Research Article

Symplegma (Asciidae: Styelidae), a non-indigenous genus spreading within the Mediterranean Sea: taxonomy, routes and vectors

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Abstract

Symplegma is a genus of compound ascidians (Fam. Styelidae) with warm water affinities and distribution in tropical and subtropical waters of the Pacific, Indian and Atlantic Oceans. The first record of this genus (as S. viride) in the Mediterranean was from 1951 in the Levantine Sea, presumably entering the basin from the Red Sea through the Suez Canal. Subsequently, it has been expanding its distributional range northward along the Levantine Sea coast, probably following the prevailing surface current direction. Recently, Symplegma has colonized the Aegean, Ionian and Tyrrhenian Seas, where it is spreading quickly, most likely mediated by shipping (i.e., hull fouling). Some specimens from the Ionian Sea (specifically from Tunisia, Malta) present opaque tunicas resembling the Indo-Pacific Symplegma bahraini; however, morphological studies suggest that the genus in the Mediterranean Sea is represented by a single species, Symplegma brakenhielmi. The taxonomy of S. brakenhielmi, as well as its spreading routes and possible introduction vectors are analysed.

Key words: alien species, colonial ascidians, color morphs, maritime traffic, distribution

Introduction

The opening of the Suez Canal, the expansion and increase in intensity of maritime traffic, aquaculture and the marine aquarium species trade are the main vectors of introduction of non-indigenous taxa in the Mediterranean Sea, accelerated and favoured by climatic change (Zibrowius 1992; Bianchi and Morri 2003; Streftaris et al. 2005; Galil 2006; Occhipinti-Ambrogi 2007; Abdulla and Linden 2008; Zenetos et al. 2012; Ferrario et al. 2017). Among the world’s seas, the Mediterranean Sea is the most invaded by non-indigenous species (NIS), at present hosting about 700 confirmed marine NIS (Zenetos et al. 2017; Galil et al. 2018). One genus of NIS
ascidians found in the Mediterranean is *Symplegma*, which normally occurs in shallow and warm waters of tropical and subtropical seas around the world (Van Name 1945; Tokioka 1967; Monniot and Monniot 1997a; Kott 2004).

Globally, there are 11 species within the *Symplegma* genus (Sanamyan 2007). Three species [*Symplegma viride* Herdman, 1886; *Symplegma brakenhielmi* (Michaelsen, 1904); and *Symplegma rubra* Monniot C., 1972] have a circumtropical distribution in the Indo-Pacific and the Atlantic Oceans, while the other *Symplegma* species occur solely in the Indian and Pacific Oceans, some of which present a wide distribution. For instance, *Symplegma reptans* (Oka, 1927) colonizes the Western and Eastern Pacific coasts (Nishikawa 1991; Lambert and Lambert 1998, 2003), while *Symplegma bahraini* Monniot C. and Monniot F., 1997 spans the whole Indo-Pacific region (Monniot 2002; Kott 2004). Other species have been reported from more restricted areas, such as the Western Pacific (*Symplegma connectens* Tokioka, 1949; *Symplegma japonica* Tokioka, 1962; *Symplegma alterna* Monniot C., 1988; and *Symplegma teruakii* Kott, 2004) and Indian Ocean (*Symplegma zebra* Monniot C., 2002).

Amongst the *Symplegma* species, only *S. brakenhielmi* has been reported from the Mediterranean. The first record was made in 1951 off Cesarea (Israel) as *S. viride* by Pérès (1958a). Since then, the genus has exhibited a slow northwards expansion (Bitar and Kouli-Bitar 2001; Çınar et al. 2006; Izquierdo-Muñoz et al. 2009; Shenkar and Loya 2009). In 2008, the species was recorded in the Ionian Sea (present work), probably introduced via shipping, since it had not been previously recorded from the east coast of Greece and southern Italy, areas which would putatively have been colonised at an earlier stage considering the prevailing surface current pathway of the Eastern Mediterranean (Hamad et al. 2005). From 2014, new records of the genus have been made within the Aegean, Ionian and Tyrrenhian Seas (Ulman et al. 2017; Aydin-Onen 2018; Mastrototaro et al. 2019; present work), indicating its rapid expansion throughout the Mediterranean, and its striking colours have also attracted attention from SCUBA divers.

The aim of this study was to clarify the taxonomy and update the distribution of the *Symplegma* genus in the Mediterranean Sea. Additionally, we discuss possible temporal changes in the distribution of the genus and the likely introduction vectors for *S. brakenhielmi* in the Mediterranean Sea. A distribution of *Symplegma* spp. at the global level is also provided.

**Materials and methods**

A comprehensive literature search on the genus *Symplegma* was conducted, including available published scientific works, “grey literature” (scientific congresses, technical reports, student theses), and web databases (Ascidian World Database, Biodiversity Heritage Library, Google Scholar,
Scopus, GBIF). Data from personal observations was also included, and first country Mediterranean records were cited according to the first actual collection/observation date, rather than the reporting date.

Colonies of *Symplegma* from different Mediterranean localities were observed and photographed at depths ranging from 0.5 to 17 m between 2005 and 2018 from natural and artificial substrates by snorkelling or SCUBA diving in the framework of different projects and studies carried out by the authors on benthic communities. Some of these colonies, collected from Egypt, Italy, Lebanon, Malta, Tunisia and Turkey (N = 14; Supplementary material Table S1) were dissected for subsequent histological studies in order to identify the specimens based on the morphological characters.

The colonies were anesthetised with menthol crystals, fixed with 4% formalin in seawater and preserved in 70º ethanol. Some zooids from each colony were dissected, stained with Masson’s haemalum, and dehydrated in ethyl and butyl alcohols for mounting on permanent slides in Canada balsam. Taxonomic identification was made considering the original and subsequent descriptions of *Symplegma* species in literature (Van Name 1945; Tokioka 1961, 1967; Monniot 1972, 1983, 1988; Kott 1985, 2004; Nishikawa 1991; Monniot and Monniot 1997a; Rocha and Costa 2005). The specimens examined in this study were deposited at the Marine Research Center of Santa Pola (CIMAR) of the University of Alicante (Spain) with the following identification codes: Sy.br-Eg01 (from Egypt), Sy.br-It01 (from Italy), Sy.br-Le01-02 (from Lebanon), Sy.br-Mt01-02 (from Malta), Sy.br-Tu01-07 (from Tunisia) and Sy.br-Tk01 (from Turkey).

**Results**

**Taxonomic identification**

In Mediterranean waters, the colonies of *Symplegma* exhibited a great variety of colour morphs (red, rose, orange, yellow, brown, cream, white, greenish-grey) which could suggest the occurrence of more than one species. The possible candidates include pantropical species, such as *S. brakenhielmi*, *S. rubra*, and *S. viride*. Other possible candidates were also considered, such as *S. reptans* from Japan (Tokioka 1949; Nishikawa 1991), which has invaded the North-eastern Pacific American coast (Lambert and Lambert 1998, 2003), and *S. bahraini* from the Indo-Pacific region (Monniot and Monniot 1997a; Kott 2004). Morphological studies led us to identify all the analysed colonies as *S. brakenhielmi*, although some of the specimens from Tunisia and Malta present morphological characters (especially an opaque tunic) resembling *S. bahraini*.

*Symplegma brakenhielmi* (Michaelsen, 1904)

(Figures 1, 2)

**Synonymies:** *Diandrocarpa brakenhielmi* var. *typica* Michaelsen (1904, p. 50); *D. brakenhielmi* var. *stuhlmanni* Michaelsen (1904, p. 52); *D. brakenhielmi* var. *ceylomica* Herdman (1906, p. 331);
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**Figure 1.** Colour morphs of *Symplegma brakenhielmi* living colonies in the Mediterranean Sea: A) Beirut airport with *Herdmania momus* (Photo by A.A. Ramos-Esplá); B) Monastir marina, Tunisia with *Botrylloides cf. nigrum* and *Styela plicata* (Photo by A.A. Ramos-Esplá); C) Fethiye, Turkey (Photo by A. Ulman); D) Kiyikislacik, Turkey (Photo by M.E. Çinar); E) wreck in front of Monastir (Photo by Y.R. Sghaier); F) Birzebbuga, Malta (Photo by A. Deidun).

*Gynandrocarpa quadricornulis*: Sluiter (1904 p. 95); *G. similis*: Sluiter (1904, p. 97). *Symplegma viride f. brakenhielmi*: Michaelsen (1918a, p. 105; Kott 1952, p. 252); *S. viride f. stuhlmanni*: Michaelsen (1918b, p. 39); *S. viride var. brakenhielmi*: Van Name (1921, p. 407); *S. oceania*: Tokioka (1961, p. 114); *S. stuhlmanni*: Kott (1998, p. 202); *S. brakenhielmi*: Monniot (1983 p. 429); part *S. viride*: (Van Name 1945, p. 232).

**Material examined:** See Table S1.

**Description:** Crusted and thin colonies, 1–2 mm thickness and up to 12 cm in diameter. Colonies were attached to ascidians, rock, seagrasses, mussels, sabellids, ropes and other artificial structures. Living colonies were light-coloured (red, rose, yellow, orange, rose, brown, white, grey; Figure 1), that turned greyish or yellowish upon fixation. The oval zooids were embedded by the tunic, depressed dorso-ventrally (rarely erect), close together, and randomly arranged without forming apparent systems. They represented different sizes (max. 3 mm), the youngest intercalated between the adults.
The thin test was rather tough in consistency and was transparent, allowing us to observe a “reticulated” pigmentation of the living zooids that concentrated in the branchial sac and the gut, although some colonies from Tunisia and Malta had a more opaque tunic. The siphons were similar, close and tubular and did not have coloured rings or a band around or between them. The thin and transparent mantle allowed the observation of the branchial sac and digestive tract. There were between 12 and 18 buccal simple tentacles, arranged in three orders, of which 6–9 were large and long and the others smaller (Figure 2A). The peribranchial area was bordered
by a narrow velum in a V-shape, where the oval dorsal tubercle, with a small aperture inside, was located (Figure 2A); and the dorsal lamina was smooth. There were very small atrial tentacles. Branchial sac was without folds, with four longitudinal vessels on each side of the body, with the first two on the left being incomplete and joining the dorsal lamina at the level of 6th–7th rows of stigmata (Figure 2B). In mature zooids, about 11–13 rows of stigmata on the left and 10–12 rows on the right were present, separated by transverse vessels of uniform size, which do not meet the medium dorsal vessel exactly opposite each other. The number of stigmata per half row was between 22 and 25, and there were usually from 4 to 5 stigmata between the internal longitudinal vessels, except next to the dorsal lamina, where 6–8 stigmata were present. The stigmata formula at the 5th row on the right side was the following: E 4-5 v 4 v 4 v 4 v 6-8 DL.

The gut occupied a third of the left part of the body and formed an open loop. The oesophagus was narrow and curved followed by a cylindrical or globular stomach. The stomach had between 9 and 13 well-marked longitudinal folds, and a short, curved and stout caecum, united by two connections to the intestine (Figure 2C). The rectum bent forward and ended in a non-lobed anus at the 5th–6th row of stigmata.

One hermaphrodite gonad was located on each side, with two lobed testes and an ovary with 1–4 ovules of different sizes (Figure 2D, E). Male and female gonads were present at the same time. The testes had 2–6 lobules, little or deeply divided, and the common spermiduct was narrow with variable length. In a particular colony (station MT-2), incubated eggs and 3–5 free larvae were concomitantly observed. The larvae presented a single organ (photolith) and three sharp papillae radially ranged (Figure 2E), with a length of 0.9 mm, corresponding to a length of 320 μm up to the trunk.

Biology and ecology (Mediterranean Sea): The colonies sampled in summer (July–September) and early autumn (October) showed fully developed gonads and free fertilized ovules; the colonies from Monastir (Tunisia) sampled at the end of August contained larvae.

*Symplegma brakenhielmi* colonised both natural (infralittoral rocky bottoms, seagrass meadows and caves) and artificial habitats (harbours, marinas), between 0.5 and 17 m of depth. It was observed attached to many different substrates: rocks, seagrasses (*Cymodocea nodosa*, *Halophila stipulacea*, known to occur in the area; Sghaier et al. 2011), ropes, pillars, ship hulls and on living invertebrates (i.e., other ascidians, bivalves, sabellids, hydrozoans).

Global distribution: Table 1 shows the distribution of the *Symplegma* spp. throughout the world. *Symplegma brakenhielmi* represents the most widely-distributed species, both in tropical (Atlantic and Indo-Pacific) and in warm-temperate waters (NE, NW and SW Atlantic; NW Pacific; Mediterranean Sea; and Australasia).
Table 1. Global distribution of the *Symplegma* spp. (regions and provinces, according to Spalding et al. 2007). Recorded as: (a) part. *S. viride*; (b) *S. viride* f. *brakenhielmi*; (c) *S. brakenhielmi*; (d) *Diandrocarpa botryllopsis*; (e) *Diandrocarpa brakenhielmi* f. *tipica*; (f) *S. viride*; (g) *Diandrocarpa brakenhielmi* f. *stuhlmanni*; (h) *S. brakenhielmi* v. *ceylonica*; (i) *S. viride* f. *stuhlmanni*; (j) *S. oceania*; (k) *Gynandrocarpa quadricornulis*; (m) *G. similis*; (n) *S. stuhlmanni*; (o) *S. reptans*; (p) *S. aff. viride*.

| Realm/province                     | *S. viride* | *S. brakenhielmi* | *S. rubra* | *S. bahraini* | *S. reptans* | Other Symplegma spp. |
|------------------------------------|-------------|-------------------|------------|---------------|--------------|---------------------|
| Temperate N Atlantic (Mediterranean Sea) | –           | See Table 2       | –          | –             | –            | –                   |
| Cold Temperate NW Pacific (Japan Sea) | –           | –                 | –          | –             | 14, 51       | *S. japonica*: 24, 51 |
| Warm Temp. NW Atlantic             | 8, 9\(^a\), 11\(^b\), 31\(^f\), 74 | 8\(^b\), 9\(^b\), 11\(^b\), 31\(^f\) | 74          | –             | –            | –                   |
| Warm Temperate NW Pacific          | –           | 61\(^i\)          | –          | –             | 14, 51       |                     |
| Warm Temperate NE Pacific          | –           | –                 | –          | –             | 60\(^a\), 71, 82 | –                   |
| Tropical W Atlantic                | 1, 2\(^d\), 9\(^o\), 10\(^q\), 11\(^r\), 22\(^w\), 39\(^u\), 42\(^w\), 43\(^u\) | 3\(^o\), 8\(^o\), 9\(^o\), 10\(^o\), 11\(^r\), 22\(^w\), 39\(^u\), 42\(^w\), 43\(^u\), 54\(^o\), 64, 67, 69, 77, 79, 86 | 33, 42, 54, 64, 67, 69, 77, 86 | –             | –            | –                   |
| Tropical E Atlantic                | –           | –                 | –          | –             | –            | –                   |
| W Indo-Pacific                     | –           | 3\(^o\), 5\(^o\), 6\(^o\), 7\(^o\), 18\(^o\), 19\(^o\), 26\(^o\), 28\(^o\), 45\(^o\), 49\(^o\), 57\(^o\), 84\(^o\), 4\(^o\), 4\(^o\), 7\(^o\), 16\(^o\), 21\(^o\), 25\(^o\), 27\(^o\), 35\(^o\), 36\(^o\), 40\(^o\), 41\(^o\), 44\(^o\), 48\(^o\), 59\(^o\), 66, 70, 72, 85 | 58, 68 | 57, 63, 65\(^o\), 68 | – | *S. zebra*: 68 |
| Central Indo-Pacific               | 84          | –                 | –          | –             | –            | *S. alterna*: 48, 72 |
| E Indo-Pacific                     | –           | 47\(^o\), 56\(^o\), 63\(^o\), 83\(^o\) | –          | –             | 63\(^o\)     | *S. arenosa*: 44, 72, 73 |
| Tropical E Pacific                 | –           | 80                | –          | –             | 82\(^o\)     | –                   |
| Warm Temperate SW Atlantic         | –           | –                 | –          | –             | –            | –                   |
| Mediterranean Sea distribution: Table 2 and Figure 3 show the spread of *S. brakenhielmi* through the Levantine, Ionian, Aegean and Trrhyrhenian Seas, with the first recorded year. |

**Discussion**

**Determination of Symplegma in the Mediterranean Sea**

The genus *Symplegma* was created by Herdman in 1886 (p. 144) and was initially represented by the single species *S. viride*. Michaelesen (1904, p. 50) regarded this genus as *nomen dubium* due to Herdman’s incomplete description and subsequently replaced it with the genus *Diandrocarpa* (Van
Table 2. Records of *Symplegma brakenhielmi* (Michaelsen, 1904) in the Mediterranean Sea by locality, year of the first record and references. Habitat: (A) artificial; (N) natural. Colour (living colonies): (b) brown; (c) cream; (g) greenish-gray; (o) orange; (p) pink; (r) red; (w) white; (y) yellow. (Id) identification by, (obs.) observed by (coll.) collected by: (AD) Alain Deidun; (AR) Alfonso Ramos-Esplá; (AU) Aylin Ulman; (GB) Ghazi Bitar; (JF) Jasmine Ferrario; (MEÇ) Melih E. Çinar; (YRS) Yassine R. Sghaier.

| Region          | Zone      | Locality     | Year     | Habitat | Colour | Source* | Observations                  |
|-----------------|-----------|--------------|----------|---------|--------|---------|------------------------------|
| Levantine Sea   | Israel    | Cesarea      | 1951     | –       | –      | 1       | as *S. viride*                |
|                  |          | Haifa Bay    | 1975     | –       | –      | 8       | det. R.H. Millar as *S. viride*|
|                  |          | Hadera, Achziv, Palmahim | 2005–2006 | A,N | r | 8 |  
|                  | Egypt     | Alexandria, Damietta | 1987–1988 | – | – | 9 |  
|                  |          | Porto Marina (Alamein) | 2013 | A | r | present work | coll. YRS (Id. AR) |
|                  | Lebanon   | Dalia (Beirut) | 1991–1992 | A | r | unpublished | Id. F. Monniot |
|                  |          | Several locat. (Beirut, Tripoli, Batroun etc.) | 1992–2016 | A,N | r,p | 2,3,4,6,7,10 | coll. GB (Id. AR); CEDRE |
| Turkey           | Yumurtaлиk | 2005 | A | r | 5,7 |  
|                  |          | Marmaris, Fethiye, Finike | 2008–2016 | A | b,w,r,p | 12,14 | coll. MEÇ, coll. AU (Id. AR) |
|                  | Cyprus    | Famagusta, Larnaca | 2016 | A | r | 11,14 | coll. AU (Id. AR) |
| Aegean Sea       | Turkey    | Akbük | 2016 | A | g | 13 |  
|                  | Greece    | Kiyikislaçik | 2016 | A | b | present work | coll. MEÇ (Id. AR) |
|                  |           | Heraklion, Agios Nicholaos (Crete) | 2015 | A | b,g | 14 | coll. AU |
|                |          | Mandraki port (Rhodes) | 2016 | A | w | 14 | coll. AU |
| Ionian Sea       | Tunisia   | Cap Monastir marina | 2008, 2014 | A,N | w,r | present work | coll. YRS (Id. AR) |
|                  |          | Ship wreck front to Monastir | 2017 | A | w,r,o,y,p,c | present work | coll. YRS (Id. AR) |
|                  | Italy     | El Kantawi port | 2015 | A | r | unpublished | coll. YRS |
|                  |          | Goulette Canal | 2015 | A | r | unpublished | coll. YRS |
|                  | Malta     | Birzebbuga (Marsaxlokk) | 2018 | A | y | present work | coll. AD, JF (Id. AR) |
|                  | Italy     | Taranto | 2016 | A | w,r,y | 15 | – |
| Tyrrhenian Sea   | Italy     | Palermo | 2016 | A | r | 12,14 | coll. AU (Id. AR) |
|                  |          | N-E Sardinian coasts | 2014, 2018 | A | w,r,y | 15 | – |

* (1) Péres (1958a); (2) Bitar and Koulī-Bītar (2001); (3) Abboud-Abi Saab et al. (2003); (4) Izquierdo-Muñoz et al. (2006); (5) Çınar et al. (2006); (6) Bitar et al. (2007); (7) Izquierdo-Muñoz et al. (2009); (8) Shenkar and Loya (2009); (9) Halim and Abdel-Messeih (2016); (10) RAC/SPA-UNEP/MAP (2015); (11) Savva and Kleitou (2017); (12) Ulman et al. (2017); (13) Aydin-Onen (2018); (14) Ulman (2018); (15) Mastrototaro et al. (2019).

Figure 3. Distribution of *Symplegma brakenhielmi* (red quadrats, new records are highlighted with an asterisk) in the Mediterranean Sea and Red Sea; year of first record (see references in Table 2), surface current direction (Hamad et al. 2005) and boundaries of the Mediterranean sub-basins (dashed lines) are also represented.

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Name, 1902) containing the single species *D. brakenhielmi*. However, Van Name (1921, 1930, 1945) considered both species synonymous, giving priority to *S. viride*, stating that *S. brakenhielmi* cannot even be maintained as a subspecies of *S. viride*. This categorical statement was supported by subsequent authors until the 1980s, when Monniot (1983) established the differences between the aforementioned two species (*S. viride* and *S. brakenhielmi*), together with *S. rubra*, in Caribbean waters. Therefore, many pre-1983 publications on *Symplegma* in the Western and Eastern Atlantic, as well as in the Indian Ocean, adopted Van Name’s description (1945) and considered these congeneric species as a single species: *S. viride*. Thus, one can infer that the first records of *Symplegma* (as *S. viride*; Herdman 1886) in the Suez Canal and Mediterranean Sea (Harant 1927; Pérès 1958a, b; Steiniz 1967; Por 1978; Koukouras et al. 1995), and Tropical Western Africa (Pérès 1949, 1951; Millar 1953; Monniot 1969; Lafargue and Wahl 1986) correspond to *S. brakenhielmi*.

*Symplegma brakenhielmi*, *S. rubra* and *S. bahraini* present lobed testes in contrast to *S. viride* and *S. reptans* without lobed testes. However, in *S. brakenhielmi*, the tunic is transparent in living colonies with the pigments localised in the blood cells, thus imparting a reticulated aspect due to the concentration in the branchial sac and gut (*S. rubra* and *S. bahraini* have opaque tunics). Additionally, *S. brakenhielmi* does not have the characteristic red rings that encircle both siphons (a characteristic of *S. rubra*); although Herdman (1906) and Tokioka (1961) do indicate the presence of red circles around the siphons in *S. brakenhielmi* var. *ceylonica* and *S. oceania* (a synonym of *S. brakenhielmi*), respectively. Other characters, such as the number of testes lobes and the division between them (more numerous and deeper in *S. rubra* and *S. bahraini*) as well as the length and width of the common spermiduct are less diagnostic (Monniot 2002; Couto 2003). The simultaneous presence of testes and ovules in *S. brakenhielmi* and *S. bahraini* separates them from *S. rubra* (Monniot 1983; Monniot and Monniot 1997a), but this is not applicable for immature zooids (Monniot et al. 2001). Rocha and Costa (2005) included the following other characters to distinguish *S. brakenhielmi* from *S. rubra*: the swollen dorsal tubercle of the former, with a small circular aperture inside; two incomplete dorsal left longitudinal vessels, reaching the dorsal lamina at the level of the 4th and 7th rows of stigmata; and only two tissue connections linking the caecum with the intestine. These characteristics have all been observed in the zooids of samples originating from the Mediterranean, and a molecular study conducted by Mastrototaro et al. (2019) corroborates the presence of *S. brakenhielmi* in the basin.

It has to be noticed that the pigmentation of the tunic and the deeply divided testicular follicles from the Maltese and Tunisian (i.e. Monastir) specimens resemble *S. bahraini* (Monniot and Monniot 1997a; Kott 2004). However, Monniot and Monniot (1987: 10) found colonies of *S. oceania*
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(synonymy of *S. brakenhielmi*) with a pigmented tunic; and Tokioka (1961, Figure 7) drew deeply divided testicular follicles for the same species. The presence of larvae in the Tunisian colonies with a single sensory organ (Millar 1953; Kott 1985; Mastrototaro et al. 2019) suggests that these colonies are more closely related to *S. brakenhielmi*, since *S. bahraini* has two sensory organs (Monniot and Monniot 1997a). Furthermore, the remaining morphological characters fall within the range of *S. brakenhielmi* and its synonyms (Monniot and Monniot 1997a; Monniot 2002; Kott 2004), such as its stomach folds, rows of stigmata, stigmata per row, pyloric caecum and its links with the intestine; longitudinal vessels on the left join the dorsal lamina, and the simultaneous presence of functional male and female gonads.

In the present study some colonies sampled in summer and autumn showed fully developed gonads or contained larvae. Larvae had also been recorded in June in Israel (Levantine Sea) by Shenkar and Loya (2009); and in July in North-eastern Sardinia (Tyrrenian Sea) by Mastrototaro et al. (2019). Generally, data on reproductive period is scarce and scattered, e.g. from January to May in the Tropical Eastern Atlantic (Senegal: Pérès 1949; Monniot 1969; Ghana: Millar 1953), as well as immature colonies in January (Pérès 1951); in May in the Southern Indian Ocean (Mauritius: Vasseur 1967); from July to October in the Northern Indian Ocean (S-India: Renganathan 1985; Bahrain: Monniot and Monniot 1997a); and in November in the Tropical Pacific (W-Australia: Kott 1985).

In conclusion, morphological studies on different color morphs (red, yellow, white, brown, pink) with transparent and opaque tunics led us to the same species in the Mediterranean Sea: *S. brakenhielmi*. In this regard, Mastrototaro et al. (2019) have not found significant morphological or genetic differences between red and yellow colonies of *S. brakenhielmi* from the Western (Sardinia) and Central (Taranto) Mediterranean Sea, thus highlighting the possible synonymy between *S. brakenhielmi* and *S. rubra* by Automatic Barcode Gap Discovery (ABGD) species delimitation analysis. However, to confirm this, it is necessary to perform genetic analysis of our specimens and compare them with *S. bahraini* and *S. rubra*.

Possible causes of spread in the Mediterranean Sea: routes and vectors

Michaelsen (1918b) recorded *S. brakenhielmi* (as *S. viride f. stuhlmanni*) in the Gulf of Suez (Red Sea) in 1914. Later in 1924, Harant (1927) found it in the Suez Canal at El Katera, 46 km away from Port Said. Pérès (1958a) reported the species (as *S. viride*) in 1951 from the Mediterranean coast of Israel, together with *Ascidia cannelata* Oken, 1920, *Phallusia nigra* Savigny, 1816 and *Hermania momus* Savigny, 1816, solitary ascidians of Indo-Pacific origin. The same author (Pérès 1958b) also argued how these new findings were probably recent introductions from the Red Sea via the Suez Canal, since he had only found them in Israel, and not in Syria, Greece or Turkey.
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In the Levantine Sea, *S. brakenhielmi* has progressively spread northwards, following the trajectory of the prevailing surface coastal current (Figure 3; Hamad et al. 2005), and has become a common species on both artificial and natural habitats in this area (Bitar and Kouli-Bitar 2001; Çinar et al. 2006; Izquierdo-Muñoz et al. 2006, 2009; Bitar et al. 2007; Shenkar and Loya 2009). In Cyprus it was found on artificial substrata in harbours in 2016 (Savva and Kleitou 2017; Ulman 2018; Ulman et al. 2019), although a study conducted in the same country in Larnaca Bay in June 2007 (UNEP-MAP RAC/SPA 2007) did not find the species; this may suggest a recent introduction mediated by shipping. Regarding the westward spread further along the coasts of Egypt, Halim and Abdel Messeih (2016) recorded the species in Alexandria in 1988–89 and in El Alamein, west of Alexandria, in 2013 (YRS pers. obs., present study), both within ports. All living colonies observed were the reddish or pink colour morphs (Figure 1A).

The recent appearance of *S. brakenhielmi* along the Aegean coasts of Turkey (in 2015–2016 at Kiyikislacik and Akbük; MEÇ pers. obs. and Aydin-Onen 2018) and Greece (Rhodes and Heraklion; Ulman 2018; Ulman et al. 2019) might suggest a further expansion of the species within the Aegean Sea through the Eastern Mediterranean surface current. However, these observed colonies had white, brown and greenish-grey morphs (not red; Figure 1C–D) and were found in harbours and rocks near fish-farm cages, which could suggest either aquaculture and/or shipping as the main introduction vectors.

In the Ionian Sea, the first record was made in October 2008 (Monastir Marina, Tunisia; AR pers. obs.), followed by new records in October 2014 (present study). The species was observed on rocks and seagrass meadows (*Cymodocea nodosa* and *Halophila stipulacea*) around the harbour with white and red colour morphs. Studies carried out in 2005 and 2006 in Hammamet, 70 km north of Monastir (Chabbi et al. 2010), and 2009–2010 in the Gulf of Gabes (Zarzis and Sfax harbours, Ramos-Esplá et al. 2011), did not find this species. In December 2014, the merchant-ship “Rochelle” sailing from Ghana to Turkey was stranded near the Kuriat Islands in front of Monastir, and was colonized by abundant colonies of *S. brakenhielmi* with different colours (red, pink, yellow, orange, cream, white; Figure 1) on the hull. Later, the species was recorded in other Tunisian localities further north (YRS pers. obs.), including the port of El Kantaoui (October 2015) and Goulette Canal (November 2015), all with red color morph. Monastir is an important marina for boat traffic, thus possibly representing a propagule seeding hub for adjacent ports. International maritime traffic by commercial vessels could be considered the most likely vector of introduction of *S. brakenhielmi*, e.g. specifically for Marsaxlokk (Malta), which represents one of the main cargo trans-shipment ports of the Mediterranean Sea; recreational boats could also be a vector (Ulman et al. 2017; Ulman 2018; Mastrototaro et al. 2019). *Symplegma brakenhielmi* was
frequently recorded within several sampled marinas, and it was also found on recreational boat hulls in Turkey (Ulman et al. 2017), suggesting that recreational boating may be an important secondary vector in its rapid expansion through the intensely sailed Mediterranean Sea (Cappato 2011).

As previously mentioned, together with \textit{S. brakenhielmi}, Péres (1958a) also identified three other solitary Lessepsian ascidians: \textit{A. cannelata}, \textit{P. nigra} and \textit{H. momus}. While the former ascidian remains localised to the south-eastern portion of the Levantine Sea (Izquierdo-Muñoz et al. 2009; Shenkar and Loya 2009), \textit{P. nigra} and \textit{H. momus} have formed proliferating populations further northward, following the same colonization dispersal pattern promoted by surface currents through the Levantine and Aegean Seas (Çinar et al. 2006; Kondylatos et al. 2010; Koutsogiannopoulos et al. 2012; Gerovasileiou and Issaris 2014; Kondylatos and Corsini-Foka 2017).

At present, only \textit{H. momus} has reached the Ionian Sea in Marsaxlokk, Malta (Evans et al. 2013), which is very close to the sampling site of \textit{S. brakenhielmi} recorded in this study and was most likely introduced through maritime traffic.

Therefore, the colonial \textit{S. brakenhielmi} appears to be spreading faster than these three solitary ascidians. The possible causes of this postulated expansion could be traced to the characteristics of its colonial strategy and larval development (Millar 1971). The rapid growth (as encrusting colonies) and short life cycle of this species are, in fact, competitive strategies for space (Jackson 1977). \textit{Symplegma brakenhielmi} exhibits two-dimensional growth, and the larva develops inside the zooids (Berrill 1940), which is advantageous for the colonization of artificial structures within fouling communities and for growth as an epibiont (Green et al. 1983; Ramos-Esplá and Ros 1990; Rocha 1991; Dijkstra et al. 2007), whereas \textit{P. nigra} and \textit{H. momus} are oviparous ascidians, with less-specialised larva (Millar 1971).

Ascidians are one of the most frequent NIS in artificial habitats due to their rapid spread and population outbreaks (Lambert 2007; Zhan et al. 2015). Generally, ascidians can be associated with multiple vectors of introduction, including ballast water, rafting, hull fouling and aquaculture (Zhan et al. 2015). While the role of ballast water in the transport of ascidians remains unclear, several studies provide evidence for the colonisation success of ascidians on recreational boat hulls as biofouling components (e.g. Darbyson et al. 2009; Lambert 2002; Ulman et al. 2017), rafting colonies living attached to seagrass leaves (Worcester 1994), and in association with shell-fish aquaculture facilities (e.g. Davis et al. 2007; Ordóñez et al. 2015). Among the \textit{Symplegma} genus, the three species recorded in two or more distant biogeographic regions (i.e., \textit{S. brakenhielmi}, \textit{S. rubra} and \textit{S. reptans}; Table 1) are likely to have been associated with the biofouling and aquaculture vectors of introduction and spread (Rocha et al. 2009; Carman et al. 2011; Mastrotroto et al. 2019; Ulman et al. 2019). In addition, colonies of \textit{S. rubra} have also been observed on free-floating
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algae and other drifting materials (Dias et al. 2006), thus could have been transported by rafting. Similarly, *Symplegma* species recorded in closer biogeographic regions (i.e. *S. viride*, *S. bahraini* and *S. japonica*; Table 1) may have locally spread through shipping or by natural means (Tamilselvi et al. 2011; Zhan et al. 2015).

Currently, regulations addressing the control of NIS only relate to aquaculture (EC 2007) and ballast water (Ballast Water Management Convention of the International Maritime Organization, IMO) vectors, while management of the biofouling vector is still unregulated and insufficiently assessed in the Mediterranean Sea. Specific management guidelines for this vector were previously formulated (IMO 2011), and an ad-hoc project (GEF-UNDP-IMO GloFouling Project) was launched in December 2018 in order to address the management of biofouling. The continuous monitoring programmes in high-risk areas of NIS introduction (e.g. ports and aquaculture facilities) and the use of preventive measures to limit NIS spread are recommended best practices against marine bioinvasions (Lehtiniemi et al. 2015). The Mediterranean Sea brings together many national jurisdictions, placing the onus for the effective management of new introductions and their related vectors on a basin-wide early detection and warning system. Finally, the dissemination of taxonomic knowledge and the elucidation of ambiguous taxonomy are essential for an accurate species identification, particularly urgent for the class Asciidiacea (Shenkar and Swalla 2011; Zhan et al. 2015).

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**References**

Abbott DP, Newberry AT, Morris KM (1997) Reef and Shore Fauna of Hawaii. 6B: Ascidians (Urochordata). Spec. Publ. Lambert G (ed), Bernice Pauahi Bishop Museum Press, Honolulu, 64 pp

Abboud-Abi Saab M, Bitar G, Harmelin JG, Harmelin-Vivien M, Romano JC, Zibrowius H (2003) Environnement côtier et biodiversité marine sur les côtes libanaises. Programme Coopération Franco-Libanais CEDRE (1999-2002), 80 pp

Abdull Jaffar Ali H, Tamilselvi M (2016) Ascidians in Coastal Water: A Comprehensive Inventory of Ascidian Fauna from the Indian Coast. Springer, 157 pp, https://doi.org/10.1007/978-3-319-29118-5

Abdulla A, Linden O (2008) Maritime Traffic Effects on Biodiversity in the Mediterranean Sea. Vol. 1: Review of impacts, priority areas and mitigation measures. IUCN Center Mediterranean Cooperation, Malaga, 168 pp

Aydin-Onen S (2018) 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* 18: 1077–1089, https://doi.org/10.4194/1303-2712-v18_9_07
Berrill NJ (1932) Ascidians of the Bermudas. Biological Bulletin 62: 77–88, https://doi.org/10.2307/1537145

Berrill NJ (1940) The development of a colonial organism: Symplegma viride. Biological Bulletin 79: 272–281, https://doi.org/10.2307/1537822

Bianchi CN, Morri C (2003) Global sea warming and ‘tropicalization’ of the Mediterranean Sea: biogeographic and ecological aspects. Biogeographia 24: 319–327, https://doi.org/10.21426/Be611029

Bitar G, Kouli-Bitar S (2001) Nouvelles données sur la faune et la flore benthiqes de la côte Libanaise. Migration Lessepsienne. Thalassia Salentina 25: 71–74

Bitar G, Ocaña O, Ramos-Espla AA (2007) Contribution of the Red Sea alien species to structuring some benthic biocenosis in the Lebanon coast (Eastern Mediterranean). Rapport Commission international Mer Méditerrané 38: 437

Cappato A (2011) Cruises and Recreational Boating in the Mediterranean. Sophia Antipolis: Plan Bleu/UNEP MAP Regional Activity Centre, 74 pp

Carlton JT, Eldredge LG (2009) Settlement and potential for transport of clubbed tunicate (Styela clava) on boat hulls. Aquatic Invasions 4: 95–103, https://doi.org/10.3391/ai.2009.4.1.10

Davis MH, Lützen J, Davis ME (2007) The spread of Symplegma viride (Tunicata, Ascidiaeae, Styelidae) encontradas no canal de Sao Sebastiao-SP. M.Sc Th., University Sao Paulo, 127 pp

Darbyson EA, Hanson JM, Locke A, Willson JM (2009) Settlement and potential for transport of clubbed tunicate (Styela clava) on boat hulls. Aquatic Invasions 4: 95–103, https://doi.org/10.3391/ai.2009.4.1.10

Costa HR (1969) Notas sobre os Ascidiacea brasileiros. VI. Atlântico Sul. Biologia Tropical 41(1): 35–38

Dias GM, Duarte LFL, Solferini VN (2006) Low genetic differentiation between isolated populations of the colonial ascidian Symplegma rubra. Marine Biology 148: 807–815, https://doi.org/10.1007/s00227-005-0111-5

Dijkstra J, Sherman H, Harris LG (2007) The role of colonial ascidians in altering biodiversity in marine fouling communities. Journal of Experimental Marine Biology and Ecology 342: 169–171, https://doi.org/10.1016/j.jembe.2006.10.035

EC (2007) Council regulation (EC) No 708/2007 of 11 June 2007 concerning use of alien and non-indigenous marine species into Pearl Harbor, Oahu, Hawaiian Islands. Marine Biology 135: 147–158, https://doi.org/10.1007/s00227-005-050612

Costa HR (1969) Notas sobre os Ascidiacea brasileiros. VI. Atlântico Sul. Biologia Tropical 41(1): 35–38

Dijkstra J, Sherman H, Harris LG (2007) The role of colonial ascidians in altering biodiversity in marine fouling communities. Journal of Experimental Marine Biology and Ecology 342: 169–171, https://doi.org/10.1016/j.jembe.2006.10.035

EC (2007) Council regulation (EC) No 708/2007 of 11 June 2007 concerning use of alien and locally absent species in aquaculture. Official Journal of the European Union, 17 pp

Evans J, Borg JA, Schembri PJ (2013) First record of Herdnania momus (Ascidiaeae: Pyuridae) from the central Mediterranean Sea. Marine Biodiversity Records 6: e134, https://doi.org/10.1017/S1755267213001127

Ferrario J, Marchini A, Caronni S, Occhipinti-Ambrogi A (2017) Role of commercial harbours and recreational marinas for the spread of fouling non-indigenous species. Biofouling 33: 651–660, https://doi.org/10.1080/08927014.2017.1351958

Galil BS (2006) The marine caravan - The Suez Canal and the Erythrean invasion. In: Gollasch S, Galil BS, Cohen AN (eds), Bridging divides - Maritime canals as invasion corridors. Springer, Dordrecht 83: 207–300, https://doi.org/10.1007/978-1-4020-5047-3_6

Galil BS, Marchini A, Occhipinti-Ambrogi A (2018) East is east and West is west? Management of marine bioinvasions in the Mediterranean Sea. Estuarine, Coastal and Shelf Science 201: 7–16, https://doi.org/10.1016/j.ecss.2015.12.021

Gerovasileiou V, Issaris Y (2014) First record of the Indo-Pacific ascidian Herdnania momus (Savigny, 1816) in Greek waters. In: S. Katsanevakis et al. New Mediterranean Biodiversity Records (October, 2014). Marine Biodiversity Records 7: 25–28, https://doi.org/10.1017/S1755267214001126

Goodbody I (1984) The ascidian fauna of two contrasting lagoons in the Netherlands Antilles: Piscadera Baai, Curaçao, and the lac of Bonaire. Studies on the Fauna of Curaçao and Other Caribbean Islands 67(202): 21–61

Goodbody I (1993) The ascidian fauna of a Jamaican lagoon: Thirty years of change. Revista de Biologia Tropical 41(1): 35–38

Ramos-Espla et al. (2020), Aquatic Invasions (in press)
Kott P (2005) Catalogue of Tunicata in Australian Waters. Canberra: Australian Biological Resources Study, 301 pp

Hartmeyer R, Michaelsen W (1928) Ascidiae Dictyobranchiae und Psychobranchiae. Fauna Sudwest-Australiens 5: 251–460

Herdman WA (1886) Report on the Tunicata collected during the voyage of H.M.S. Challenger during the years 1873-1876. Part II. Ascidiae compositae. Reports of Science Research Voyage of H.M.S. Challenger Zoology 14(38): 1–432

Herdman WA (1906) Report on the Tunicata collected by Prof. Herdman at Ceylon in 1902. Report to the Government of Ceylon on the Pearl Oyster Fisheries

IMO (2011) Guidelines for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species. Annex 26 Resolution MEPC.207(62). Adopted on 15 July 2011, 25 pp

Izquierdo-Muñoz A, Diáz-Valdés M (2006) Contribución al estudio de la clase Ascidiacea (Chordata: Tunicata) en los puertos del Mar Mediterráneo. XIV Simposio Ibérico de Estudios de Biología Marina, Barcelona, September 2016: 147

Izquierdo-Muñoz A, Diáz-Valdés M, Ramos-Esplá AA (2009) Recent non-indigenous ascidians in the Mediterranean Sea. Aquatic Invasions 4: 59–64, https://doi.org/10.3391/ai.2009.4.1.5

Jackson JBC (1977) Competition on marine hard substrata: the adaptive significance of solitary and colonial strategies. American Naturalist 111: 743–767, https://doi.org/10.1086/283203

Kondylatos G, Corsini-Foka M (2017) Two aliens new to the Hellenic Aegean waters: Parupeneus forskali (Perciformes, Mullidae) and Herdmania momus (Tunicata). In: Stamouli C et al. New Mediterranean Biodiversity Records (December 2017). Mediterranean Marine Science 18, p 544, https://doi.org/10.12681/mms.15823

Kondylatos G, Corsini-Foka M, Pancucci-Papadopoulou MA (2010) Occurrence of the first non-indigenous ascidian Phallusia nigra Savigny, 1816 (Tunicata: Ascidiacea) in Greek waters. Aquatic Invasions 5: 181–184, https://doi.org/10.3391/ai.2010.5.2.08

Kott P (1952) The Ascidians of Australia. I. Stolidobranchiata Lahille and Phlebobranchiata. Lahille, Australian Journal of Marine and Freshwater Research 3: 205–333, https://doi.org/10.1071/MF9520205

Kott P (1964) Stolidobranch and Phlebobranch Ascidians of the Queensland Coast. University of Queensland Papers 2(7): 127–152

Kott P (1972) The ascidians of South Australia II. Eastern sector of the Great Australian Bight and Investigator strait. Transactions of the Royal Society of South Australia 96(4): 165–195

Kott P (1975) The ascidians of South Australia III. Northern sector of the Great Australian Bight and additional records. Transactions of the Royal Society of South Australia 99(1): 1–19

Kott P (1976) The ascidian fauna of Western Port, Victoria and a comparison with that of Port Phillip Bay. Memoirs of the National Museum of Victoria 37: 53–95, https://doi.org/10.24199/mvnc.1976.37.06

Kott P (1981) The Ascidians of the Reefs Flats of Fiji. Proceedings of the Linnean Society of New South Wales 105(3): 147–212

Kott P (1985) The Australian Ascidiae Part I, Phlebobranchia and Stolidobranchia. Memoirs of the Queensland Museum 23: 1–440

Kott P (1988) Tunicata. In: Wells A, Houston WWK (eds), Zoological Catalogue of Australia. Hemichordata, Tunicata, Cephalochordata. Melbourne, CSIRO Publ, Australia 34, pp 51–259

Kott P (2004) Ascidiae (Tunicata) in Australian waters of the Timor and Arafura Seas. Beagle: Records of the Museums and Art Galleries of the Northern Territory 20: 37–81

Kott P (2005) Catalogue of Tunicata in Australian Waters. Canberra: Australian Biological Resources Study, 301 pp

Kott P, Goodbody I (1982) The Ascidians of Hong Kong and Southern China. In: Morton BS, Tseng CK (eds), The marine Flora and Fauna of Hong Kong and Southern China. Proceedings of the first international marine biological workshop. Hong Kong University Press, Hong Kong, pp 503–554
Koutouzianniopoulos D, Zenotos A, Ramos-Esplá AA (2012) New ascidian records (Chordata: Tunicata) for the Aegean Sea (Eastern Mediterranean). In: Thessalou-Legaki M et al. New Mediterranean Biodiversity Records (December 2012). Mediterranean Marine Science 13, pp 321–322, https://doi.org/10.12681/mms.313

Koukouras A, Voultsiadiou-Koukoura E, Kevrekidis T, Vafidis D (1995) Ascidian fauna of the Aegean Sea with a check list of the Eastern Mediterranean and Black Sea species. Annales de l’Institut oceanographique de Paris 71(1): 19–34

Lafargue F, Vasseur P (1989) Ascidies des récifs du N.E. du Canal de Mozambique (Campagne ‘Benthedi’ du N.O. “Suroit”, 17 mars-14 avril 1977). Mésogée 49: 59–66

Lafargue F, Wahl M (1986) Contribution to the knowledge of littoral ascidians (Asciadia, Tunicata) of the Senegalese coast. Bulletin de l’Institut Fonndamental d’Afrique Noire Cheikh Anta Diop: Série A, Sciences de la vie, sciences de la terre 46(3–4): 385–402

Lambert G (2002) Nonindigenous ascidians in tropical waters. Pacific Science 56: 291–298, https://doi.org/10.1353/pac.2002.0026

Lambert G (2003) Marine biodiversity of Guan: the Asciadia. Micronesica 35–36: 584–593

Lambert G (2007) Invasive sea squirts: a growing global problem. Journal of Experimental Marine Biology and Ecology 342: 3–4, https://doi.org/10.1016/j.jembe.2006.10.009

Lambert CC, Lambert G (1998) Non-indigenous ascidians in southern California harbors and marinas. Marine Biology 130: 675–688, https://doi.org/10.1007/s002270050289

Lambert CC, Lambert G (2003) Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. Marine Ecology Progress Series 259: 145–161, https://doi.org/10.3354/meps259145

Lambert G, Faulkcs Z, Lambert CC, Scofield VL (2005) Ascidians of South Padre Island, Texas, with a key to species. Texas Journal of Science 57(3): 251–262

Lee SSC, Chan JYH, Teo SLM, Lambert G (2016) State of knowledge of ascidian diversity in other Caribbean Islands. Marine Biology and Ecology 324: 718–743

Lehtiniemi M, Ojaveer H, David M, Galil B, Gollasch S, McKenzie C, Minchin D, Occhipinti-Ambrogi A, Olenin S, Pederson J (2015) Dose of truth-Monitoring marine non-indigenous species to serve legislative requirements. Marine Policy 54: 26–35, https://doi.org/10.1016/j.marpol.2014.12.015

Lotufo TMC (2002) Asciadia (Chordata: Tunicata) do Littoral Tropical Brasileiro. PhD Thesis, University of Sao Paulo, 183 pp

Mastrotorto F, Montesanto F, Salonna M, Greico F, Trainito E, Chimienti G, Gissi C (2019) Hitch-hikers of the sea: concurrent morphological and molecular identification of Symplegma brakenhiemli (Tunicata: Asciadia) in the western Mediterranean Sea. Mediterranean Marine Science 20: 197–207, https://doi.org/10.12681/mms.19390

Michaelsen W (1904) Revision der compositen Styeliden oder Polyzoien. Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten, Hamburg 21(2): 1–124

Michaelsen W (1918a) Ascidie Psychobranchiae und Diktyobranchiae des Roten Meeres, Denkschriften der Kaiserlichen Akademie der Wissenschaften. Mathematisch-Naturwissenschaftlichen Classe 95(10): 1–122

Michaelsen W (1918b) Die phychobranchen und diktyobranchen Ascidien des westlichen Indischen Ozeans. Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten, Hamburg 35: 75 pp

Millar RH (1953) On a collection of ascidians from the Gold Coast. Proceedings of the Zoological Society of London 123: 277–325, https://doi.org/10.1111/j.1096-3642.1953.tb00176.x

Millar RH (1955) On a collection of ascidians from South Africa. Proceedings of the Zoological Society of London 125: 169–221, https://doi.org/10.1111/j.1096-3642.1955.tb00597.x

Millar RH (1956) Ascidians from Mozambique, East Africa. Annals and Magazine of Natural History 12: 913–932, https://doi.org/10.1080/00222935608655917

Millar RH (1958) On some Ascidians from Brazil. Annals and Magazine of Natural History 13: 497–514, https://doi.org/10.1080/00222935808650975

Millar RH (1962) Some Ascidians from the Caribbean. Studies on the Fauna of Caraçao and other Caribbean Islands 59: 61–77

Millar RH (1971) The Biology of Ascidians. Advances in Marine Biology 9: 1–100, https://doi.org/10.1016/S0065-2881(08)60341-7

Millar RH (1975) Ascidians from the Indo-west-Pacific region in the Zoological Museum, Copenhagen (Tunicata Asciadiae). Steenstrupia 3: 205–336

Millar RH (1977) Ascidians (Tunicata: Asciadiae) from the Northern and North-eastern Brazilian shelf. Journal of Natural History 11: 169–223, https://doi.org/10.1080/00222937700770131

Monniot C (1969) Sur une collection d’ascidies de Dakar (Philipobranches et Stolidobranches). Bulletin du Musée National d’Histoire Naturelle 41(3): 622–654

Monniot C (1972) Ascidies Stolidobranches des Bermudes. Bulletin du Muséum National d’Histoire Naturelle 57(3): 617–643

Monniot C (1983) Ascidies littorales de Guadelupe IV Styelidae. Bulletin du Muséum national d’histoire naturelle. Section A, Zoologie, biologie et écologie animales 5(2): 423–456

Monniot C (1988) Ascidies de Nouvelle-Caledonie IV Styelidae (suite). Bulletin du Muséum National d’Histoire Naturelle 10A(2): 163–196
Monniot C (2002) Stolidobranch ascidians from the tropical western Indian Ocean. Zoological Journal of the Linnean Society 35: 65–120, https://doi.org/10.1006/jzls.2002.0017

Monniot C, Monniot F (1987) Les ascidies de Polynésie française. Memoires du Muséum National d'Histoire Naturelle 136: 1–155

Monniot C, Monniot F (1997a) Records of ascidians from Bahrain, Arabian Gulf with three new species. Journal of Natural History 31: 1623–1643, https://doi.org/10.1080/00222939700770871

Monniot F, Monniot C (1997b) Ascidians collected in Tanzania. Journal of East African Natural History 86: 1–35, https://doi.org/10.2982/0012-8317(1997)61:ACIT2.CO;2

Monniot F, Monniot C (2001) Ascidians from the tropical western Pacific. Zoosystema 23: 201–383

Monniot C, Monniot F, Griffiths CL, Schleyer M (2001) South African Ascidians. Annals of South African Museum 108(1): 1–141

Nishikawa T (1991) The Ascidians of the Japan Sea II. Publications of the Seto Marine Biological Laboratory 35: 25–170, https://doi.org/10.5314/176172

Occhipinti-Ambrogi A (2007) Global change and marine communities: alien species and climate change. Marine Pollution Bulletin 55: 342–352, https://doi.org/10.1016/j.marpolbul.2006.11.014

Ordóñez V, Pascual M, Fernández-Tejedor M, Pineda MC, Tagliapietra D, Turon X (2015) Ongoing expansion of the worldwide invader Didemnum vexillum (Ascidiaeae) in the Mediterranean Sea: high plasticity of its biological cycle promotes establishment in warm waters. Biological Invasions 17 : 2075–2085, https://doi.org/10.1007/s10530-015-0861-1

Pérès JM (1949) Contribution à l’étude des ascidies de la côte occidentale d’Afrique. Bulletin de l’Institut Français d’Afrique Noire 11: 159–207

Pérès JM (1951) Nouvelle contribution à l’étude des ascidies de la côte occidentale d’Afrique. Bulletin de l’Institut Français d’Afrique Noire 13: 1051–1071

Pérès JM (1958a) Ascidies récoltées sur les côtes méditerranéennes d’Israël. Bulletin of the Research Council of Israel 7(B): 143–150

Pérès JM (1958b) Ascidies de la baie de Haifa collectées par E. Gottlieb. Bulletin of the Research Council of Israel 7(B): 151–164

Plante R, Vasseur P (1966) Sur une collection d’ascidies de la région de Tuléar (côte Sud-Ouest de Madagascar). Annales de l’Université de Madagascar. Série A: Sciences de la Nature et Mathématiques 4: 143–157

Plough HH (1978) Sea Squirts of the Atlantic Continental Shelf from Maine to Texas. Johns Hopkins University Press Ltd, London, 132 pp

Por FD (1978) Lessepsian migration: The influx of Red Sea biota into the Mediterranean by way of the Suez Canal. Springer-Verlag Berlin and New York, 244 pp

RAC/SPA-UNEP/MAP (2015) Ecological characterization of sites of interest for conservation in Lebanon. Ramos-Espá AA, Bitar G, Khalaf G, El Shaer H, Forcada A, Limam A, Ocaña G, Sghaiyer YR, Valle C. MedMPAnet Project, Tunis, 146 pp

Ramos-Espá AA, Ros JD (1990) Tipos biológicos en ascidias litorales de sustratos duros. Bentos 6: 283–299

Ramos-Espá AA, Ayadi H, Mouelhi S, Hattour S, Drira A, Elaouani Z, El Lakhrach J, Monniot F, Monniot C (2001) Ascidians from the tropical western Pacific. Zoosystema 23: 201–383

Rocha RM (1991) Replacement of the compound ascidian species in a southeastern Brazilian fouling community. Boletim do Instituto Oceanográfico 39: 141–153, https://doi.org/10.1590/S0101-81752005000200005

Rocha RM, Faría SB (2005) Ascidians at Currais Islands, Paraná, Brazil: taxonomy and distribution. Biota Neotropica 5: 1–20, https://doi.org/10.1590/S1676-060320050000300013

Rocha RM, Costa LVG (2005) Ascidians (Urochordata: Ascidiaeae) from Arraial do Cabo, Rio de Janeiro, Brazil. Heringia. Série Zoologia 95: 57–64, https://doi.org/10.1590/S0073-47122005000100009

Rocha RM, Faría SB, Moreno TR (2005a) Ascidians from Bocas del Toro, Panama. I. Biodiversity. Caribbean Journal of Science 43(3): 600–612

Rocha RM, Moreno TR, Metri R (2005b) Ascidias (Tunicata, Ascidiaeae) da Reserva Biológica Marinha do Arvoredo, Santa Catarina, Brasil. Revista Brasileira de Zoologia 22: 461–476, https://doi.org/10.1590/S0101-81752005000200024
Rocha RM, Kremer LP, Baptista MS, Metri R (2009) Bivalve cultures provide habitat for exotic tunicates in southern Brazil. Aquatic Invasions 4: 195–205, https://doi.org/10.3391/ai.2009.4.1.20

Rocha RM, Guerra-Castro E, Lira C, Marquez Pauls S, Hernández I, Pérez A, Sardi A, Pérez J, Herrera C, Carbonini AK, Caraballo V, Salazar D, Diaz MC, Cruz-Motta JJ (2010) Inventory of ascidians (Tunicata, Ascidiae) from the National Park La Restinga, Isla Margarita, Venezuela. Biota Neotropica 10: 209–218, https://doi.org/10.1590/S1676-0603201000100021

Rocha RM, Dias GM, Lotufo TMC (2011) Checklist das ascidias (Tunicata, Ascidiae) do Estado de são Paulo, Brasil. Biota Neotropica 11: 749–759, https://doi.org/10.1590/S1676-06032011000500036

Rodrigues SA (1962) Algumas ascidias do littoral sul do Brasil. Boletim Facultad de Filosofía Ciencia y Letra Universidad Sao Paulo 261: 193–215, https://doi.org/10.11606/issn.2526-3382.bflczoologia.1962.120585

Rodrigues SA, Rocha RM (1993) Littoral compound ascidians (Tunicata) from Sao Sebastiao, Estado de Sao Paulo, Brazil. Proceedings of the Biological Society of Washington 106: 728–739

Rodrigues SA, Rocha RM, Lotufo TMC (1998) Guia Ilustrado para Identificaçao das Ascidias do Estado Sao Paulo. IBUSP/FAPEST, 190 pp

Sanamyan K (2007) Symplegma Herdman, 1886. In: Shenkar N, Gittenberger A, Lambert G, Rius M, Rocha RM, Swalla BJ, Turon X (2015) Ascidiae World Database. Symplegma Herdman, 1886. Accessed through: World Register of Marine Species. http://www.marine.species.org/aphipa.php?taxid=206885 (accessed 2019-09-02)

Savva I, Kleito P (2017) On three alien species from Cyprus (eastern Mediterranean Sea). In: Gerovasileiou V et al. New Mediterranean Biodiversity Records (July 2017) Mediterranean Marine Science 18(2), pp 355–384, https://doi.org/10.12681/mms.13771

Sghaier Y, Zakhama-Sraieb R, Benamer I, Charfi-Cheikhrouha F (2011) Occurrence of the seagrass Halophila stipulacea (Hydrocharitaceae) in the southern Mediterranean Sea. Botanica Marina 54: 575–582, https://doi.org/10.1515/BOT.2011.061

Shenkar N, Loya Y (2009) Non-indigenous ascidians (Chordata: Tunicata) along the Mediterranean coast of Israel. Marine Biodiversity Records 2: e166, https://doi.org/10.1017/S1755276209990753

Shenkar N, Swalla BJ (2011) Global diversity of Ascidiae. PLoS ONE 6: e20657, https://doi.org/10.1371/journal.pone.0020657

Sluiter CP (1904) Die Tunicaten der Siboga-Expedition. Die Socialen and Holosomen Ascidien. Die Tunicaten der Siboga-Expedition. Die Socialen and Holosomen Ascidien. Botanica Marina 54: 575–582, https://doi.org/10.1515/BOT.2011.061

Steinitz H (1967) A tentative list of immigrants via the Suez Canal. Israel Journal of Zoology 16(3): 166–169

Streftaris N, Zenetos A, Papathanassiou E (2005) Globalisation in marine ecosystems: the story of non-indigenous marine species across European seas. In: Gibson RN, Atkinson RJA, Gordin JDM (eds), Oceanography and Marine Biology. An Annual Review 43: 419–453, https://doi.org/10.10120/9781420037449.ch8

Tamilselvi M, Sivakumar V, Abdul Jaffar Ali H, Thilaga RD (2011) Distribution of alien tunicates (Ascidians) in Tuticorin coast, India. World Journal of Zoology 6(2): 164–172

Tokioka T (1949) Contribution to Japanese ascidian fauna II. Notes on some ascidians collected chiefly along the coast of Kii Peninsula. Publication of the Seto Marine Biological Laboratory 1: 39–64, https://doi.org/10.5134/174434

Tokioka T (1961) Ascidians collected during the Expedition of the Osaka Museum of Natural History. I. Ascidians presented by Dr R. L. A. Catala of the Aquarim of Noumea. Publication of the Seto Marine Biological Laboratory 9: 104–138, https://doi.org/10.5134/174659

Tokioka T (1962) Contribution to Japanese ascidian fauna XVIII. Ascidians from Sado Island and some records from Sagami Bay. Publication of the Seto Marine Biological Laboratory 1: 39–47, https://doi.org/10.5134/174434

Tokioka T (1967) Pacific Tunicata of the United States National Museum. Bulletin of the United States National Museum 251: 1–247, https://doi.org/10.5479/si.03629236.251.1

Tokioka T, Nishikawa T (1975) Contributions to the Japanese ascidian fauna XXVII. Some ascidians from Okinawa, with notes on a small collection from Hong Kong. Publication of the Seto Marine Biological Laboratory 22: 323–341, https://doi.org/10.5134/175896

Tovar-Hernández MA (2012) Tunicata . In: Low-Pfeng AM, Peters EM (eds), Invertebrados marinos exóticos en el Pacífico mexicano Cap. VI. Geomare, AC, Inesemarnat, México, pp 85–106

Ulman A (2018) Recreational boating as a major vector of spread of non-indigenous species around the Mediterranean. PhD Thesis, Universities of Sorbonne and Pavia, 219 pp

Ramos-Esplá et al. (2020), Aquatic Invasions (in press)
Ulman A, Ferrario J, Occhipinti-Ambrogi A, Arvanitidis C, Bandi A, Bertolino M, Bogi C, Chatzigeorgiou G, Çiçek BA, Deidun A, Ramos-Esplá A, Koçak C, Lorenti M, Martinez-Laiz G, Merlo G, Princisgh E, Scribano G, Marchini A (2017) A massive update of non-indigenous species records in Mediterranean marinas. PeerJ 5: e3954, https://doi.org/10.7717/peerj.3954

Ulman A, Ferrario J, Forcada A, Arvanitidis C, Occhipinti-Ambrogi A, Marchini A (2019) A hitchhiker’s guide to Mediterranean marinas travel for alien species. Journal of Environmental Management 241: 329–339, https://doi.org/10.1016/j.jenvman.2019.04.011

UNEP-MAP RAC/SPA (2007) Integrated Coastal Area Management in Cyprus: Biodiversity Concerns on the Coastal Area Management Programme of Cyprus. AA Ramos, D Cebrián and A Dementropoulos. Ed. RAC/SPA, Tunis, 69 pp

Van der Sloot CJ (1969) Ascidians of the Family Styelidae from the Caribbean. Studies on the Fauna of Curaçao and other Caribbean Islands 30(110): 1–57

Van Name WG (1902) Ascidians from Bermuda Islands. Transaction of the Connecticut Academy of Arts and Sciences 5(11): 325–412

Van Name WG (1921) Ascidians of the West Indian region and Southeastern United States. Bulletin of the American Museum of Natural History 44: 284–494

Van Name WG (1930) The ascidians of Porto Rico and the Virgin Islands. Scientific Survey of Puerto Rico 10: 403–512

Van Name WG (1945) The North and South American ascidians. Bulletin of the American Museum of Natural History 84: 1–476

Vasseur P (1967) Contribution à l’étude des ascidies de l’Île Maurice (Archipel des Mascareignes, Océan Indien). Recueil des Travaux de la Station marine d’Endoume 6: 101–139

Worcester SE (1994) Adult rafting versus larval swimming: dispersal and recruitment of a botryllid ascidian on eelgrass. Marine Biology 121: 309–317, https://doi.org/10.1007/BF00346739

Zenetos A, Gofas S, Morri C, Rosso A, Viollandi D, Garcia Rasò JE, Çinar ME, Almogi-Labin A, Ates AS, Azzurro E, Ballesteros E, Bianchi CN, Bilecenoglou M, Gambi MC, Giangrande A, Gravili C, Hyams-Kaphzan O, Karachle PK, Katsanevakis S, Lipej L, Mastrototaro F, Mineur F, Panucci-Papadopoulou MA, Ramos-Esplá A, Salas C, San Martin G, Sfriso A, Streftaris N, Verlaque M (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 Sciences 13: 328–352, https://doi.org/10.12681/mms.327

Zenetos A, Çinar ME, Crocetta F, Golani D, Rosso A, Serpellon G, Shenkar N, Turon X, Verlaque M (2017) Uncertainties and validation of alien species catalogues: The Mediterranean as an example. Estuarine, Coastal and Shelf Science 191: 171–187, https://doi.org/10.1016/j.ecss.2017.03.031

Zhan A, Briski E, Bock DG, Ghabooli S, MacIsaac HJ (2015) Ascidians as models for studying invasion success. Marine Biology 162: 2449–2470, https://doi.org/10.1007/s00227-015-2734-5

Zibrowius H (1992) Ongoing modification of the Mediterranean marine fauna and flora by the establishment of exotic species. Mésogée 51: 83–107