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**Status of the “Mangrove tunicate” *Ecteinascidia turbinata*
 (Ascidiacea: Perophoridae) in the Mediterranean Sea**

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Keywords:	non-indigenous species, marine fouling, cryptogenic species, biodiversity
Abstract:	The ascidian <i>Ecteinascidia turbinata</i> is reported from Maltese waters for the first time. Mature colonies were recorded on artificial substrata at two different sites (and on natural substrata at one of these), 4 km apart, during the summer months. The appearance of this ascidian is expected to be seasonal as the winter temperature in Malta may fall below that required for the maintenance of zooid growth. A second species, <i>E. moorei</i> , which was described in 1890 is here confirmed to be the same as <i>E. turbinata</i> , meaning that the species has existed in the Mediterranean since at least ca 1880. The possibility that the Mediterranean population is a relic one from warmer periods cannot yet be excluded, so it is best described as being cryptogenic. The species appears to be spreading slowly, perhaps as a result of the rise in surface sea temperature. The Maltese sites offer an opportunity to monitor the species as they are accessible dive sites. This will allow assessment of whether this species remains restricted to these sites, or if it spreads perhaps to impact other species.

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3 Status of the “Mangrove tunicate” *Ecteinascidia turbinata*
4 (Ascidiacea: Perophoridae) in the Mediterranean Sea

5

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29 ABSTRACT

30 *The ascidian Ecteinascidia turbinata is reported from Maltese waters for the first time.*
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45

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47 INTRODUCTION

48

49 *Ecteinascidia turbinata* (Herman, 1882) has attracted much attention from within the
50 biological and medical fields as it has been found to be a source of several metabolites, some
51 of which may act as anti-cancer agents (Rinehart *et al*, 1990). Foremost amongst these is ET-
52 743 which has a unique mode of action, targeting DNA repair (D’Incalci & Galmatini, 2010).
53 ET-743 is now authorised by the European Commission under the name ‘Trabectedin’ for use
54 to treat ovarian cancer. It is now known that ET-743 is not produced by the tunicate itself but
55 rather is the product of the intracellular bacterium *Endoecteinascidia frumentensis* (Moss *et*
56 *al*, 2003; Pérez-Matos *et al*, 2007; Elbaz, 2009).

57 The main world-wide population of *E. turbinata* is centred on the tropical Western Atlantic
58 (Caribbean) region with what are presumed to be satellite populations occurring elsewhere.
59 However, according to Van Name (1945) “Reports of it from Old World localities ... are to
60 be mistrusted, as very probably referring to allied species”. It is now known that this is the
61 same species which occurs in West Africa at Joal and Dakar in Senegal (Pérès, 1948, 1949),
62 the Cape Verde Islands (Rennie & Wiseman. 1906), the Suez Canal and into the Red Sea
63 (Elbaz, 2009), and there are also populations in the Mediterranean (Pérès, 1958) (Figure 1).
64 This observation has led to the assumption that *E. turbinata* has expanded its area of
65 distribution, probably through human activity, from the Caribbean area but this has not been
66 confirmed. The Mediterranean Sea has long been a major route for maritime traffic and
67 around 680 alien species have successfully colonized it (Galil *et al*, 2014), with 66 alien
68 species colonizing the Maltese Islands alone (Evans *et al*, 2014). It is generally assumed that
69 vessels have facilitated the transport of these species (Zenetos *et al*, 2012), as components of
70 fouling communities on the hulls (Galil *et al*, 2012) and perhaps more recently through ballast
71 water discharge. Either way, harbours have become hotspots for alien species (Hulme, 2009).

72
73 Ascidians are particularly good colonisers and many alien ascidians are now found in the
74 Mediterranean, some of which have become invasive (Izquierdo-Muñoz *et al*, 2009). The
75 opening of the Suez Canal in 1869 has allowed immigration from the Red Sea, and another
76 member of the genus, *E. thurstoni* has colonised locations in the eastern Mediterranean
77 presumably through this route (Shenkar & Loya, 2009). This colonisation process has been
78 enhanced by temperature increases in the Mediterranean (Nykjaer, 2009; Raitos *et al*, 2010).
79 The enlargement of the Canal in 2015 is likely to increase the invasion rate further (Galil *et*
80 *al*, 2015). It is not completely clear if alien ascidians in the Mediterranean represent a
81 significant threat to indigenous species or if they may result in inconvenience to human
82 activities as has been the case with many other ascidian species across the world (Adams *et*
83 *al*, 2011).

84
85 METHODS AND MATERIALS

86 *Ecteinascidia turbinata* was discovered at three sites in Malta. Several colonies of this species
87 were found and photographed on the 10th September 2015 by SCUBA diving at depths of
88 approximately 20 m. The mature colonies (Figure 2) were attached to the wreck of the SS
89 ‘Margit’ at Kalkara Creek (35.8907° N, 14.5224° E), in the Grand Harbour, Valletta. The
90 wreck lies on a sand and silt bottom and the water was turbid, with visibility estimated at
91 between 3 and 4 meters. The water temperature at 20 meters was measured to be 26°C. This
92 finding prompted a retrospective investigation of photographs recorded at various other sites;

these included images in the authors' libraries or published online by local divers. We also contacted several underwater nature photographers directly to check if they had ever encountered this species but only considered records as 'confirmed' if supported by photographic evidence. Through this exercise, we came across additional images of *E. turbinata* taken on the wreck of the "Tug 2" tugboat off Exiles, Sliema (35.9197° N, 14.4988° E) and from St. George's Bay (35.9264° N, 14.4891° E), with the earliest record being made at the latter site in August 2008 (Table 1). The 'Tug 2' wreck is located some 4 km away from that of the SS 'Margit' and was scuttled as a SCUBA diving attraction in June 2013, so the tunicates appeared on the wreck within 16 months of its sinking. This wreck lies over a sandy bottom at a depth of 21m, with a rocky reef in the vicinity; the area is generally characterised by clear waters with a typical visibility of 15–25m.

Twelve sampled colonies were fixed with 10% formalin in seawater. Some zooids of each colony have been dissected, stained with Masson haemalum and mounted on permanent slides. Taxonomic identification was carried out using original descriptions (Herdman, 1882, 1890, 1891, Van Name, 1945). The specimens have been deposited in the Department of Marine Sciences of the University of Alicante (DCMBA, ET-Mt01-12).

RESULTS

In general the tunicate described here corresponded closely to former descriptions of *E. turbinata* (Herdman, 1882; 1890; Berrill, 1932; Van Name, 1945). However some of the zooids investigated microscopically were similar to the original description of *E. moorei* (Herdman, 1890). This finding explains the disagreement in the published descriptions of *E. moorei* and *E. turbinata* and confirms their synonymy. The most striking features of these colonial tunicates are the bright orange siphons and the translucent tunic of the zooids. The colonies are made of spherical or hemispherical (depending on attachment) bunches of tight erect zooids, attached by a web of anastomosed stolons (Figure 2a and 2b). The zooids from the sample taken from the 'Tug 2' site are 6–15 mm long and have gonads in different stages of development. The tunic is naked and transparent. The siphons are close together, with some of the orange colour remaining after formalin fixation. When contracted, the siphons are still apparent (Figure 2c), (not invaginated as is typical in *E. thurstoni*). The siphonal musculature is marked (not weak as *E. thurstoni*); the transverse muscles do not cross the intersiphonal space (as in *E. thurstoni*). The muscles are not interrupted between the two sides before the eleventh row of stigmata, but extend farther on the right side. In agreement with others (e.g. Van Name, 1921) there are about 40–50 tentacles present in three to four distinct lengths. The prepharyngeal band has a high anterior blade and a posterior ribbon. There is no dorsal indentation. The dorsal tubercle is small and oval (as in *E. thurstoni*), located under the prepharyngeal band (Figure 3a). The dorsal lamina has elongated and flattened languets with or without a membrane (Figure 3a,b).

The branchial sac has 15–16 rows of stigmata on the left side and 16–17 on the right. Flat or cylindrical papillae (Figure 3c) are united by 15–16 longitudinal vessels on each side, but some papillae, such as those closest to the dorsal lamina, lack vessels. The vessel close to the endostyle is made of T-shaped papillae, sometimes arranged two by two. The longitudinal

vessels are very regular, the meshes containing 2-4 stigmata. The gut forms an open loop without a flat secondary curve (unlike *E. thurstoni*) (Figure 2c). The oval stomach shows two wide oblique bands (Figure 2c) and there is a post-stomach. The intestine ascends at first along the dorsal side but does not become parallel to the dorsal lamina (as in *E. thurstoni*). The anus has two lobes at the level of the 6-7th stigmata row. The male gonad is developed with small and numerous vesicles in concentric rings around the ovary (Figure 2c). The sperm duct converges towards the left side of the rectum at the level of 10-11th stigmata row, and ends close to the anus (Figure 3a). Some zooids presented fertilised ovules and one early larva was observed.

DISCUSSION

Classification of *E. turbinata*.

The genus *Ecteinascidia* was formed by Herdman (Herdman, 1882) from specimens obtained during the 'Challenger' expedition. In this initial report, Herdman reported that tunicate samples from Alexandria harbour (Egypt) were the same species, *E. turbinata*, as he had found in the Caribbean. However, he later (Herman, 1890), reported that the tunicate from Alexandria was in fact a related but distinct species, which he named *E. moorei* in honour of Mr T.J. Moore, curator of the Liverpool Free Public Museum, who had passed the sample to him. There is confusion as to the taxonomic status of *E. moorei* as some authors state that *E. moorei* is a junior synonym of *E. turbinata*. Harant (1927a) includes it as a synonym stating that he had studied both and concluded that they were the same species but did not provide the evidence. Later Harant & Vernieres (1933) stated the synonymy but the reference given for this (Van Name, 1930) was incorrect. However, Van Name (1921: 378) makes the following statement about the dorsal lamina in *E. turbinata*: "... a continuous membrane which is extended into a well-developed languet at each transverse vessel. Or it may be described as a series of languets connected by a basal membrane. It lies turned over toward the right side of the body". One of the characteristics that separate *E. turbinata* of *E. moorei* is the development of the dorsal membrane, but in *E. turbinata* the two types of development of the membrane occur.

In Herdman's dichotomic key (1890: 160), the main characters that separate *E. moorei* from *E. turbinata* and *E. thurstoni* are: dorsal languets and connecting ducts are wide and membranous; whereas *E. turbinata* and *E. thurstoni* present dorsal languets and connecting ducts that are narrow and papilliform. Moreover, Kott (1952: 316) adds the following characters to *E. moorei*: zooids clustered into dense hemisphere, vas deferens ends ventral to rectum; whereas in *E. turbinata* and *E. thurstoni*: zooids clustered into irregular mass, vas deferens ends dorsal to rectum. The specimens from Malta can form dense masses of zooids (Figure 2), present dorsal languets and connecting ducts both narrow and wide (Figure 3a-c), and have a spermiduct that ends ventral or lateral to rectum (Figure 3a).

Others treated *E. moorei* as a full species (Garstang, 1891; Sluiter, 1905; Michaelsen, 1918; Harant, 1927b), a few after it had been synonymised (Kott, 1952; Kott, 1964; Por, 1978). To further complicate the matter, Monniot & Monniot (1997) are of the opinion that the *E. moorei* of Sluiter (1905) is in fact *E. thurstoni* while Pérès (1954) considers another species, *E. herdmanni* as young specimens of *E. turbinata*. Our finding that some zooids within the

same colony conform to the descriptions of both *E. turbinata* and *E. moorei*, confirms that *E. moorei* is a junior synonym of *E. turbinata*. This explains why Herdman (1890) apparently changed his mind about the identity of the sample from Alexandria and why there is no presently known population of *E. moorei*. It remains to be seen if *E. moorei*-like zooids also exist in *E. turbinata* colonies in the Caribbean population.

Two different colour varieties have been identified in *E. turbinata* across the range in the Caribbean, Atlantic and the Mediterranean (López-Legentil & Turon, 2007). Colonies occur as “orange” or “white” especially when photographed against a white background and all those in the Spanish Mediterranean so far described have been the white variety. The colonies that we have found were also of the white (translucent) type. Colony colour may be determined by environmental factors as it has been noted that the colour of *E. thurstoni* colonies was dependent on the available light with poor illumination resulting in colourless animals (Por & Dor 1975). The natural situation in which *E. turbinata* is found in its Caribbean range and in the Red sea, is the submerged roots of mangrove trees where ambient conditions are similar to those found in the SS ‘Margit’ site in Kalkara Creek, where low light levels were caused by a high silt sediment burden with relatively high temperatures; however, in Cuba it is also found in clean water attached to hard coral between 22 and 38 metres depth (Hernández-Zanuy *et al*, 2007), which is closer to the conditions found in St George’s Bay and on the ‘Tug 2’ wreck (Table 1). *E. turbinata* is also found on wrecks in Bermuda (Berrill, 1932).

The status of *E. turbinata* in the Mediterranean

It is now evident that *E. turbinata* has existed in the Mediterranean since at least the late 1800s but was listed as a non-indigenous species (as *E. moorei*) in the Mediterranean (Streftaris *et al*, 2005) and as alien in the WoRMS database (Sanamyan, 2015). However, it was not included in the lists of recent non-indigenous ascidians by Izquierdo-Munoz *et al*. (2009) and Zenetos *et al*. (2010) on the basis that there are old records of this species and that it is circumtropical. On the other hand, some have described *E. turbinata* as a “probably native species” (Chebbi *et al*, 2010). The EASIN database gives this species as ‘cryptogenic’ and we feel that this is the safest description. It is also possible that *E. turbinata* survives as relic populations such as those we have found in Malta, from warmer periods in the distant past.

Distribution of *E. turbinata* in the Mediterranean and neighbouring areas

E. turbinata has been described as being rare in the Mediterranean (Pérès, 1949) but it is known (Figure 1) from many localities as follows. The Western Mediterranean: Balearic Islands (Rodríguez, 1922; Pérès, 1957; Ramos *et al* 1991; Carballo *et al* 1997), Alicante (Cornet & Ramos, 1980; Ramos, 1988; Luján, 1995), and Marsala in Sicily (Pérès, 1954) and Bizerte in Tunisia (Pérès, 1956). Central Mediterranean (Ionian Sea) in southern and eastern Tunisia, in the Gulf of Gabès (Pérès, 1954, 1956; Zaouali, 1976; Ben Mustapha *et al*, 2002; Meliane, 2002; Chebbi *et al* 2010), and Kuriat Islands (Ramos & Ben Mustapha, 2010). Aegean Sea (Monniot, 1983; Koutsogiannopoulos *et al*, 2012). With respect to the Levantine basin (Eastern Mediterranean), apart from the old records from Alexandria (Herdman, 1882, 1891; Harant, 1939), no further citations have been made, despite a number of studies carried out in the area (Sedra & Khalil, 1972; Abdel-Messeih, 1982; Ghobashy & Abdel-Messeih, 1991; Ramadan *et al*, 2006; Shenkar & Loya, 2009). Regarding the NW Mediterranean,

Harant (1927a) reported *E. turbinata* from Cap Creus (NE Spain). However, no later studies in this Mediterranean sector have observed this species (Cornet & Ramos, 1980; Ramos, 1984; Lafargue *et al*, 1986; Turón, 1987). *E. turbinata* has also been reported in areas neighbouring the Mediterranean Sea (Fig. 2): the Bay of Cadiz (Ramos, 1988; Ramos *et al*, 1992; Naranjo, 1995), quite near to the Gibraltar Strait; and the Suez Canal and the Red Sea (Harant, 1927b; Elbaz, 2009).

Prospects for the Mediterranean *E. turbinata* populations

Despite the fact that *E. turbinata* larvae are relatively large (4.5 mm), active swimmers that are protected to some extent from fish predation through toxins and aposematic colouration (Young & Bingham, 1987), long range dispersal of this tunicate is reported to be primarily due to rafting of adult colonies (Bingham & Young, 1991), with larval dispersal limited to a few hundred metres (Carballo, 2000). The nearest previously known location of *E. turbinata* to Malta is in the Gulf of Gabès, Tunisia (Figure 1). However it is not likely to have spread to Malta from this location due to the limited autonomous larval dispersion and the hydrographic isolation of Gulf waters. It is possible that this tunicate may have been transported to its present Maltese site by shipping, as two other ascidians are proposed to have done (Evans *et al*, 2015), either in ballast water or as adult colonies with fouling assemblages (Izquierdo Muñoz *et al*. 2009).

Whereas *E. turbinata* colonies grow all year round in the El Bibane lagoon, Tunisia (Chebbi, 2010; Chebbi *et al*, 2010), populations in Alicante and the island of Formentera are seasonal (Luján, 1995; Carballo, 2000) and survive winter months as quiescent bodies derived from the stolons. The crucial temperature below which zooids regress appears to be around 17–18°C (Carballo *et al*, 1997). It remains to be determined if the recently discovered colonies are seasonal in Malta, where fertilised ova and larvae have been observed in late October; in Alicante this happens a month before, in late September (Luján, 1995). However, the data for the SS ‘Margit’ site (Table 2) indicate that the temperature drops below the threshold for the winter months making it very likely that *E. turbinata* is seasonal. It is of course possible that this particular population has adapted to growth in colder waters (but we note that this has not occurred in the Spanish populations). The colonies present at the Peloponnese site in Greece were large and numerous in July 2011, but very small and sparse in July 2012 (Koutsogiannopoulos *et al*, 2012), indicating that conditions (probably temperature) result in these populations too being seasonal and perhaps often marginal.

CONCLUSIONS

We have recorded several live colonies of *E. turbinata* on two Maltese shipwrecks and in one case in a neighbouring inlet. This is the first report of this ascidian in Maltese waters. All our records so far have been made in the summer months (mid-July to late October) but we suspect that this species will reproduce seasonally around Malta due to the winter water temperatures falling below the 17–18°C threshold below which zooids regress to resting stolons. We expect that conditions will prove suitable for *E. turbinata* across Malta and that

the ‘Tug 2’ site will be particularly valuable for monitoring its establishment success over time and studying how the presence of this tunicate influences existing species assemblages. Since around 2009, *E. turbinata* seems to be expanding into new areas, and it has now colonised *Posidonia oceanica* meadows and the infralittoral rock of Mar Menor, a coastal lagoon in SE Spain outside the previously recorded areas (A.A.R.E. pers. obs.), the Aegean Sea (Koutsogiannopoulos *et al*, 2012) and Malta (present work). The question as to the origins of the present day populations of Mediterranean *E. turbinata* may be resolved by phylogeographic analysis (López-Legentil & Turon 2005; Elbaz, 2009) comparing variations in suitable genes from across its present range. The available information indicates that the status of *E. turbinata* in Malta and the Mediterranean is best described as being cryptogenic, and yet the possibility of it being a relic population cannot be ruled out.

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REFERENCES

- Adams C.M., Shumway S.E., Whitlatch R.B., and Getchis T.** (2011) Biofouling in marine molluscan shellfish aquaculture: a survey assessing the business and economic implications of mitigation. *Journal of the World Aquaculture Society*. 42, 242-252.
- Abdel-Messih M.K.** (1982) Studies of ascidians in Alexandria waters. MSc. Thesis, University of Alexandria.
- Ben Mustapha K., Komatsu T., Hattour A., Sammari Ch., Zarrouk S., Souissi A., and El Abed A.** (2002) Tunisian mega benthos from infra (*Posidonia* meadows) and circalittoral (coralligenous) sites. *Bulletin de l'Institut National es Sciences et Technologies de la Mer de Salammô* 29, 23-36.
- Berrill N.J.** (1932) Ascidians of the Bermudas. *Biological Bulletin*. 62, 77–88.
- Bingham B.L., and Young C.M.** (1991) Larval behavior of the ascidian *Ecteinascidia turbinata* Herdman; an *in situ* experimental study of the effects of swimming on dispersal. *Journal of Experimental Marine Biology and Ecology* 145, 189–204.
- Carballo J.L., Naranjo S., Hernández-Zanuy A., and Kukurtzii B.** (1997) Estudio de la poblacion de *Ecteinascidia turbinata* Herdman 1880 (Ascidacea) en la isla de Formentera

- (Mediterraneo. España). Distribución, densidad y crecimiento. *Ciencias del Mar UAS* 15, 7-15.
- Carballo J.L.** (2000) Larval ecology of an ascidian tropical population in a Mediterranean enclosed system. *Marine Ecology Progress Series* 195, 159-167.
- Carballo J.L., Naranjio S., Kukurtzü B., De La Calle F., and Hernández-Zanuy A.** (2000) Production of *Ecteinascidia turbinata* (Ascidacea: Perophoridae) for obtaining anticancer compounds. *Journal of the World Aquaculture Society* 31(4), 481-490.
- Cornet C., and Ramos A.A.** (1980) Ascidacea. In: Estudio sistemático y ecológico de las esponjas y ascidias del Mediterráneo español. *Memoria Beca Fundación Juan March*, 362-499.
- Chebbi N.** (2010) Etude systematique, bioecologique et chimique des ascidies de Tunisie. Environmental Sciences. Université du 7 Novembre a Carthage; Institut National Agronomique de Tunisie. PhD Thesis.
- Chebbi N., Mastrototaro F., and Missaoui H.** (2010) Spatial distribution of ascidians in two Tunisian lagoons of the Mediterranean Sea. *Cahiers de Biologie Marine*. 51, 117-127.
- D'Incalci M. and Galmarini C.M.** (2010) A review of trabectedin (ET-743): A unique mechanism of action. *Molecular Cancer Therapy*. 9(8), 2157-2163.
- Elbaz M.A.** (2009) Bacterial communities associated with the sea squirts of the genus *Ecteinascidia* from the Red Sea and Florida. *Scholar Archive*. Paper 376. (MSc Oregon Health & Science University).
- Evans J., Barbara J., and Schembri P.J.** (2015) Updated review of marine alien species and other 'newcomers' recorded from the Maltese Islands (Central Mediterranean). *Mediterranean Marine Science*, 16(1), 225-244.
- Galil B.S.** (2012) Truth and consequences: the bioinvasion of the Mediterranean Sea. *Integrative Zoology* 7, 299-311.
- Galil B.S., Marchini A., Occhipinti-Ambrogi A., Minchin D., Narščius A., Ojaveer H., and Olenin S.** (2014) International arrivals: widespread bioinvasions in European Seas. *Ethology Ecology & Evolution*. 26 (2-3), 152-171.
- Galil B., Boero F., Frascchetti S., Piraino S., Campbell M., Hewitt C., Carlton J., Cook E., Jelmert A., Macpherson E., Marchini A., Occhipinti-Ambrogi A., Mckenzie C., Minchin D., Ojaveer H., Olenin S., and Ruiz G.** (2015) The enlargement of the Suez Canal and introduction of non-indigenous species to the Mediterranean Sea. *Limnology and Oceanography Bulletin* 24, 43-45.
- Garstang W.** (1891) Report on the Tunicata of Plymouth. I. Clavelinidae, Perophoridae, Diazonidae. *Journal of the Marine Biological Association of the UK*. 2, 47-67.
- Ghobashy A.F.A., and Abdel-Messeih M.K.** (1991) Ascidians in Egypt waters. *Journal of the Egyptian German Society of Zoology*. 4, 313-326.

- Goodbody I., and Cole L.** (2006) The tropical western Atlantic Perophoridae (ascidiacea) II. The genus *Ecteinascidia*. *Bulletin of Marine Science*. 79(1), 49-69.
- Harant H.** (1927a) La Faune Ascidiologiques de Banyuls et de Cette, essai de révision des ascidies de la Méditerranée occidentale. *Annales de l'Institut Océanographique* IV-V, :209-251.
- Harant H.** (1927b) Rapport sur les Tuniciers XXIV. Cambridge Expedition to the Suez Canal, 1924. *Transactions of the Zoological Society of London* 22, 365-374.
- Harant H.** (1939) Les fonds de pêche près d'Alexandrie. Ascidiacea. *Notes et Mémoires, Institut Fouad I Hydrobiologie et Pêche*, 28, 1-12.
- Harant H., and Vernières P.** (1933) Tuniciers. Fascicule 1: Ascidies. *Faune de France*, 27, 99.
- Herdman W.A.** (1882) Report on the Tunicata collected during the voyage of HMS Challenger during the years 1873-76. *Report on the scientific results of the voyage of H.M.S. Challenger*. 6, 1-297.
- Herdman W.A.** (1890) On the Genus *Ecteinascidia* and its Relations, with descriptions of two new species, and a classification of the Family Clavelinidae. *Transaction of the Biological Society of Liverpool*. 5, 160-161.
- Herdman W.A.** (1891) A revised classification of the Tunicata, with definitions of the orders, suborders, families, subfamilies, and genera, and analytical keys to the species. *Journal of the Linnean Society of London, Zoology*. 23, 558-652.
- Hernández-Zanuy A., Carballo J.L., García-Cagide A., Naranjo S. and Esquivel M.** (2007) Distribución y abundancia de la ascidia *Ecteinascidia turbinata* (Ascidiacea: Perophoridae) en Cuba. *Revista de Biología Tropical*. 55, 247-254.
- Hulme P.E.** (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*. 46, 10-18.
- Izquierdo-Muñoz A., Díaz-Valdés M. and Ramos-Esplá A.A.** (2009) Recent non-indigenous ascidians in the Mediterranean Sea. *Aquatic Invasions* 4, 59-64.
- Kott P.** (1952) Ascidians of Australia. 1 Stolidobranchiata and Phlebobranchiata. *Australian Journal of Marine and Freshwater Research* 3(3), 206-333.
- Kott P.** (1964) Stolidobranch and phlebobranch ascidians of the Queensland coast. Pap. Dep. Zool. Univ. Qd2(1), 127-52.
- Koutsogiannopoulos D., Zenetos A. and Ramos-Esplá A.A.** (2012) New ascidian records (Chordata: Tunicata) for the Aegean Sea (Eastern Mediterranean). In: Thessalou-Legaki M. et al. [Collective article] New Mediterranean Biodiversity Records (December 2012), pp. 321-322, *Mediterranean Marine Science* 13(2), 312-327.

- Lafargue F., Ramos A.A, Turon X., Banaigs B. & Whal M.** (1986) The littoral ascidians of the Spanish Mediterranean I. From Port Bou to the Islas Medas. *Vie Milieu* 36 (2), 133-139.
- López-Legentil S. & Turon X.** (2007) Lack of genetic variation in mtDNA sequences over the amphiatlantic distribution range of the ascidian *Ecteinascidia turbinata*. *Molecular Phylogenetics and Evolution* 45, 405–408.
- Luján I.** (1995) Biología y autoecología de *Ecteinascidia turbinata* Herdman, 1880 (Tunicata: Ascidiacea) en una zona templado cálida (SE Península Ibérica). M.Sc Thesis, University of Alicante.
- Meliane I.** (2002) Contribution to the knowledge of the ascidian fauna in the south-east of Tunisia. MSc. Thesis, University of Alicante.
- Michaelsen W.** (1918) Die Ptychobranchen und Diktyobranchen Ascidien des westlichen Indischen Ozeans. *Mitteilungen aus dem naturhistorischen Museum in Hamburg*. 35, 1-73.
- Monniot C. & Monniot F.** (1997) Records of ascidians from Bahrain, Arabian Gulf with three new species. *Journal of Natural History* 31(11), 1623-1643.
- Monniot C.** (1983) Ascidiés littoraux de Guadeloupe. II. Phlébobranches. *Bulletin du Muséum national d'histoire naturelle Paris*, 4 sér. 5, 51-71.
- Moss C., Green D.H., Perez B., Velasco A., Henriquez R. and McKenzie J.D.** (2003) Intracellular bacteria associated with the ascidian *Ecteinascidia turbinata*: phylogenetic and *in situ* analysis. *Marine Biology* 143, 99-110.
- Naranjo S.A.** (1995) Taxonomía, zoogeografía y ecología de las Ascidiás del Estrecho de Gibraltar. Implicaciones de su distribución bionómica en la caracterización ambiental de áreas costeras. Ph.D. Thesis, University of Sevilla.
- Nykjaer L.** (2009) Mediterranean Sea surface warming 1985–2006. *Climate research*. 39, 11–17.
- Pérès J. -M.** (1948) Sur une collection d'ascidiés de la zone intercotidale de Dakar. *Bulletin du Muséum national d'histoire naturelle Paris*. 20(1), 87-95.
- Pérès J. -M.** (1949) Contribution à l'étude des Ascidiés de la côte occidentale d'Afrique. *Bulletin de l'institut fondamental d'afrique noire*. XI (1-2).
- Pérès J. -M.** (1954) Contribution à l'études des Ascidiés de Tunisie. *Bull. Stat. Océanog. Salammbo*. 49, 3-21.
- Pérès J.M.** (1956) Résultats scientifiques des campagnes de la “Calypso” II. Ascidiés. *Annales Institute de Océanographie Paris*, 32, 267-304.
- Pérès J.M.** (1957) Ascidiés récoltées dans les parages des Baléars par le ‘Professeur Lacaze-Duthiers’ (deuxième partie : Ivisa et San Antonio). *Vie Milieu*, Suppl. 6 , 223-234.

- Pérès J. -M.** (1958) Origine et affinités du peuplement en ascidies de la Méditerranée. Rapp. P-v. *Comm. Int'l. Expl. Sci. Med.* 14, 493–502.
- Pérez-Matos A.E., Rosado W. and Govind N.S.** (2007) Bacterial diversity associated with the Caribbean tunicate *Ecteinascidia turbinata*. *Antonie van Leeuwenhoek* 92, 155–164.
- Por F.D.** (1978) Lessepsian Migration: the influx of Red Sea biota into the Mediterranean by way of the Suez Canal. Berlin: Springer-Verlag; 228 pp.
- Por F.D. and Dor I.** (1975) Ecology of the metahaline pool of Di Zahav, Gulf of Elat, with notes on the Siphonocladacea and the typology of near-shore marine pools. *Marine Biology*. 29, 37–44.
- Raitsos D.E., Beaugrand G., Georgopoulos D., Zenetos A., Pancucci-Papadopoulou A.M., Theocharis A. & Papathanassiou E.** (2010) Global climate change amplifies the entry of tropical species into the Eastern Mediterranean Sea. *Limnology and Oceanography*, 55, 1478–1484.
- Ramadan S.E., Kheirallah A.M. & Abdel-Salam K.H.** (2006) Marine fouling community in the Eastern harbour of Alexandria, Egypt compared with four decades of previous studies. *Mediterranean Marine Science* 7(2), 19–29.
- Ramos A.A.** (1984) Els ascidis de les Illes Medes. In: J. Ros, I. Olivella & J.M. Gili (eds.). *Els Sistemes Naturals de les Illes Medes*. Inst. Estud. Cat. Arx. Sec. Cienc. Barcelona, 73, 581–596.
- Ramos A.A.** (1988) Ascidias litorales del Mediterráneo Ibérico: Faunística, ecología y biogeografía. PhD Thesis I, University of Barcelona.
- Ramos A.A., Turon X., Wahl M., Banaigs, B. and Lafargue, F.** (1991) The littoral ascidians of the Spanish Mediterranean II. Balearic Islands. Species collected by the R/V “Professeur Georges Petit”. *Vie Milieu*, 41(2/3), 153–163.
- Ramos A.A., Buencuero V., Vázquez E. & Lafargue F.** (1992) Some biogeographical remarks about the Ascidian littoral fauna of the Straits of Gibraltar Iberian sector). *Bulletin de l'Institut Océanographique de Monaco*. 9, 125–132.
- Ramos A.A & Ben Moustapha K.** (2010) Etude des habitats marins et des principales espèces des Iles Kuriat (Tunisie). Rapport de la mission. APAL, RAC-SPA, Tunisie.
- Rennie J. and Wiseman R.** (1906) On collections of the Cape Verde Island marine fauna, made by Cyril Crossland of St. Andrew University, July to September 1904. *Proceedings of the Zoological Society of London*, 903–911.
- Rinehart K.H., Holt T.G., Fregeau N.L., Stroh J.G., Keiper P.A., Sun F., Li L.H. and Martin. D.G.** (1990) Ecteinascidins 729, 743, 745, 579A, 759B, and 770 potent antitumor agents from the Caribbean tunicate *Ecteinascidia turbinata*. *Journal of Organic Chemistry* 55, 452–455.

- Rodriguez E.** (1922) Fauna balear: tunicados existentes en la coleccion del laboratorio biologico marino de Baleares. *Boletin del Pescas.* 68, 97-111.
- Sanamyan K.** (2015) *Ecteinascidia turbinata* Herdman, 1880. In: Shenkar, N.; Gittenberger, A.; Lambert, G.; Rius, M.; Moreira Da Rocha, R.; Swalla, B.J.; Turon, X. (2015) Ascidiacea World Database.
- Sedra S.N. & Khalil S.H.** (1972) Studies on the Protochordates of the Alexandria district. *Bulletin of the Entomological Society of Egypt* 23, 75-89.
- Shenkar N. and Loya Y.** (2009) Non-indigenous ascidians along the Mediterranean coast of Israel. *Marine Biodiversity Records* 2, 1-7.
- Sluiter C.Ph.** (1905) Tuniciers rcueillis en 1904 par M. Ch. Gravier, dans le Golfe de Tadjourah (Somalie Franaise). *Mmoires de la Socit zoologique de France*, 18, 1-21+2pl.
- Streftaris N., Zenetos A., and Papathanassiou E.** (2005) Globalisation in marine ecosystems: The story of non-indigenous marine species across European seas. *Oceanography and Marine Biology: An Annual Review*. 43, 419-453.
- Turon X.** (1987) Estudio de las ascidias de las costas de Catalua e Islas Baleares. PhD Thesis, University of Barcelona
- Van Name W.G.** (1921) Ascidians of the West Indian Region and Southeastern United States. *Bulletin of the American Museum of Natural History*. 44, 283-494.
- Van Name W.G.** (1930) The ascidians of Porto Rico and the Virgin Islands. *Scientific Survey. Porto Rico and Virgin islands. New York Academy of Science*, 10, 401-512,
- Van Name W.G.** (1945) The North and South American Ascidians. *Bulletin of the American Museum of Natural History*. 84, 1-476.
- Young C.M. and Bingham B.L.** (1987) Chemical defence and aposematic coloration in larvae of the ascidian *Ecteinascidia turbinata*. *Marine Biology* 96, 539-544.
- Zaouali J.** (1976) Contribution ltude de la bionomie benthique de la mer de Bou Grara. *Archives Institute Pasteur Tunis* 3, 261-270.
- Zenetos A., Gofas S., Verlaque M. Cinar M.E., Garca Raso J.E., et al** (2010) 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. *Mediterranean Marine Science*, 11(2), 381-493.
- Zenetos A., Gofas S., Morri C., Rosso D., Violanti D., et al** (2012) Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part 2. Introduction trends and pathways. *Mediterranean Marine Science*, 13(2), 328-352.

Table 1. A summary of the sites at which *Ecteinascidia turbinata* was recorded in Malta.

Date	Site	Conditions
17 th August 2008	Old boat mooring line at St. George's Bay. Depth 6 m.	Good visibility (15-20m)
12 th July 2014	'Tug 2' wreck. Depth 15–20m.	Good visibility (15-20m)
10 th October 2014	Exiles reef St Georges Bay. Depth 10–15m.	Good visibility (15-20m)
18 th October 2014	'Tug 2' wreck. Depth 15–20m.	Good visibility (ca. 20m)
18 th July 2015	'Tug 2' wreck. Depth 15–20m.	Good visibility (15-20m)
10 th September 2015	Wreck of SS 'Margit', Kalkara creek, Valletta Harbour. Depth 20 m.	Low light, high suspended particle/silt. Temp 26°C
28 th October 2015*	'Tug 2' wreck. Also on a mooring block c200m from wreck. Depth 15–20m.	Good visibility (15-20m) Temp 22°C

* samples taken

Table 2. Temperature and salinity measurements from outer Kalkara Creek, site of the wreck of the SS 'Margit' measured in 2010 (Gianella Pisani, unpublished).

Date	Temperature (°C)		Salinity (ppt)	
	Surface	at 5 meters	Surface	at 5 meters
January	16.4	16.1	37.4	37.6
April	18.5	18.4	37.3	37.2
July	28.0	27.9	38.1	38.3
December	20.4	20.2	41.5	41.5

Figure 1 A map of the Mediterranean Sea and the immediate areas, showing the positions of known *Ecteinascidia turbinata* populations (Black circle - previous reports, Grey circles - this study). Two main areas are highlighted (dashed ovals). **A**, Alexandria, Egypt (Herdman 1880); **Al**, Alicante (Cornet & Ramos, 1980; Carballo *et al*, 1997); **B**, Balearics, Spain including Majorca (Ramos *et al*, 1991; Carballo *et al* 1997); **Bi**, Bizerte; **Ca**, Cadiz (Ramos, 1988; López-Legentil & Turon, 2007); **Cc**, Cape Creus (Harant & Vernières, 1933); **F**, Formentera Island (Ramos *et al*, 1991; Carballo *et al*, 1997); **G**, Gulf of Gabès (Pérès & Picard, 1956; Ben Mustapha *et al*, 2002); **Mo**, Monastir (Ben Mustapha *et al*, 2002); **K**, Kuriat Island, Tunisia (this study); **M**, Malta (this study); **Ma**, Marsala (Pérès, 1954); **Mu**, Murcia, Spain (this study); **P**, Argolikos Gulf, Peloponnese, Greece (Monniot, 1983; Koutsogiannopoulos *et al*, 2012); and **S**, the Suez Canal (Harant, 1927; Elbaz, 2009).

Figure 2. a,b) Two mature colonies of the ascidian *Ecteinascidia turbinata* photographed on the wreck of the SS ‘Margit’ in Kalkara Creek, Grand Harbour, Malta on 10 September 2015 (left), and a sample taken from “tug 2” on 28th October 2015 (right). **c) A single mature zooid with testes and ovary.** The external appearance of the colonies match the differential descriptions for the genus (Goodbody & Cole, 2006). The main distinguishing features include the close position of the orange coloured **exhalant** and **inhalant** siphons at the top of the zooid, the lack of red pigment spots between the lobes of the siphon edge and the relative straightness of the intestine (Berrill, 1932). Each individual zooid in both photographs is between 2 and 3 cm in length.

Figure 3. Branchial sac of *Ecteinascidia turbinata* from Malta: a) anterior-dorsal part of the zooid showing perypheral band (bp), dorsal tubercle (dt), neural gland (ng), dorsal languets (dl), anus (a) and sperm duct (sp.); b) dorsal continuous membrane (dm) with languets (dl); c) papillae (p) cylindrical and triangular. Bars: 1mm (a); 0.5mm (b,c).

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Only