mathrm{N}$	$32^{\circ}21'\mathrm{E}$	
2	El-Kantara	$31^\circ 51' \mathrm{N}$	$32^{\circ}18'\mathrm{E}$	
3	El-Danvah Club	$30^\circ 33' \mathrm{N}$	$32^{\circ}18'\mathrm{E}$	
4	El-Ta'awen	$30^{\circ}24'\mathrm{N}$	$22^{\circ}18'\mathrm{E}$	
5	Deversoir	$30^{\circ}24'\mathrm{N}$	$32^{\circ}21'\mathrm{E}$	
6	Abu Sultan	$30^{\circ}22'\mathrm{N}$	$32^{\circ}18'\mathrm{E}$	
7	Fayed	$30^{\circ}19'\mathrm{N}$	$32^{\circ}19'\mathrm{E}$	
8	Fanara	$30^\circ 17' \mathrm{N}$	$32^{\circ}18'\mathrm{E}$	
9	Kabreet	$30^{\circ}15'\mathrm{N}$	$32^{\circ}28'\mathrm{E}$	

Table 2. The sites surveyed along the Red Sea and their GPS positions

No.	Site	Position		
1	El-Monkata'a	$28^{\circ}12'N$	$34^{\circ}25'\mathrm{E}$	
2	El-Rawisia	$28^{\circ}11'\mathrm{N}$	$34^{\circ}27'\mathrm{E}$	
3	Marsa Abu-Zabad	$28^{\circ}09'\mathrm{N}$	$34^{\circ}27'\mathrm{E}$	
4	El-Ghargana	$28^{\circ}07'\mathrm{N}$	$34^{\circ}27'\mathrm{E}$	
5	Ras Mohamed	$27^{\circ}44'\mathrm{N}$	$34^\circ 15' \mathrm{E}$	
6	Al-Gonah	$27^{\circ}24'\mathrm{N}$	$33^{\circ}41'\mathrm{E}$	
7	Abu-Monkar	$27^{\circ}13'\mathrm{N}$	$33^\circ 52' \mathrm{E}$	
8	South Safaga	$25^{\circ}38'\mathrm{N}$	$33^\circ 59' \mathrm{E}$	
9	Wadi Abu-Hamrah	$26^{\circ}21'\mathrm{N}$	$34^{\circ}09'\mathrm{E}$	
10	Sharm El-Bahari	$25^{\circ}52'\mathrm{N}$	$34^{\circ}24'\mathrm{E}$	
11	Sharm El-Qebly	$25^{\circ}50'\mathrm{N}$	$34^{\circ}26'\mathrm{E}$	
12	Marsa Sagara	$25^{\circ}40'\mathrm{N}$	$34^{\circ}35'\mathrm{E}$	
13	Wadi El-Gemal	$24^{\circ}40'\mathrm{N}$	$35^{\circ}05'\mathrm{E}$	
14	Wadi El-Gemal Island	$24^{\circ}40'\mathrm{N}$	$35^{\circ}10'\mathrm{E}$	
15	Ras Baghdadi	$24^{\circ}39'\mathrm{N}$	$35^{\circ}06'\mathrm{E}$	
16	Wadi Masturah	$24^{\circ}23'\mathrm{N}$	$35^{\circ}16'\mathrm{E}$	
17	Wadi Qala'an	$24^{\circ}21'\mathrm{N}$	$35^{\circ}19'\mathrm{E}$	
18	Wadi Rawad El-Adaiah	$24^{\circ}20'\mathrm{N}$	$35^{\circ}20'\mathrm{E}$	
19	Wadi El-Ra'da	$24^{\circ}19'\mathrm{N}$	$35^{\circ}20'\mathrm{E}$	
20	Shawareet Island	$24^{\circ}21'\mathrm{N}$	$35^{\circ}24'\mathrm{E}$	
21	Wadi Lahmy	$24^{\circ}13'\mathrm{N}$	$35^{\circ}26'\mathrm{E}$	
22	Qora'at Hartway	$24^{\circ}06'\mathrm{N}$	$35^{\circ}30'\mathrm{E}$	
23	El-Hamirah Mangrove	$23^{\circ}29'\mathrm{N}$	$35^{\circ}29'\mathrm{E}$	
24	Shalateen Island	$23^{\circ}08'N$	$35^{\circ}41'\mathrm{E}$	
25	Sharm El-Madfa'a	$22^{\circ}57'N$	$35^{\circ}40'\mathrm{E}$	
26	Marsa Sha'ab	$22^{\circ}50'N$	$35^{\circ}45'\mathrm{E}$	
27	Marsa Abu-Fasi	$22^{\circ}41'\mathrm{N}$	$36^{\circ}00'\mathrm{E}$	
28	El-Hoor	$22^{\circ}38'\mathrm{N}$	$36^{\circ}13'\mathrm{E}$	
29	Adal Deep	$22^{\circ}33'\mathrm{N}$	$36^{\circ}17'\mathrm{E}$	

At the surveyed sites, ecological data including depth, salinity [PSU], temperature [°C], pH and oxygen concentration [mg dm⁻³], as well as the presence or absence of *E. thurstoni* were recorded in situ. At the sites where *E. thurstoni* was present, samples were collected and kept fresh in tanks containing sea water. Back at the laboratory (Marine Sciences Department at Ismailia, Sharm El-Sheikh Environmental School, Marine Parks at Hurghada), other biological parameters were measured: maximum colony diameter [mm], maximum number of zooids per colony, maximum weight of colony [g], maximum length of zooid [mm], and maximum weight of zooid [g]. These data were used to evaluate the sites with larger populations of *E. thurstoni*.

For the data analysis, means and standard deviations were calculated for replicate determinations of the various parameters investigated. Analysis of variance (one-way ANOVA) was used to evaluate the significance of differences between groups of measured parameters at different stations with the significance level set at $p \leq 0.05$.

3. Results

The environmental conditions (temperature, salinity, oxygen content and pH) recorded along the Suez Canal (Table 3) and Red Sea coast (Table 4) during the investigation period were more or less typical. The first value in the following sets refers to the sites along the Suez Canal, the second to the Red Sea sites: temperature – no less than $27 \pm 2^{\circ}$ C and $27 \pm 1^{\circ}$ C; salinity – no less than 42 ± 1 PSU and 42 ± 2 PSU; oxygen content 5.2 ± 0.5 mg dm⁻³ and 5.9 ± 0.3 mg dm⁻³ (in situ measurements showed all the sites surveyed to be well aerated); pH – 7.2 ± 0.4 and 7.5 ± 0.3 (readings were normally slightly higher than neutral). Statistical analysis (one-way ANOVA) of these four parameters (Table 7, see page 249) showed no significant differences between the different sites.

Examination of the collected specimens showed that a colony of *Ecteinascidia thurstoni* (Figure 2a) consists of thick clusters of individual zooids connected at their bases by a network of stolons adhering to the surface of the object, e.g. the pneumatophores of mangroves, on which the colony grows. Figure 2b shows that the community of *E. thurstoni* in the Suez Canal usually consists of large colonies, whereas the Red Sea community consists of small colonies at most sites, as shown in Figure 2d. Nonetheless, medium-size or dense colonies were recorded at Wadi El-Ra'ada, as shown in Figures 2e and 2f, respectively.

During the field survey in summer 2005, *E. thurstoni* was found in mangrove habitats of the Red Sea, whereas in other habitats – coral reefs,

No.	Site	Salinity [PSU]	Temp. [°C]	рН	Oxygen [mg dm ⁻³]	Habitat	E. thurstoni present/ absent
1	Port Said	43 ± 1	27 ± 1	7.5 ± 0.3	6.5 ± 0.3	cement banks of Suez Canal	_
2	El-Kantara	43 ± 1	27 ± 1	7.9 ± 0.3	6.9 ± 0.4	cement banks of Suez Canal	_
3	El-Danva Club	42 ± 2	28 ± 2	8.2 ± 0.2	6.2 ± 0.4	cement banks of Suez Canal	+
4	Tosun	43 ± 1	27 ± 1	7.9 ± 0.2	7.1 ± 0.3	metal pilings of jetties	+
5	Deversoir	44 ± 2	28 ± 1	7.8 ± 0.2	7.5 ± 0.3	metal pilings of jetties	+
6	Abu -Sultan	43 ± 1	27 ± 1	7.8 ± 0.3	6.9 ± 0.5	metal pilings of jetties	++
7	Fayed	43 ± 1	28 ± 2	8.2 ± 0.3	6.3 ± 0.3	metal pilings of jetties	+ + +
8	Fanara	43 ± 1	28 ± 2	8.3 ± 0.3	5.9 ± 0.3	cement banks of Suez Canal	++
9	Kabreet	44 ± 2	29 ± 1	8.5 ± 0.2	7.2 ± 0.2	cement banks of Suez Canal	+

Table 3. Occurrence of *Ecteinascidia thurstoni* at different sampling sites along the Suez Canal during the summers of 2006 and 2007

- absent, + rare, ++ common, +++ abundant

sea grasses, and artificial hard substrates such as jetties, cement and metal pilings – individuals or colonies of this tunicate were absent. On the other hand, along Suez Canal coasts, colonies of E. thurstoni were found to be flourishing on the pilings of jetties, and the metal and cement banks of the Canal, especially at the Bitter Lakes (Table 5), where the colonies of the species were more frequent than in the Red Sea.

During the summer seasons of 2006 and 2007, the survey along the Red Sea concentrated mainly on mangroves, where 29 sites were investigated (Table 4). This showed that *E. thurstoni* was present at only 4 sites out of 29. One of these sites was in the north – El-Rawisia at the Nabq Protected Marine Park, Gulf of Aqaba – and the three others were in the south – Wadi El Qala'an, Wadi Rawad El-Adaiah, and Wadi El-Ra'ada. At the first three sites, *E. thurstoni* was represented by colonies with very few, small zooids, usually found on the pneumatophores of the mangrove *Avicennia marina*, or on the thalli of the seaweed *Laurancia obtusa* (at El-Rawisia). At the fourth site (Wadi El-Ra'ada), *E. thurstoni*

No.	Site	Salinity [PSU]	Temp. [°C]	pН	$\begin{array}{c} Oxygen \\ [mg \ dm^{-3}] \end{array}$	Species	E. thurstoni present/		
	absent								
Nab	q & Ras Mohamed								
1	El-Monkata'a	44 ± 2	28 ± 1	7.5 ± 0.2	6.1 ± 0.3	Avicennia marina	-		
2	El-Rawisia	44 ± 2	28 ± 1	7.5 ± 0.3	6.2 ± 0.2	A. marina	+		
3	Marsa Abu -Zabad	44 ± 1	28 ± 1	7.7 ± 0.3	6.1 ± 0.1	A. marina	-		
4	El-Ghargana	44 ± 1	27 ± 2	7.5 ± 0.2	6.3 ± 0.2	A. marina	_		
5	Ras Mohamed	44 ± 1	27 ± 2	7.6 ± 0.3	6.2 ± 0.2	A. marina	_		
Hur	ghada								
6	Al-Gonah	45 ± 1	28 ± 1	7.7 ± 0.3	5.3 ± 0.4	A. marina	_		
7	Abu-Monkar	45 ± 1	27 ± 1	7.8 ± 0.1	5.4 ± 0.4	A. marina	_		
8	South Safaga	44 ± 1	27 ± 1	7.8 ± 0.1	5.3 ± 0.3	A. marina	_		
Qus	eir								
9	Wadi Abu-Hamrah	44 ± 1	27 ± 2	7.9 ± 0.1	5.9 ± 0.3	A. marina	-		
10	Sharm El-Bahari	44 ± 2	27 ± 2	8.1 ± 0.2	5.6 ± 0.5	A. marina	_		
11	Sharm El-Qebly	43 ± 2	27 ± 2	8.1 ± 0.2	5.8 ± 0.3	A. marina	_		
12	Marsa Sagara	43 ± 2	29 ± 1	7.6 ± 0.3	5.7 ± 0.4	A. marina	_		
13	Wadi El-Gemal	43 ± 1	27 ± 1	7.7 ± 0.3	5.6 ± 0.4	A. marina	_		
14	Wadi El-Gemal Island	43 ± 1	28 ± 1	8.1 ± 0.2	5.5 ± 0.3	A. marina	_		
15	Ras Baghdadi	44 ± 2	28 ± 1	8.2 ± 0.3	6.1 ± 0.2	A. marina	-		
Han	nata								
16	Wadi Masturah	42 ± 1	27 ± 2	7.2 ± 0.4	5.9 ± 0.3	A. marina	_		
17	Wadi Qala'an	43 ± 1	27 ± 1	7.3 ± 0.5	5.8 ± 0.3	A. marina	+		
18	Wadi Rawad El- Adaiah	43 ± 1	28 ± 1	7.5 ± 0.5	6.3 ± 0.2	A. marina	+		
19	Wadi El-Ra'da	44 ± 2	27 ± 1	7.6 ± 0.4	6.2 ± 0.2	A. marina	++		
20	Shawareet Island	43 ± 1	28 ± 1	7.7 ± 0.4	5.7 ± 0.3	A. marina	_		
21	Wadi Lahmy	44 ± 1	27 ± 2	7.5 ± 0.5	5.6 ± 0.4	A. marina	_		
22	Qora'at Hartway	44 ± 1	28 ± 2	7.8 ± 0.3	6.0 ± 0.3	A. marina	_		
Sha	lateen								
23	El-Hamirah Mangrove	44 ± 1	28 ± 1	7.6 ± 0.2	6.1 ± 0.3	A. marina	-		
24	Shalateen Island	43 ± 1	27 ± 1	7.7 ± 0.4	5.8 ± 0.4	A. marina	_		
25	Sharm El-Madfa'a	44 ± 2	27 ± 1	7.8 ± 0.4	5.7 ± 0.4	Rhizophora mucronata	_		
26	Marsa Sha'ah	43 ± 1	28 ± 1	7.7 ± 0.3	6.2 ± 0.3	A. marina, R. mucronata	-		
Hala	Halayeeb								
27	Marsa Abu-Fasi	44 ± 1	27 ± 1	7.9 ± 0.4	5.9 ± 0.4	A. marina, R. mucronata	_		
28	El-Hoor	43 ± 1	28 ± 1	8.1 ± 0.2	5.2 ± 0.5	A. marina, R. mucronata	—		
29	Adal Deep	44 ± 1	29 ± 1	8.2 ± 0.2	5.3 ± 0.5	A. marina, R. mucronata	-		

Table 4. Occurrence of *Ecteinascidia thurstoni* at different sampling sites along the Red Sea during the summers of 2006 and 2007

- absent, + rare, ++ common



Medium-sized colony, Wadi Ra'ada, Red Sea

Large colony, Wadi Ra'ada, Red Sea

Figure 2. Colonies of *Ecteinascidia thurstoni* collected from the Suez Canal and Red Sea

was represented by colonies with relatively numerous zooids on the basal part of the pneumatophores of *A. marina*. Wadi El-Ra'ada was the most productive site along the Red Sea coast as regards collection of this tunicate. Moreover, the field survey showed that the roots of the mangrove *Rhizophora mucronata* in the Shalateen – Halayeeb sector did not support any colonies of this tunicate (Table 4). On the other hand, investigation of nine sites along Suez Canal during the same season showed that the species usually flourishes on the pilings of jetties, especially at Fayed on the Bitter Lakes (Table 3), where large colonies with large zooids were found and collected.

Table 5. Occurrence and frequency of *Ecteinascidia thurstoni* in different habitatsof the Suez Canal and Red Sea (summer 2005)

Habitat	Occurrence	Frequency	
Red Sea coral reef seagrass	absent absent	- - loss froquent	
Suez Canal seagrass pilings of jetties metal & cement banks	absent present present	frequent frequent	

Table 6. Morphometric characteristics of *Ecteinascidia thurstoni* along the Suez Canal and Red Sea coasts (samples collected during the summers of 2006 and 2007)

No.	Site	Maximum diameter or length of colony	Maximum number of zooids/	Maximum weight of colony	Maximum length of zooid	Maximum weight of zooid
		[mm]	colony	[g]	[mm]	[g]
Suez	c Canal					
3	El-Danva Club	70	110	90	17	1.0
4	Tosun	70	150	120	17	1.0
5	Deversoir	80	155	140	18	1.1
6	Abu -Sultan	90	140	130	18	1.1
7	Fayed	100	250	300	20	1.3
8	Fanara	70	170	150	20	1.2
9	Kabreet	50	120	110	19	1.1
Red	Sea					
2	El-Rawisia	very patchy, separate, on thallus of <i>Laurancia</i> <i>obtusa</i> , growing on pneumatophores	20	1.00	7	0.15
17	Wadi Qala'an	very patchy, separate, on basal part of pneumatophores	5	1.00	6	0.25
18	W. Rawad El- Adaiah	very patchy, separate, on basal part of pneumatophores	6	1.00	7	0.25
19	Wadi El- Ra'ada	130 mm, on basal part of pneumatophores	163	30	11	0.26

Table 6 lists the maximum morphometric characteristics of *E. thurstoni* colonies at different sites. The data show that the Suez Canal population is more productive in terms of biomass: the colonies at Fayed (Bitter Lakes), which usually grow in shallow calm water, achieved a maximum weight of 300 g. The greater weight of these colonies was related mainly to colony diameter, zooid length, weight and number per colony. Table 6 makes it clear that the colonies with the maximum diameter (100 mm) were collected from Fayed. Also, colonies with the maximum number of zooids were collected from that same site (250 zooids/colony) on the Suez Canal, and from Wadi El-Ra'ada (163 zooids/colony) on the Red Sea coast. Zooids with a maximum length (20 mm) were recorded at Fayed and Fanara on the Suez Canal and on the mangroves of Wadi El-Ra'ada (11 mm). Zooids with maximum weights of 1.3 g and 1.2 g were recorded at Fayed and Fanara, respectively, whereas they weighed 0.26 g at Wadi El-Ra'ada.

One-way ANOVA revealed highly significant differences between the Suez Canal and Red Sea sites with respect to different morphometric characteristics (zooid length, weight, and number per colony, as well as colony weight) (Table 7).

 Table 7. One way analysis of variance for different ecological parameters and morphometric characteristics of *Ecteinascidia thurstoni* between different stations

Source of variation	df	Sum of	Mean	F-value	p-value
		squares	square		
salinity	5	216.658	43.332	0.319	0.898
temperature	5	83.511	16.702	0.119	0.987
pH	17	946.500	55.676	0.307	0.991
O_2	25	3615.500	144.620	1.819	0.140
weight of zooid	6	200.667	33.444	25.948	0.0001^{*}
weight of colony	10	217.250	21.725	86.900	0.0001^{*}
length of zooid	6	213.000	35.500	76.071	0.0001^{*}
no. zooids per colony	16	220.000	13.750	55.00	0.0001^{*}

df – degree of freedom, p-value $\leq 0.05\%$, *significantly different

4. Discussion

Our results regarding the distribution of the ascidian *Ecteinascidia* thurstoni along the Suez Canal and the Egyptian Red Sea coast showed that it is like other species of *Ecteinascidia* in that it has a seasonal pattern, usually being present during the summer months. Its distribution is related mainly to seasonal fluctuations in temperature; at low temperatures, colonies usually regress to the stolons. These then normally bud again the following summer to form new zooids and then colonies by asexual reproduction. Such stolon-generated asexual growth by budding is a primary characteristic of *Ecteinascidia* species (Satoh 1994). Budding results in the colony's rapid development and ensures the animals survival during adverse environmental conditions in winter (Carballo 2000a).

Elsewhere in the world, the distribution of *Ecteinascidia* has been described from the Estany des Peix Lagoon on Formentera Island, Spain (Carballo et al. 1997), the mangals of the Gulf of Mexico and the Yucatan Peninsula (Carballo 2000b), and in Cuba (Hernández-Zanuy et al. 2007), where it was found in shallow waters of 1-3 m depth. Along the Suez Canal and Egyptian Red Sea coast, the most interesting aspect regarding its distribution was its occurrence at the most northerly point of the Red Sea (Gulf of Aqaba – El-Rawisia) and 600 km farther south, at the Hamata mangroves; in between these two sites the coastal waters did not support any Ecteinascidia colonies. This could be due to the settlement behavior of the larvae. *Ecteinascidia* populations are highly gregarious, due in part to low larval dispersal, and so become very localized (Bingham & Young 1991, Carballo 2000a); the larvae tend to settle close to their parent colonies. The short dispersal distance of the larvae, caused by their short life span (a few hours) and the location of the settlement structures (e.g. pneumatophores) close to the population nucleus provide some guarantee of success for larval capture and settlement, and prevent advection to inappropriate sites. This could partially explain the patchy distribution of *Ecteinascidia* species along the Red Sea. Other environmental factors, e.g. currents, tidal patterns, predators, could additionally affect their distribution. Long-distance exchange between isolated islands probably occurs by the rafting of adult colonies on fragmented mangrove roots rather than through larval dispersal (Bingham & Young 1991).

The other interesting feature is that the species has different morphometric characteristics (zooid length, weight, and number per colony, in addition to colony weight) at different locations on the Suez Canal and Red Sea. The morphometric characteristics of the Suez Canal population were very much greater than those of the Red Sea population (Table 6). Zooid length (height) ranged from 6 to 20 mm: minimum and medium zooid lengths were recorded at Red Sea sites, while maximum zooid lengths or heights were recorded at Suez Canal sites. In a Mediterranean population of *E. turbinata*, zooid height or length ranged from 12.3 to 20.65 mm at the Estany des Peix Lagoon on Formentera Island, Spain (Carballo et al. 1997). In Phuket Province, on the Andaman Sea coast of southern Thailand, this tunicate is found only in one reef area at 1–3 m depth. The zooid varies between 8 and 12 mm in height (Chavanich et al. 2005).

These populations with different morphometric characteristics could be studied for their bacterial communities, chemistry and genetics. The resulting information could be implemented in the development of the biosynthesis of ET-743. The development of this drug has been hampered in recent years by the difficulty in securing an inexpensive supply of the compound. Margo Hygood of the Oregon Health and Science University (personal communication) hypothesizes that ET-743 is in fact microbial in origin, as it shows a remarkable similarity to other microbial secondary metabolites. Characterizing the bacterial community as well as the chemistry of *Ecteinascidia* species from different locations will therefore help to identify the bacterial species common to all tunicates containing bioactive metabolites. Furthermore, identification of a non-bioactive population of *Ecteinascidia* would be extremely valuable in determining the true symbionts of the animal, as well as potential sources of the compound. Ultimately, this will aid in the further development of the compound in the laboratory and hence its biosynthesis on a commercial scale.

Finally, it is recommended to study the distribution of *E. thurstoni* in order to locate its different populations along the Suez Canal and the Red Sea. This will assist in the study of their genetics, as well as their chemistry and bacterial communities at different isolated locations, which will in turn help to define sources of ET-743. Its biosynthesis on an economically viable scale will then become less expensive than its chemical synthesis.

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References

- Bingham B. L., Young C. M., 1991, Larval behavior of the ascidian Ecteinascidia turbinata Herdman; an in situ experimental study of the effects of swimming on dispersal, J. Exp. Mar. Biol. Ecol., 145 (2), 189–204.
- Bingham B. L., Young C. M., 1995, Stochastic events and dynamics of a mangrove root epifaunal community, Mar. Ecol.-PSZNI, 16 (2), 145–163.
- Carballo J.L., 2000a, Larval ecology of an ascidian tropical population in a Mediterranean enclosed ecosystem, Mar. Ecol.-Prog. Ser., 195, 159–167.
- Carballo J.L., 2000b, Distribución de Ecteinascidia turbinata (Ascidiacea: Perophoridae) en los manglares del la Península de Yucatán (México), Rev. Biol. Trop., 48 (2/3), 365–369.

- Carballo J. L., Hernández-Zanuy A., Naranjo S., Kukurtzü B., García Cagide, A., 1999, Recovery of Ecteinascidia turbinata Herman 1880 (Ascidiacea: Perophoridae) populations after different levels of harvesting on a sustainable basis, Bull. Mar. Sci., 65 (3), 755–760.
- Carballo J.L., Naranjo S., Hernández-Zanuy A., Kukurtzü B., 1997, Estudio de la población de Ecteinascidia turbinata Herdman 1880 (Ascidiacea: Perophoridae) en la Isla de Formentera (Mar Mediterráneo. España): Distribución, densidad y crecimiento, Cienc. Mar-UAS, 15, 7–15.
- Carballo J. L., Naranjo S., Kukurtzü B., De La Calle F., Hernández-Zanuy A., 2000, Production of Ecteinascidia turbinata (Ascidiacea: Perophoridae) for obtaining anticancer compounds, J. World Aqua. Soc., 31 (4), 481–490.
- Chavanich S., Koeysin P., Viyakarn V., Piyatiratitivorakul S., Menasveta P., Suwanborirux K., Poovachiranon S., 2005, A tunicate from a Thai coral reef: a potential source of new anticancer compounds, Coral Reefs, 24, 621.
- Cuevas C., Pérez M., Martín M.J., Chicharro J.L., Rivas C.F., Flores M., Francesch A., Gallego P., Zarzuelo M., de la Calle F., García J., Polanco C., Rodríguez I., Manzanares I., 2000, Synthesis of Ecteinascidin ET-743 and Phthalascidin Pt-650 from Cyanosafracin, Bull. Org. Lett., 2 (16), 2545–2548.
- Garcia-Rocha M., Garcia Gravalos M.D., Avila J., 1996, Characterization of antimitotic products from marine organisms that disorganize the microtubule network: Ecteinascidin 743, isohomohalichondrin-B and LL-15, Brit. J. Cancer, 73 (8), 875–883.
- Hernández-Zanuy A. C., Carballo J. L., García-Cagide A., Naranjo S., Esquivel M., 2007, Distribución y abundancia de Ecteinascidia turbinata Herdman (Ascidiacea-Perophoridae) en Cuba, Rev. Biol. Trop., 55 (1), 247–254.
- Jimeno J. M., Faircloth G., Cameron L., Meely K., Vega E., Gomez A., Fernandez-Sousa Faro J. M., Rinehart K., 1996, Progress in the acquisition of new marinederived anticancer compounds: development of Ecteinascidin-743 (ET-743), Drug. Future, 21 (11), 1155–1165.
- Moss C., Green D. H., Pérez B., Velasco A., Henríquez R., McKenzie J. D., 2003, Intracellular bacteria associated with the ascidian Ecteinascidia turbinata: phylogenetic and in situ hybridization analysis, Mar. Biol., 143 (1), 99–110.
- Naranjo S. A., Kukurtzü H. B., Barbero C., Martin S., Carballo J. L., 2001, Aquaculture of Ecteinascidia turbinata Herdman, 1880 as source of marine anticancer agents, [in:] The biology of ascidians, H. Sawada, H. Yokosawa & C. C. Lambert (eds.), Springer-Verlag, Tokyo, 355–360.
- Pharmamar, 2008, http://www.pharmamar.com
- Rinehart K. L., 2000, Antitumor compounds from tunicates, Med. Res. Rev., 20 (1), 1–27.
- Rinehart K. L., Holt T. G., Fregeau N. L., Stroh J. G., Keifer P. A., Sun F., Li L. H., Martin D. G., 1990, Ecteinascidins 729, 743, 745, 579A, 579B and 770: Potent antitumor agents from the Caribbean tunicate Ecteinascidia turbinata, J. Org. Chem., 55 (15), 4512–4515.

- Satoh N., 1994, Developmental biology of ascidians, [in:] Developmental and cell biology series, P. W. Barlow, D. Bray, P. B. Green & J. M. W. Slack (eds.), Cambridge Univ. Press, Cambridge, U.K.
- Suwanborirux K., Charupant K., Amnuoypol S., Pummangura S., Kubo A., Saito N., 2002, Ecteinascidins 770 and 786 from the Thai Tunicate Ecteinascidia thurstoni, J. Nat. Prod., 65 (6), 935–937.
- Young C. M., Bingham B. L., 1987, Chemical defense and aposematic coloration in larvae of the ascidian Ecteinascidia turbinata, Mar. Biol., 96 (4), 539–544.
- Youssef D. T. A., 2007, Chemistry part, [in:] Symbionts and chemistry of the Et-743 producing tunicate Ecteinascidia turbinata. Annual progress Report No 2, M. G. Haygood & A. A-F. Gab-Alla (eds.), U.S.–Egypt Science and Technology Joint Fund, Grant Number BIO8-002-006, (unpublished).