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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Sponge species from ports of the inner and middle parts of İzmir Bay (Aegean Sea, Eastern Mediterranean)

İzmir iç ve orta körfezi limanlarından sünger türleri (Ege Denizi, Doğu Akdeniz)

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Abstract: Within a surveillance programme for the detection of invasive alien species, many benthic samples were randomly collected from several ports located in the inner and middle parts of Izmir Bay. A total of four sponge species (*Sycon raphanus*, *Sycon ciliatum*, *Paraleucilla magna* and *Dysidea fragilis*) were found on the artificial hard substrata. *Paraleucilla magna*, which is being newly recorded from the Aegean coast of Turkey, is an invasive alien species and very abundant almost at all ports, covering maximally 35% of surfaces sampled. The morphological and distributional features of these species are described.

Keywords: Porifera, Demospongiae, Calcarea, Invasive Alien Species, biodiversity, distribution, Aegean Sea, Mediterranean Sea

Öz: İstilacı yabancı türlerin tespiti için yapılan bu araştırmada, İzmir Körfezi'nin iç ve orta kısımlarında bulunan çeşitli limanlardan birçok bentik örnek rastgele toplanmıştır. Yapay sert substrat üzerinde toplam dört sünger türü (*Sycon raphanus*, *Sycon ciliatum*, *Paraleucilla magna* ve *Dysidea fragilis*) tespit edilmiştir. Türkiye'nin Ege kıyılarından yeni kaydedilmekte olan *Paraleucilla magna*, istilacı yabancı bir türdür, örneklenen yüzeylerin maksimum % 35'ini kaplamakta olup neredeyse tüm limanlarda bol miktarda tespit edilmiştir. Bu çalışma, bu türlerin morfolojik ve dağılım özelliklerini açıklamaktadır.

Anahtar kelimeler: Porifera, Demospongiae, Calcarea, İstilacı yabancı tür, biyoçeşitlilik, dağılım, Ege Denizi, Akdeniz

INTRODUCTION

Port environments are separated from nearby coastal systems due to the artificial structures they have and continuous shipping activities (Awad et al., 2014). Such activities make the environments more prone to invasion by alien species. It is well known that one of the main pathways for the introduction of alien species is the transport by vessels, especially in ballast waters (Carlton, 1985; Galil, 2009). However, the organism transfer by hull fouling is also widespread in the world's oceans (Bax et al., 2003). The polluted environments in ports favor the establishment of alien species of wide niche breadths. For example, on polluted hard- and soft-bottoms of Alsancak and İskenderun Harbours, which are located in the eastern Mediterranean Sea, alien species (ship-mediated species in Alsancak Harbour, lessepsian invaders in İskenderun Harbour) comprised more than 70% of total zoobenthic populations (Çinar, 2006; Çinar, et al., 2006). Many alien invertebrates and fish were reported on floating docks and other artificial surfaces in ports rather than adjacent natural substrates (Glasby and Connell, 2001; Paulay et al., 2002; Çinar et al., 2006; Perçin, 2018). Therefore, port surveys are very important for the detection and surveillance of alien species.

The Mediterranean Sea is known to be a hot spot area for the introduction of alien species as it has more than one pathway including the Suez Canal (Çinar et al., 2011). More than 900 alien species have been recorded up to date in the Mediterranean Sea (Zenetos et al., 2012). Among alien species, a total of 603 alien species have become established in the region (Zenetos et al., 2017). The majority of alien species (almost 75%) belonging to different taxonomic groups are known from the eastern part of the Mediterranean. However, there is a lack of reliable information regarding alien sponge species in the Mediterranean (Zibrowius, 2002). Acording to Zenetos (2010) eight alien sponge species might be considered as Lessepsian species in the Mediterranean Sea. However, the presence of these species in the Mediterranean was regarded as questionable. It is now apparent that only *Paraleucilla magna* was considered as an established alien species in the Mediterranean, but its origin is still unknown (Longo et al., 2007; Zammit et al., 2009; Guardiola et al., 2011). This species was also previously reported on the natural substrata in the Sea of Marmara (Topaloğlu et al., 2016).

The present study aims to determine the diversity of sponge species inhabiting ports of İzmir Bay and to present their distributional and morphological features

MATERIAL AND METHODS

The sponge specimens were collected on artificial substrata (concrete surface, buoys, submerged ropes and tyres) at 0–3 m depth at 8 ports located in the inner and middle parts of İzmir Bay between March and May 2016 (Figure 1).

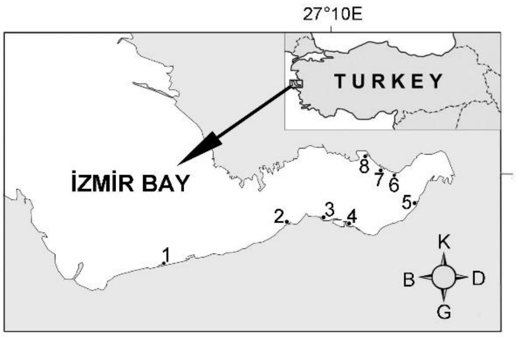

Figure 1. Locations of sampling sites: 1. Güzelbahçe Fishing Port, 2. Sahilevleri Fishing Port, 3. İnciraltı Fishing Port, 4. Levent Marina, 5. Pasaport Ferry Terminal, 6. Karşıyaka Sailing Club, 7. Karşıyaka Fishing Port, 8. Bostanlı Fishing Port

All sponges were scraped from surfaces and fixed in 4% formaldehyde. To estimate the percent coverage of *Paraleucilla magna* at ports, three-replicated quadrates (25 × 25 cm) were put randomly on surfaces sampled. In the laboratory, all sponge specimens were washed under tap water and then transferred to vials including 70% ethanol.

For the examination of skeletal structures, skeleton preparations were made by hand dissection under a stereomicroscope. Spicule slides were prepared according to the method proposed by Klautau and Valentine (2003). A minimum of 25 spicules of each type were measured with an ocular micrometer. Some specimens were dehydrated in ethanol and embedded in paraffin wax and sectioned with a microtome at various thicknesses in order to analyse the shape and size of the choanocyte chambers. The specimens identified are deposited at the Museum of the Faculty of Fisheries, Ege University (ESFM).

RESULTS

Examination of sponge specimens collected from the ports of İzmir Bay yielded a total of 4 sponge species (*Sycon raphanus*, *S. ciliatum*, *Paraleucilla magna* and *Dysidea fragilis*) belonging to 3 families (Sycettidae, Amphoriscidae and Dysideidae) and 2 classes (Calcarea and Demospongiae). The invasive alien species *P. magna*, which is being recorded for the first time from the Aegean coast of Turkey, was found in all ports with high percent coverages.

Class: CALCAREA Bowerbank, 1864
Family: Sycettidae Dendy, 1893
***Sycon ciliatum* (Fabricius, 1780)** (Figure 2)

Spongia ciliata Fabricius ,1780: 448.
Sycandra ciliata; Haeckel, 1870: 296, pls. 151, 58, 51.
Sycon ciliatum; Borojevic, et al., 1968: 33.

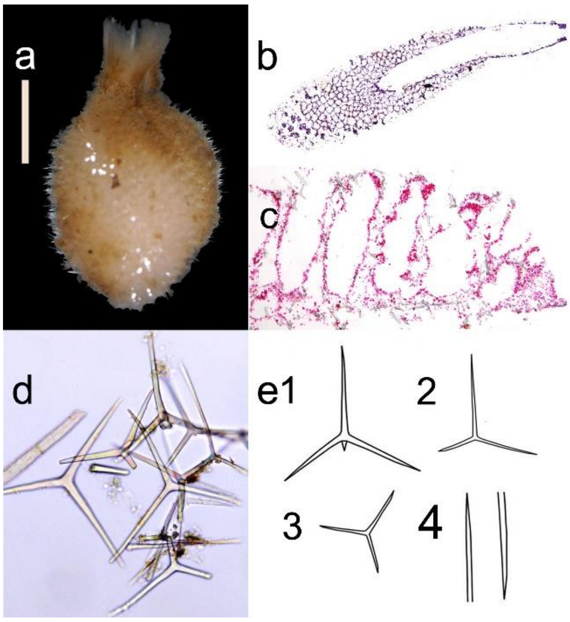

Figure 2. *Sycon ciliatum*. a. General view (scale: 3 mm) b. longitudinal median section of an entire specimen (scale 3 mm), c. Choanocyte chambers (scale: 100 µm), d. Overview of spicules (scale: 50 µm) e1. Subregular tetractine (scale: 40 µm), e2. Large subatrial triactine (scale: 100 µm), e3. Small tubar triactine (scale: 120 µm), e4. Diactine (scale: 100µm).

Material examined: ESFM–POR/2016–1, station 1, May 2016, 0.5 m, on tyre, 8 specimens; ESFM–POR/2016–2, station 2, May 2016, 1 m, on tyre, 6 specimens; ESFM–POR/2016–3, station 3, May 2016, 0.5 m, on tyre, 18 specimens.

Description: Living members of this species have a soft consistency. The body is vase-shaped. Specimens high 2.5 cm diameter up to 0.8 cm wide. The terminal large osculum is surrounded by a fringe of long stiff diactines (Figure 2a). The surface is hairy. In addition, spicules are visible on the surface of body. Specimens have a short stalk. Specimens are usually solitary or occur in small groups. Colour is grey, white and occasionally brown in life and in ethanol. The aquiferous system is syconoid (Figure 2b). The choanocyte chambers are partially cylindrical (100–220 µm), entirely free from each other (Figure 2c). The skeleton consists of tiractines, tetractines and diactines (Figure 2d). The atrial skeleton is composed of sagittal triactines and tetractines. Subregular tetractines (basal actines: 40–80 X 8–10 µm, apical actines: 50–110 X 9–12 µm) are regularly overlapping in the atrial skeleton. Actines are slightly conical and sharp (Figure 2, e1). Subatrial skeleton consists of brushes of larger triactines. Actines are slightly conical and sharp. The paired actines are straight or slightly curved. The unpaired actine is frequently longer than the paired ones (paired actines: 100–220 X 5–12 µm, unpaired actines: 90–260 µm X 8–16 µm) (Figure 2, e2). The tubar skeleton consists of small triactines with long and thin diactines. Triactines is subregular to sagittal (paired actines: 80–120 x 4–8 µm unpaired actines: 90–140 x 5–10 µm). Diactines are sharp and slightly curved (900–2800 x 6–20 µm) (Figure 2, e3-e4).

Habitat and Distribution: It is a common species in the shallow sublittoral zone, rarely in depths of more than 150 m, in the Mediterranean, the Atlantic and the Arctic (Longo and Pronzato, 2011; Van Soest et al., 2017b; Rapp, 2013). Along the coasts of Turkey, this species was previously reported from the Sea of Marmara by Ostroumoff (1894; 1896) and Topaloğlu et al., (2016).

Sycon raphanus Schmidt, 1862 (Figure 3)

Sycon raphanus Schmidt, 1862: 14–15, pl. i, Figure 2; Burton, 1956: 115; Tsurnamal, 1975: 146, Figure 4.

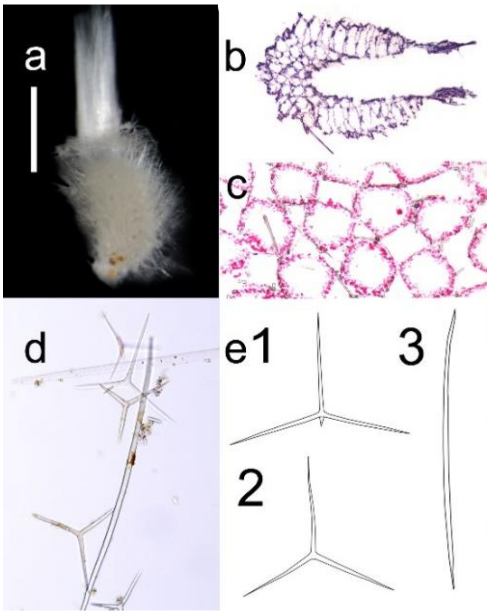

Figure 3. *Sycon raphanus*. a. General view (scale: 2 mm), b. longitudinal median section of an entire specimen (scale 2.5 mm), c. Choanocyte chambers (scale: 100 µm) d. Overview of spicules (200 µm), e1.Atrial tetractines (scale: 60 µm), e2. Tubar triactines (scale: 100 µm), e3. Diactine (scale: 300 µm)

Material examined: ESFM–POR/2016–4, station 1, May 2016, 1 m, on tyre, 8 specimens; ESFM–POR/2016–5, station 2, May 2016, 0–1 m, on tyre, 6 specimens.

Description: Alive specimens of this species have a fragile tissue. Color is white in life and in ethanol. Specimens have globular or tube in shaping up to 0.9 cm high and 0.5 cm wide with short stalk. Numerous diactines on the surface make it very hispid (Figure 3a). Specimens are usually solitary or occur in small groups. The aquiferous system is syconoid and the atrium is central (Figure 3b). The choanocyte chambers (range from 60–150 µm) are almost fused in the distal cone (Figure 3c). The skeleton consists of triactines, tetractines and diactines (Figure 3d). Tubar skeleton and distal cones are composed of triactines with slightly curved paired actines (paired actines 90–180 X 10–12 µm and unpaired actines 150–250 X 10–12 µm) and diactines. The atrial skeleton is composed of subregular to regular (rarely sagittal) tetractines and triactines. Tetractines of the atrial skeleton are similar to triactines. (apical actines 55–90 x 6–10 µm, basal actines 100–210 x 8–12 µm) (Figure 3, e1, e2). Diactines are slightly curved (900–2800 x 20–25 µm) (Figure 3e, 3).

Habitat and Distribution: This species is very common in the shallow water benthic habitats of the Mediterranean Sea and the eastern Atlantic coasts. It occasionally occurs in depths down to 300 m depth (Longo and Pronzato, 2011; Van Soest et al., 2017b). It was previously reported from the coasts of Turkey, except for the Black Sea coast (Topaloğlu and Evcen 2014).

Family: Amphoriscidae Dendy, 1893
Paraleucilla magna Klautau, Monteiro & Borojevic, 2004 (Figure 4,5)

Paraleucilla magna Klautau et al., 2004: 1–8, Figure 2 (a-c); Longo et al., 2007: 1749–1755, Figure 2-5; Topaloğlu et al., 2016: 54, Figure 3.

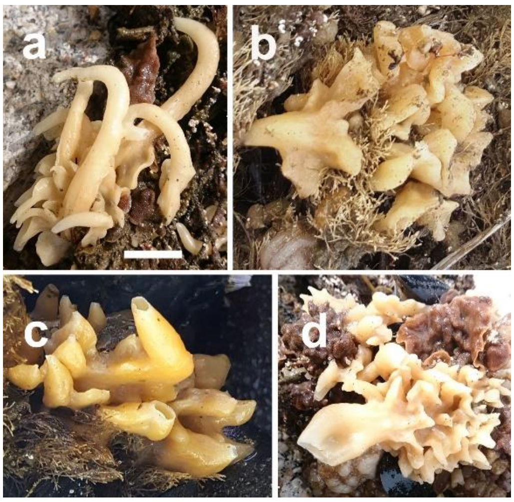

Figure 4. Different morphologies of *Paraleucilla magna* in İzmir Bay. Scale bar: a= 2 cm, b= 2.5 cm, c= 4 cm, d= 4 cm

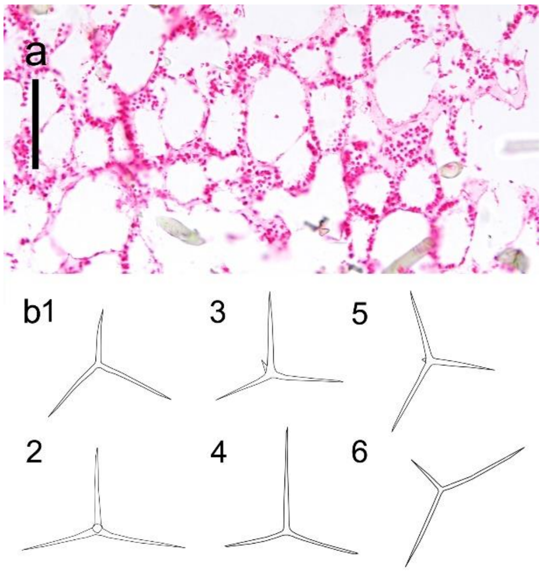

Figure 5. Cross section through the choanocyte chamber of *P. magna* (scale: 120 µm), b. Spicules, b1. Cortical triactine (scale 300 µm), b2, 3. Cortical tetractine (scale: 320 µm), b4. Subatrial triactine (scale: 200 µm), b5. Subatrial tetractine (scale: 300 µm), b6. Atrial triactine (scale: 150 µm).

Material examined: ESFM–POR/2016–6, station 1, March 2016, 0.3 m, on tyres, 4 specimens; ESFM–POR/2016–7, station 2, March 2016, 0.3–1 m, on tyres and concrete walls (particularly on Mytilus galloprovincialis Lamarck, 1819), 13 specimens; ESFM–POR/2016–8, station 3, March 2016, 0.5 m, on used tyre and buoys, 3 specimens; ESFM–POR/2016–9, station 4, March 2016, 0.3 m, on tyres, buoys and walls, 15 specimens; ESFM–POR/2016–10, station 5, March 2016, 1–3 m, on rope, 2 specimens; ESFM–POR/2016–11, station 6, April 2016, 1 m, on rope and tyres, 3 specimens; ESFM–POR/2016–12, station 7, April 2016, 0.5 m, on tyre, 2 specimens; ESFM–POR/2016–012, station 8, April 2016, 0.3 m on tyres and buoys, 3 specimens.

Additional material examined: ESFM-POR/2004-02, identified as Porifera (sp.), Izmir Bay, June 2004, 1 specimen.

Description: It is massive, thickly encrusting body. Consistency of living specimens is a friable and very fragile. Color is cream or light brown in life and white in ethanol. The specimens show different morphological features, but they are usually massive or tubular structure reaching a height of 10–15 cm (Figure 4). The oscules are terminal on erect tubes, 10–15 mm in diameter. The surface is smooth.

The aquiferous system is leuconoid and the atrium is large. Choanocyte chambers are spherical and sub-spherical, ranging from 60 to 110 µm (Figure 5a). Skeleton is inarticulate, consisting of triactines and tetractines. Cortical triactines can be equiangular, but usually sagittal with paired and unpaired actines (cortical triactines paired actines 180–560 µm and unpaired ones 190–580 µm, thickness of the actine at its base 10–40 µm (Figure 5, b1). Cortical tetractines are equiangular; their apical actin is longer than basal actines (length of the apical actine 300–810 µm (mean 265 µm) and basal ones 180–600 µm, thickness of the actine at its base 16–45 µm (Figure 5, b2, b3). Subatrial skeleton contains different kind of triactines and tetractines. Subatrial triactines usually have unpaired actines, which are longer than other actines (length of the paired ones 200–600 µm [mean 270.2 µm] and unpaired one 280–690 µm, thickness of the actine at its base 15–50 (Figure 5, b4). Subatrial tetractines have apical actines shorter than others (length of the paired actines 170–600 and unpaired actine 200–610 µm, thickness of the actine at its base 15–50 µm (Figure 5, b5). Atrial triactines are sagittal and their unpaired actines are shorter than paired ones (length of the paired actines 180–470 µm and unpaired ones 30–180 µm, thickness of the actine at its base 5–20 µm.

Habitat and Distribution: This species was found at all stations in the study area. Its highest coverage (35%) was encountered at station 2, while the lowest coverage (5-10%) at stations 4, 5 and 8.

The invasive alien sponge *Paraleucilla magna* may become very abundant in eutrophic environments and shows seasonality (Longo et al., 2007). This species was originally described from the Atlantic coast of Brazil based on the specimens collected in 2001 (Klautau et al., 2004). This species was later reported from different parts of the Mediterranean Sea with its first collection dating in the area dated back to 2001 (Longo et al., 2007). This species was especially abundant on mussel farms (on rows) and artificial substrata in Mar Piccolo and Mar Grande, Taranto (North-west Ionian Sea) (Longo et al., 2007).

In addition, it abundantly occurred on other coasts of Italy (Ionian Sea, Tyrrhenian Sea and Adriatic Sea) and on the coast of Malta (Zammit et al., 2009). It was reported to have been introduced to the region by shipping (Longo et al., 2007). It was suggested that mussel farming activities were the main reason for its regional introduction (secondary introduction) within the Mediterranean Sea (Longo et al., 2007). *Paralaucilla magna* was also observed from the different regions in the Mediterranean Sea: Spain (Frotscher et al., 2008; Guardiola et al., 2011), Adriatic Sea Ploče harbor (Cvitković et al., 2013), Brač Island in Croatia (Klautau et al., 2016), Gulf of Olbia in Italy (Baldacconi and Trainito, 2013), Portugal, Madeira and Acores (Guardiola et al., 2016), Tivat in Montenegro (Mačić et al., 2016) and Gulf of Thessaloniki (Greece), in 2014 in a mussel farm (Gerovasileiou et al., 2017) northern Cyprus, marinas of Crete and Rhodes Island (Ulman et al., 2017) and later from Marina Cap Monastir in Tunisia (Sghaier, et al., 2019).

This species was first identified as Porifera (sp. 1) along the coasts of Turkey, based on the material collected on a mussel bed (*M. galloprovincialis*) in June 2004 in the inner part of İzmir Bay (İnciraltı) (Çinar et al., 2008). It was later reported from natural substrata (rocks) at 10 m depth from the coast of Büyükada (Sea of Marmara) (Topaloğlu et al., 2016).

Class: DEMOSPONGIAE Sollas, 1885
Family: Dysideidae Gray, 1867
***Dysidea fragilis* (Montagu, 1814)** (Figure 6)

Spongia fragilis Montagu, 1814: 114
Dysidea fragilis Manconi et al., 2013: 20, 21, Figure 9(a-c).

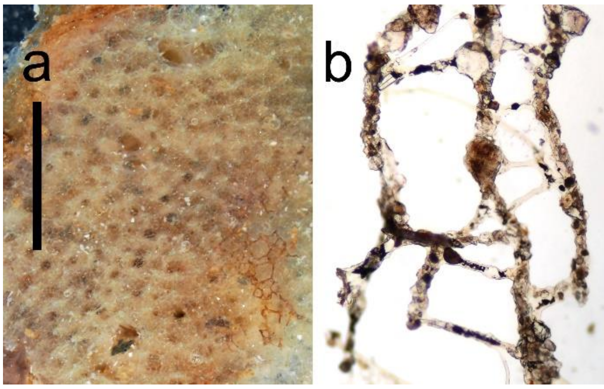

Figure 6. *Dysidea fragilis*. a General view of the specimen (scale: 0.4 cm) with conules; b. The reticulate skeletal network with scanty spongin and irregular meshes of primary and secondary fibers (scale: 200 µm).

Material examined: ESFM–POR/2016–14, station 1, March 2016, 0.3 m, on *M. galloprovincialis*, 1 specimen.

Description: Colour is grey to light brown in life and white in ethanol. Surface is smooth and conulose (Figure 6a). Oscules are scattered on the surface, which is up to 2–3 mm in length. Its consistency is fragile and soft. Inhalant apertures are 70–110 µm in diameter. Skeleton is reticulate and extremely fragile due to scanty spongin. Primary and secondary fibers are not distinguishable from each other (50–200 µm) (Figure 6b). Spongin fibres are reticulated sometimes covered by sand grains.

Habitat and Distribution: This species is very common in the benthic habitat and Atlanto-Mediterranean taxon (Van Soest et al., 2017a). It was originally described from the British coast and wea reported in various regions and biotopes of the Mediterranean Sea between 1 and 200 m depth (Manconi et al., 2013). It was also reported from the coasts of Turkey, except for the Black Sea (Topaloğlu and Evcen, 2014).

DISCUSSION

Among the species found in the present study, *Sycon ciliatum* is very similar to *S. raphanus* in terms of its external shape: both species are ovoid and hispid with apical oscula ornate by a specular fringe. In addition, the morphology of spicules in *S. raphanus* is very close to that in *S. ciliatum*. This high morphological affinity between these species has caused confusion over the years. *Sycon raphanus*, which was originally described from the Adriatic Sea by Schmidt (1862), is characterized by having a stalk and a bulb shaped body, which are the main characters that distinguish it from the closely similar species. Afterwards, Haeckel (1872) disapproved the presence of *S. ciliatum* in the Mediterranean. According to him, all specimens identified as *S. ciliatum* in the region in fact belonged to *S. raphanus* (Klautau et al., 2016). However, Longo and Pronzato (2011) postulated that the external morphology is not a good taxonomic character to separate these two species. At the present time, histological preparation is required to distinguish these species.

Table 1. Comparative data for the dimensions of spicules (means in µm) of the *Paraleucilla magna* specimens from different parts of the world

Studies	Cortical triactine (Length x wide) (mean)		Cortical tetractines (Length x wide) (mean)		Subatrial triactines (Length x wide) (mean)		Subatrial tetractines (Length x wide)		Atrial triactines (Length x wide)	
	Paired	Unpaired	Basal Ones	Apical Ones	Paired	Unpaired	Paired	Unpaired	Paired	Unpaired
Klautau *et.* al. (2004) (holotype)	292.8 x 24.0 (n=30)	289.6 x 24.0 (n=30)	434.4 x 34.4 (n=30)	468.8 x 31.2 (n=30)	266.4 x 24.0 (n=25)	358.4 x 25.6 (n=25)	394.4 x 33.6 (n=30)	338.4 x 31.2 (n=30)	371.2 x 22.4 (n=30)	164.0x 21.6 (n=30)
Longo et al, (2007)	374.9 x 27.9 (n=25)	427.9 x 27.9 (n=25)	388.9 x 36.6 (n=25)	572.1 x 36.6 (n=25)	399.6 x 35.9 (n=25)	584.1 x 35.9 (n=25)	344.4 x 30.6 (n=25)	383.6 x 30.6 (n=25)	236.5 x 7.4 (n=25)	64.2 x 7.4 (n=25)
Zammit et al, (2009)	202.8 x 22.8 (n=25)	178.2 x 22.8 (n=25)	257.4 x 33.6 (n=25)	342.6 x 33.6 (n=25)	240 x 28.2 (n=25)	299.8 x 28.2 (n=25)	286.2 x 32.4 (n=25)	286.2 x 32.4 (n=25)	195.6 x 19.8 (n=25)	91.8 x 19.8 (n=25)
This study	224.0 x 25.0 (n=25)	240.0 x 25.0 (n=25)	265.4 x 34.0 (n=25)	350.8 x 32.8 (n=25)	270.2 x 30.0 (n=25)	320.4 x 30.2 (n=25)	310.0 x 31.2 (n=25)	302.0 x 31.2 (n=25)	200.8 x 18.0 (n=25)	120.4 x 18.0 (n=25)

According to Van Soest et al. (2017b), the important difference between these species is the degree of fusion of the choanocyte chambers (radial tubes). In *S. raphanus*, they are almost entirely fused just up to the distal cone, while in *S.*

ciliatum, they are entirely free from each other (Van Soest et al., 2017b). Longo and Pronzato (2011) indicated that *S. raphanus* occasionally has a short stalk, but all of our specimens have short stalks. In addition, *S. ancora* Klautau et al. (2016), which was originally described from the Adriatic Sea, resembles closely to *S. raphanus* and *S. ciliatum*, but differs from them in the shapes of the atrial triactines and the presence of anchor-like tetractines at the base (Klautau et al., 2016).

The spicule lengths of *Paraleucilla magna* show a similar dimension in different places (Table 1). However, the spicule lengths of our specimens are closely related to those given by Zammit et al. (2009) from the coasts of Malta. As this species exhibits a fast growing and spread potential, monitoring studies should be implemented to assess its impacts on the hard benthic communities prevailing inside and outside of ports in the Mediterranean Sea.

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