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The effects of silica dissolution on the experimented spicules, studied by SEM analysis, resulted in an enlargement of the axial canal sometimes resulting in empty spicules. While in demosponges the axial canal wall of eroded spicules was perfectly smooth or slightly rough, the hexactinellid *Rossella racovitzae* showed a cavernous, well recognizable pattern of dissolution.

The dissolution rates were determined evaluating the decrease in outer diameter and in the expansion of the axial channel of about 300 spicules for each considered species and locality. The spicules from the Mediterranean *Geodia cydonium* did not show any detectable dissolution in both sites, while those from *Tethya citrina* showed a loss of silica of about 23% in the Mediterranean and 47% in the Celebes Sea. *Paratetilla bacca* from the Red Sea decreased the silica content of about 30% in both the localities. *Tetilla leptoderma* from Mar del Plata lost about 8% and 42% of silica respectively in Mediterranean and Celebes Sea. Finally, the hexactinellid spicules from the Antarctic *Rossella racovitzae* showed highest dissolution rates in both the experimented sites (37% and 66% in the Mediterranean and Celebes Sea, respectively).

The different levels of dissolution can be related to the different taxonomic position as well as to the temperatures at which the spicules have been deposited and exposed. In fact, spicules from the same species showed a dissolution rate generally higher in tropical waters than in Mediterranean.

Siliceous sponge spicule dissolution: in field experimental evidences from temperate and tropical waters

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Abstract

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Introduction

Siliceous sponge spicules are among the most fascinating skeletal features of marine invertebrates. In the last three decades, an impressive amount of work was dedicated to the elucidation of spicule structure and mechanism of silica deposition, also with applicative purposes (e.g. Müller et al., 2006, 2007a, 2007b). Biogenic silica (bSi) precipitated by sponges (but also by diatoms, radiolarian and silicoflagellates) is in the form of opal-A which is highly disordered and almost completely amorphous (DeMaster, 2003).

In spite of the wide interest in spicule formation, very few data are available about the role of siliceous sponges in the silica balance of the oceans (Maldonado et al., 2005, 2010). When sponges decay, their spicules become free in the environment and a certain proportion of bSi is sunk for a relevant span of time, because dissolution rate is considered low (Maldonado et al., 2005; Gutt et al., 2013).

The first attempt to study these siliceous structures lost in the sediments was due to Rützler and McIntyre (1978), who evaluated the amount of spicules present in the coralline sand from Carrie Bow Key (Belize). Those spicules showed, on their surface, a typical pitting pattern of dissolution that was artificially replicated in laboratory by treating spicules coming from living specimens with hydrofluoric acid 1 N. This peculiar kind of spicule erosion pattern was already been described from Jamaica and tentatively attributed to some biological agents (Land, 1976).

More recently, sponge spicules have been shown to be a component, although with variable amounts, of the sediments of some coastal areas all around the world (Inoue, 1985; Bavestrello et al., 1996; Andri et al., 2001; Bertolino et al., 2012; Chu et al., 2011). Moreover, also the sediments entrapped inside the Mediterranean coralligenous concretions, with an estimated age of thousands of years, include several spicules. Although these structures are sometimes deeply etched and damaged, they are still recognisable and, in several cases, allowed the identification of the original old sponge assemblage (Bertolino et al., 2014).

On the other hand, Maldonado et al. (2005) demonstrated in lab that free spicules re-dissolve into silicic acid at far slower rates than those recorded for diatom frustules, thus determining a bSi sink. This phenomenon is particularly intense in some geographic areas, mainly polar waters (Cattaneo-Vietti et al., 2000; Van Wagoner et al., 1989) where spicules can produce impressive mats as thick as 1.5 m (Koltun, 1968; Gutt, 2007) but no quantitative data are until now available about their dissolution rates.

The aim of this research is to evaluate, by means of SEM and morphometric analysis, the fate of free

spicules deriving from different sponge species coming from different latitudes, left for six months at sea both in temperate and tropical localities.

Materials and Methods

The spicular material used in the field experiments came from different species (*Geodia cydonium* (Jameson, 1811), *Tethya citrina* Sarà & Melone, 1965, *Paratetilla bacca* (Selenka, 1867), *Tetilla leptoderma* Sollas, 1886, and *Rossella racovitzae* Topsent, 1901) collected around the world (Tab. 1). Spicules were obtained from dry specimens by dissolving their tissues in boiling nitric acid. The spicules were further cleaned by rinsing several times in distilled water and then dried at 50 °C.

Small packs, containing 1 g of spicules from each sponge specimen, were prepared using plankton nets (50 µm mesh). Two sets of packs (each one in triplicate) were then left for six months in superficial sediments of two localities characterised by different water average temperatures: a detritic bottom of the north-Mediterranean (Paraggi Bay, 15 m depth) (from June to December 2013) and a coralline sandy bottom of Celebes Sea (Banka Is. North Sulawesi, Indonesia, 10 m depth) (from July 2014 to January 2015). The average water temperatures at the sites of the field tests were 18.4 °C and 28.2 °C, respectively for the north-Mediterranean and the Celebes Sea. The packs were fixed to plastic pegs and covered by about 1 cm of sediments.

After the permanence at sea, the content of each pack was dried and weighted.

To describe possible changes in the spicular morphological features due to the putative dissolutive processes, SEM and light microscopy preparations were made before and after the permanence at sea. For the morphometric analysis, three microscopy slides were prepared for each spicule pack, before and after the field tests. For each slide, the external and axial canal diameters of 100 spicules were measured. From these data the average surface of the annular spicule section was calculated as difference between the surface referred to the spicular diameter and the surface of the axial canal (Fig. 1).

A gross estimation of the total dissolution was obtained, for each species and locality, by calculating the percent variation of the spicular average section vs the section of the untreated spicules.

Statistical analyses were carried out to test for significance of differences in the average spicular and axial channel diameters as well as the annular surface of the spicule section among the three considered conditions (NT=No Treatment, M=Treatment in the Mediterranean Sea, I=Treatment in Celebes Sea) for each of the 5 species separately. As the normal distribution of data after a square root-transformation was verified by the Shapiro-Wilk's test, a one-way analysis of variance (ANOVA) was

performed followed by a Tukey's pairwise comparison. Analyses were performed using PAST for Windows version 1.91 (Hammer et al., 2001).

Results

SEM analysis has shown evident morphological changes between untreated and treated spicules, mainly due to the enlargement of their axial canal. While the external surface remained smooth in all tested spicules in both experimental sites, without any evident mark of etching, the inner canal showed different levels and patterns of dissolution (Figs. 2, 3).

In *Geodia cydonium*, the axial canal of treated trienes maintained the original triangular shape, with a slight enlargement in comparison to untreated spicules (Fig. 2A-C). No morphological differences were recognisable between spicules tested in the two localities. In the strongyloxeas of *Tethya citrina* (Fig. 2D), a huge enlargement of the canal (Fig. 2E, F) occurred in both sites after six months of permanence at sea and the canal wall showed a rough surface (Fig. 2F). Similar results occurred for *Paratetilla bacca* (Fig. 2G-I) and *Tetilla leptoderma* (Fig. J-M) although in these two species the canal wall remained perfectly smooth (Fig. 2I, M).

In the spicules of the hexactinellid *Rossella racovitzae* (Fig. 3A), the small square axial canal (Fig. 3B) showed a huge enlargement (Fig. 3 C-D), sometimes resulting in the formation of “empty” spicules (Fig. 3E). The wall of the canal was heavily etched, with a characteristic cavernous pattern of dissolution (Fig. 3F).

To evaluate the silica dissolution, the weight loss of the total amount of the tested spicules resulted unsuitable due to high loss of spicules through the plankton net probably under the effect of the water movement. However, the morphometric analysis of the average spicule thickness, of the axial canal diameter and of the estimated annular spicule section, indicated different intensity of dissolution according to the different seawater temperatures to which they were tested (Fig. 4).

The spicules of the atlanto-mediterranean demosponge *G. cydonium*, after six months of permanence at sea in both sites, were not subjected to reduction of the spicular thickness (Fig. 4A; Tabs. 2, 3), while the diameter of the axial canal showed an increasing with significant differences in the two tested temperatures (Fig. 4B; Tabs. 2, 3). The calculated transversal surface of the spicules was not subjected to significant variations after the permanence in both sites (Fig. 4C; Tabs. 2, 3) resulting in a slight percent of silica dissolution (3.9 and 7.5%, respectively in the Mediterranean and Celebes sites) (Fig. 4D).

The strongyloxeas of the Mediterranean demosponge *T. citrina*, showed a significantly decreasing of

their thickness in the spicules tested in Mediterranean and Celebes Sea (Fig. 4A; Tabs 2, 3) as well as a significant increasing of axial canal diameter between treated and untreated spicules (Fig. 4B; Tabs. 2, 3). The spicular annular section showed a progressive reduction from spicules untreated, treated in Mediterranean and in Celebes Sea (Fig. 4C; Tabs 2, 3) resulting in an estimated silica lost of about 23% in the Mediterranean and 47% in the Celebes Sea (Fig. 4D).

The variation of all recorded spicular parameters in *P. bacca* from Dalahk Is (Red Sea) were significant in untreated vs treated spicules, while no significant differences were recorded between spicules tested in Mediterranean and Celebes Sea. From these data, it was possible to estimate a silica dissolution of about 30% (Fig. 4; Tabs. 2, 3).

In *T. leptoderma* from Mar del Plata, the main recorded differences were observed after the treatment in the tropical waters, producing a silica dissolution of about 40% while no differences were recorded between untreated material and spicules experimented in the Mediterranean Sea (Fig. 4; Tabs. 2, 3).

Finally the spicules from the Antarctic hexactinellid *R. racovitzae* showed highest dissolution rates in both the experimented sites (37 and 66% in the Mediterranean and Celebes Sea, respectively) due to significant reductions of the external diameter and significant increasing of the axial canals (Fig. 4; Tabs. 2, 3).

Discussion

Forty years ago Rützler & McIntyre (1978), in their seminal paper about the presence of sponge spicules in Caribbean sediments, quantified the amount of siliceous spicules and described, through SEM imaging, their peculiar pattern of dissolution in natural conditions. Although numerous other papers have identified spicules in sediments of several marine or fresh-water basins (Inoue, 1985; Bavestrello et al., 1996; Andri et al., 2001; Bertolino et al., 2012; Chu et al., 2011), no field data are until now available about their natural dissolution rates and about the environmental factors affecting this process. The amount of silica up-taken by sponges is not negligible in the global silica balance and therefore a clarification of its turnover is necessary for a correct estimation of the bSi balance of the ocean (Kamatani, 1971).

Sponge spicules are more resistant to dissolution, particularly in comparison with diatoms (Maldonado et al., 2005; Kamatani, 1971): Maldonado et al. (2005), in fact, found no detectable dissolution of demosponge spicules and a 5% of dissolution in hexactinellid spicules after 8 months of lab experiments at room temperature, in contrast to a severe dissolution of the diatom frustules. On this base, several authors considered the silica uptake of the sponges as a sink of available silica from the

marine system (Maldonado et al., 2005, 2010; Chu, 2011).

Here we demonstrate, through field experiments that, although with differences among different species, the dissolution rate of spicules lost in the sediments is not negligible. Among the tested spicules, only those of *G. cydonium*, in fact, showed a slight dissolution rate, while all other spicules lost, in 6 months, from 8 to 37% of their volume in temperate waters, and from 34 to 66% in tropical ones. In particular, in the tested conditions, the spicules of the hexactinellid *Rosella racovitzae*, dissolve at a rate significantly faster than that of all the tested demosponges.

The recorded total dissolved silica resulted both from a reduction of the external diameter and an enlargement of the axial canal. While the erosion of the external surface was always homogeneous, without any marks of etching in all tested spicules, the enlargement of the axial canal resulted in different types of dissolution process with different microtextures, according to the examined species. The phenomenon is particularly evident in the hexactinellid *R. racovitzae*, in which the wall of the axial canal showed a cavernous etching pattern. On the contrary, in the demosponge spicules, the axial canal is slightly etched in *Tethya* or completely smooth in *Paratetilla* and *Tetilla*. These differences could be related to the complex, not homogeneous silica deposition around the axial filament (Mueller et al., 2007b; 2009).

From a compositional point of view, it is already stated that the spicules of demosponges and hexactinellids are very similar (Standford, 2003): both are composed almost completely by silica, deposited around a central axial filament which harbours the silicatein. Nevertheless, the recorded differences in IR spectra between the spicules of the two classes (Standford, 2003) were considered related to different molecular configurations, involving Si-O-Si or other linkages, that may result in different dissolution patterns. This structural difference could result in the higher dissolution rates of the hexactinellid *R. racovitzae* in comparison with the demosponges, at both tested temperatures.

It was already stated that the solubility of bSi is highly temperature dependent (Lawson et al., 1978; Kamatani, 1982) and this laboratory evidence was supported by ecological observations: spicules from living specimens of *Tethya*, collected in a marine cave from Oman (Persian Gulf) characterised by very warm waters, showed deep marks of dissolution already inside the living sponges (Bavestrello et al., 1996)). The significant differences recorded in the two tested localities confirmed the role of the water temperature on the silica dissolution: spicules from the same species showed in Mediterranean the maximal dissolution rate which was about the minimum in tropical waters.

These results indicate that the sponge spicules, and in particular those from hexactinellids, can play a significant role in the sinking process of silica only in cold waters, supporting the geographic

differences of the presence of spicules in sediments (Kamatani, 1971; Kratz et al., 1991; Conley and Schelske, 1993). For example, in the Antarctic Ocean, where the cold waters prevent the silica dissolution, sponge spicules can form thick mats (Koltun, 1968; Gutt et al., 2013; Bonci et al., 1989; Barthel, 1992), while at Tropics, with comparable sponge biomasses, spicule mats were never recorded. The evidence that the spicules of several Mediterranean species are completely dissolved in a couple of years is not in agreement with the finding of recognisable spicules, although partially etched, in sediment buried in the *Posidonia oceanica* mat with an estimated age of tens of years (Bertolino et al., 2012) or in sediments included in the growing coralligenous conglomerate, datable thousands of years (Bertolino et al., 2014). Probably, the peculiar features of these habitats, characterised by high concentration of organic matter, anoxic conditions and low pH values, were able to preserve bSi from the dissolution process.

Although other researches will be necessary at this subject, our results seem to suggest that the dissolution rate can be related to the temperature at which the spicule was formed. It is already known that the sponge spicule size varies along a latitudinal gradient, suggesting an inverse relationship with water temperature and silica deposition (Topsent, 1917; Hentschel, 1929; Hartman, 1958; Simpson, 1978; Hooper, 1991) and this trend was attributed to a more efficient entrapment of silicon rather than to effects upon silicon transport (Simpson, 1978). In our experiment, the spicules of two related demosponges, *P. bacca* and *T. leptoderma*, respectively secreted in warm and cold waters, showed different patterns of dissolution. Those from the warm waters of the southern Red Sea dissolved at the Mediterranean temperature, while those secreted in the cold south Atlantic waters, significantly dissolved only in the tropical condition, suggesting a more compact and resistant structure in spicules coming from cold waters.

However, also the role of the silica concentration in the waters cannot be ignored to determine different structures of the silica secreted, considering that, both at global (Treguer et al., 1995) and local level, according to the seasonal cycles (Stone, 1970), the silica concentration in the seawater is inversely correlated with the temperature, a condition which could play a major role in determining the morphological and structural characteristics of the spicule.

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Figure legends

Figure 1 – The spicule dissolution was evaluated by measuring the annular surface of the spicule section before (A, B) and after (C, D) a six month of permanence in the field. a-b, external diameter; a'-b', diameter of the axial canal.

Figure 2 – A-C, *Geodia cydonium*. A-B, Trienes showing the axial canal of normal size before the treatment and (C) slightly enlarged after the permanence at sea. D-F, *Tethya citrina*. D, Untreated fractured stroglyoxea showing the small axial canal). E-F, Enlarged axial canals after the field test. While the external surface remains smooth (E), the wall of the axial canal shows a rough pattern of dissolution (F). G-I *Paratetilla bacca*. G, Untreated triaene; H, enlargement of the axial canal after the treatment (H); I, the perfectly smooth wall of the axial canal. J-M, *Tetilla leptoderma*, J, untreated triaene, L-M, different stages of the enlargement of the axial canal after the permanence at the sea.

Figure 3 – *Rossella racovitzae*. A, untreated spicules; B, section of an untreated spicule showing the normal size of the axial canal. C-D, spicules of the mat with an enlarged axial canal after the permanence in the sea. E-F, spicules with the enlarged canal wall characterised by a cavernous etching pattern.

Figure 4 – Average external diameter (A); average diameter of the axial canal (B), average annular surface of the spicule section (C) of 300 spicules per species and per site. Black bars, untreated spicules; grey bars, spicules left for six month in the Mediterranean; white bars, spicules left for six month in the Celebes Sea. D, estimated percent dissolution of the spicules tested in Mediterranean (grey bars) and Celebes Sea (white bars) vs untreated spicules.

Tab. 1 – Tested species, localities of collection with depth and temperature range of the localities of collection; spicule types

Class	Species	Locality	Depth (m)	SWT (°C)	Spicule type
Demospongiae	<i>Geodia cydonium</i>	North Adriatic Sea (Mediterranean)	20	7 - 25	Oxeas Orto-Anatriaenes
Demospongiae	<i>Tethya citrina</i>	North Adriatic Sea (Mediterranean)	30	7 - 25	Strongyloxea
Demospongiae	<i>Paratetilla bacca</i>	Dalhak Is. (Red Sea)	6	26 - 32	Oxeas Anatriaenes Protriaenes
Demospongiae	<i>Tetilla leptoderma</i>	Mar del Plata (SW Atlantic)	360	8	Oxeas Anatriaenes Protriaenes
Hexactinellida	<i>Rossella racovitzae</i>	Ross Sea (Antarctica)	200	-1.5	Oxeas

Tab. 2 – Average values (\pm SE) of external spicule diameter, axial canal diameter and surface of the annulus of the spicule section after and before six months of permanence at sea in temperate and tropical localities.

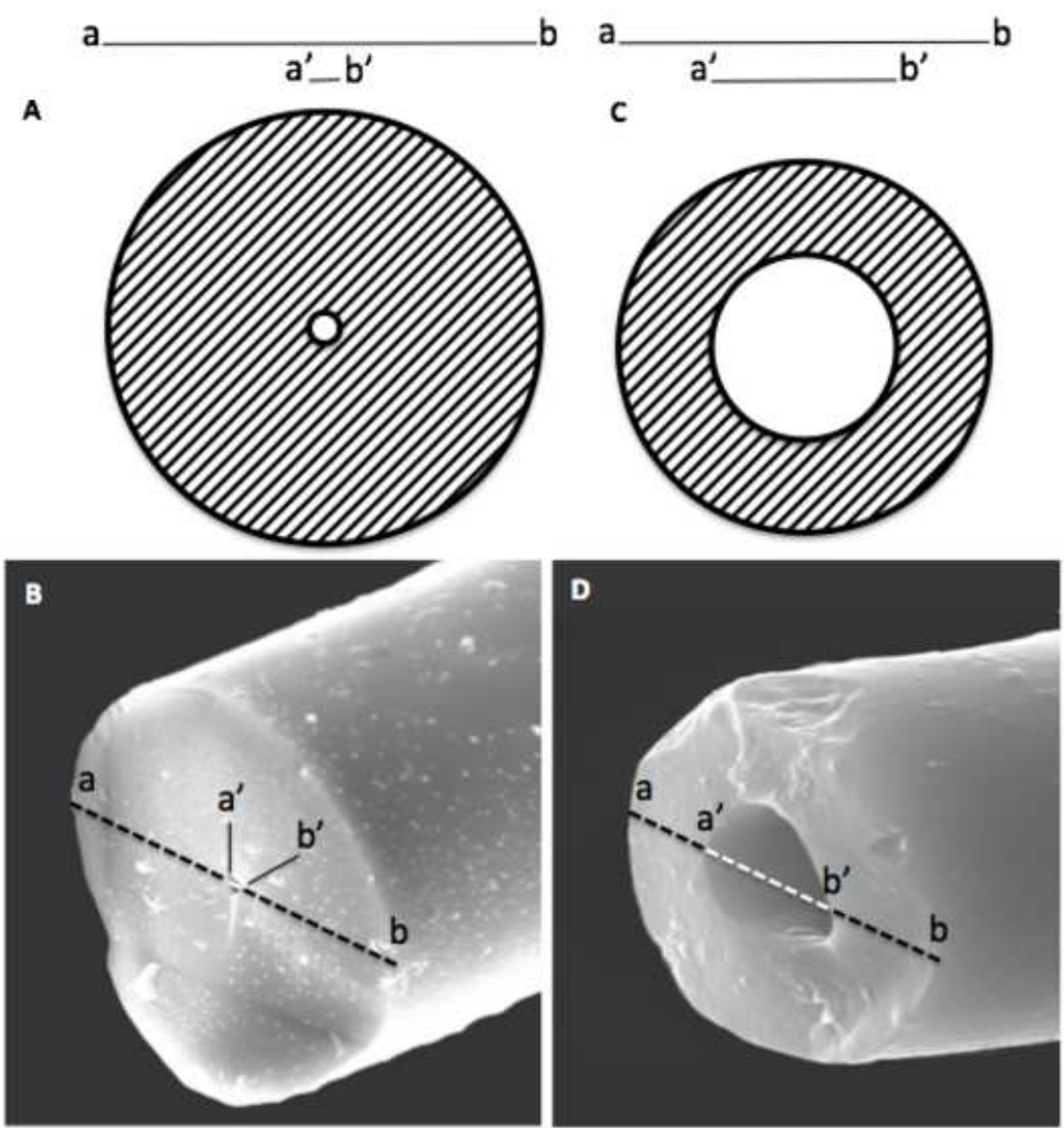
Species	Treatment	External diameter (μm)	Axial canal diameter (μm)	Annular section surface (μm^2)
<i>Geodia cydonium</i>	untreated	24,59 \pm 0,70	1,21 \pm 0,03	589,98 \pm 30,52
	6 months Medit	24,12 \pm 0,71	1,41 \pm 0,03	566,86 \pm 30,51
	6 month Celebes	23,52 \pm 0,76	1,71 \pm 0,05	545,33 \pm 33,53
<i>Tethya citrina</i>	untreated	21,54 \pm 0,38	3,17 \pm 0,09	381,97 \pm 13,12
	6 months Medit	19,03 \pm 0,28	3,84 \pm 0,09	293,81 \pm 9,18
	6 month Celebes	15,91 \pm 0,23	4,14 \pm 0,08	201,01 \pm 6,34
<i>Paratetilla bacca</i>	untreated	35,25 \pm 0,67	3,67 \pm 0,15	1046,45 \pm 36,48
	6 months Medit	30,05 \pm 0,51	5,98 \pm 0,20	743,13 \pm 24,88
	6 month Celebes	29,16 \pm 0,41	5,58 \pm 0,16	694,43 \pm 18,49
<i>Tetilla leptoderma</i>	untreated	20,97 \pm 0,84	1,09 \pm 0,04	479,21 \pm 42,92
	6 months Medit	19,70 \pm 0,63	1,38 \pm 0,05	459,46 \pm 29,24
	6 month Celebes	16,20 \pm 0,46	1,33 \pm 0,05	278,47 \pm 19,66
<i>Rossella racovitzae</i>	untreated	12,72 \pm 0,36	0,99 \pm 0,04	151,34 \pm 10,07
	6 months Medit	10,40 \pm 0,22	1,63 \pm 0,05	95,54 \pm 5,03
	6 month Celebes	7,97 \pm 0,13	2,14 \pm 0,05	51,77 \pm 2,25

Tab 3 - Results of the one-way ANOVA tests.

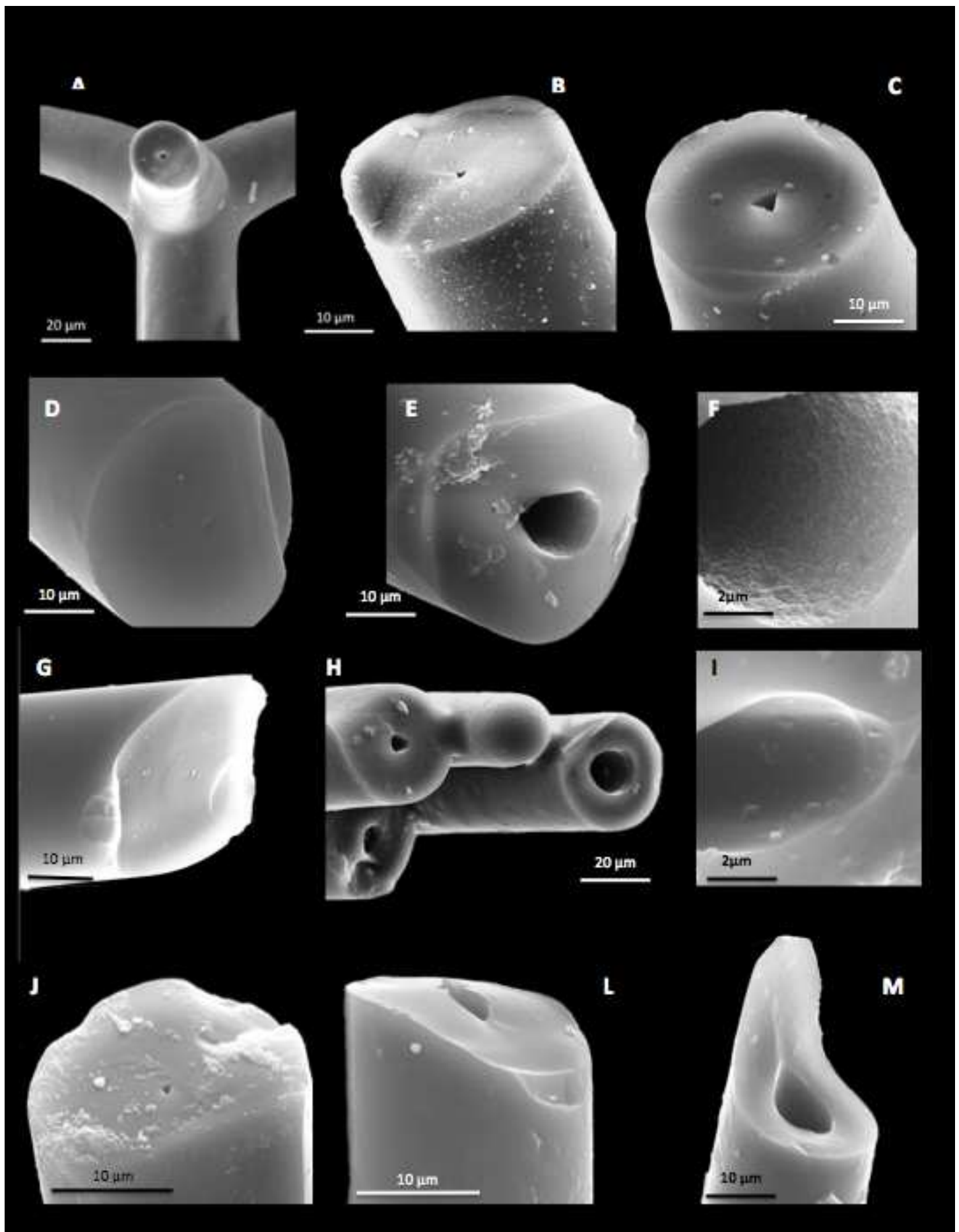
Effect of treatment on the spicule external diameter for each species (Fig. 4A). NT=Untreated, M=Mediterranean Sea, I=Celebes Sea			
Comparison	df	F	p
<i>Geodia cydonium</i>			
Between groups	2	0.6455	p=ns (NT=M=I)
Within groups	865		
Total	867		
<i>Tethya citrina</i>			
Between groups	2	89.66	p<0.001 (NT>M>I)
Within groups	1025		
Total	1027		
<i>Paratetilla bacca</i>			
Between groups	2	28.7	p<0.001 (NT>M=I)
Within groups	974		
Total	976		
<i>Tetilla leptoderma</i>			
Between groups	2	16.81	p<0.001 (NT=M>I)
Within groups	1033		
Total	1035		
<i>Rossella racovitzae</i>			
Between groups	2	120.4	p<0.001 (NT>M>I)
Within groups	1035		
Total	1037		

Effect of treatment on the spicule axial canal diameter for each species (Fig. 4B). NT= Untreated, M=Mediterranean Sea, I= Celebes Sea			
Comparison	df	F	p
<i>Geodia cydonium</i>			
Between groups	2	51.72	p<0.001 (NT<M<I)
Within groups	865		
Total	867		
<i>Tethya citrina</i>			
Between groups	2	29.07	p<0.001 (NT<M=I)
Within groups	1025		
Total	1027		
<i>Paratetilla bacca</i>			
Between groups	2	38.37	p<0.001 (NT<M=I)
Within groups	974		
Total	976		
<i>Tetilla leptoderma</i>			
Between groups	2	5.631	p<0.01 (NT<M=I)
Within groups	1033		
Total	1035		
<i>Rossella racovitzae</i>			
Between groups	2	88.51	p<0.001 (NT<M<I)
Within groups	1035		
Total	1037		

Effect of treatment on the spicule annular section surface for each species (Fig. 4C). NT= Untreated, M=Mediterranean Sea, I= Celebes Sea			
Comparison	df	F	p
<i>Geodia cydonium</i>			
Between groups	2	0.7229	p=ns (NT=M=I)
Within groups	865		
Total	867		
<i>Tethya citrina</i>			
Between groups	2	99.84	p<0.001 (NT>M>I)
Within groups	1025		
Total	1027		
<i>Paratetilla bacca</i>			
Between groups	2	38.57	p<0.001 (NT>M=I)
Within groups	974		
Total	976		
<i>Tetilla leptoderma</i>			
Between groups	2	15.59	p<0.001 (NT=M>I)
Within groups	1033		
Total	1035		
<i>Rossella racovitzae</i>			
Between groups	2	124	p<0.001 (NT>M>I)
Within groups	1035		
Total	1037		



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Figure(s)

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