RESEARCH ARTICLE

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Comparative biometry of *Eunicella singularis* (Gorgonian) sclerites at East Mediterranean Sea (North Aegean Sea, Greece)

Abstract The two forms of *Eunicella singularis* sclerites, spindles and clubs, were examined in two sites in East Mediterranean Sea, Greece. The comparison of the sclerites size was based on the hydrodynamic profile of the sites (the site of Arethoussa was exposed and the Phidonissi site was sheltered), on the sites bathymetry (AR 5–8 m, AR 9–13 m, PH 5–10 m, PH 11–13 m) and on the position occupied by the sclerites on the gorgonian colonies (top, middle and base). With the usage of image system analysis the following microfeatures were measured or calculated: projection, length, width, elongation factor, compactness factor, length × width and length/width. The present study confirms the hypothesis that spindles are involved in the recovering of the gorgonian colonies and clubs contribute to the flexion capacity of the colonies. The spindles of the shallow *E. singularis* colonies (AR 5–8 m) were significantly bigger (length, width) compared with the others, in order to assure the best recovery of the colonies after their flexion, induced by higher water current velocity in shallow water. The clubs occupy the intern part of the mesoglea and their size (length, width) increased in colonies from the shallow exposed site in order to facilitate the gorgonian flexion. The present study demonstrates the gorgonian adaptation (in a microscopic scale) to the environmental pressure of hydrodynamic conditions.

Introduction

Gorgonians are sedentary colonies exposed to the environmental hydrodynamic conditions. Their axes

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constitute a robust structure attached to the substratum. The architecture of this macroscopic structure is based on the presence of microscopic unities, the spicules or sclerites. The presence of sclerites is not an exclusive characteristic of the gorgonian colonies, as some other forms with a different constitution have been observed in several zoological groups as Porifera, Hexacorallia and Echinodermata. The gorgonian axis is composed by gorgonin, containing modified collagen (Goldberg 1976). The chemical constitution of sclerites and their formation mechanisms have been the object of several studies (Goldberg 1981; Goldberg and Benayahu 1987a, b; Kingsley 1984; Majoran 1987).

The mechanic properties of gorgonian axes are modified by the presence of the sclerites in the coenenchyme (Muzik and Wainwright 1977; Esford and Lewis 1990; Vogel 1988, 1981). The gorgonian colonies profit by water currents for their feeding. However, an increase of water velocity can damage the colonies and as a result the gorgonian distribution depends on the water current intensity (Jeyasuria and Lewis 1987; Yoshioka and Yoshioka 1989). Several studies during the last years deal with the mechanic role of the sclerites (Esford and Lewis 1990; Lewis and Von Wallis 1991; West 1997). Except their contribution in the mechanic properties of the colonies, the sclerites are also implicated in the colonies defense. As sclerites increase in size, gorgonians become less palatable for predators such as *Cypfoma gibbosium* in tropical waters and *Simnia spelta* in Mediterranean Sea (Van Alstyne and Paul 1992; West et al. 1993; West 1998).

Eunicella singularis or White gorgonian is a very common Mediterranean species. However, very little is known about the sclerites adaptation to the water current velocity with an exception of a study concerning the species of the same genus *E. cavolinii* (Velimirov 1976). The aim of the present study was to investigate whether the sclerites size is related to their position on the colony, to the bathymetry of the site and to the water current intensity.

Materials and methods

Gorgonian colonies of *E. singularis* were collected from Arethoussa and Phidonissi. The sampling sites are located in North Aegean Sea, in Kavala Bay (Greece) (Fig. 1). The gorgonian populations from each site were subdivided into two subpopulations, the shallow and deep one. Samples were collected from Arethoussa between 5 and 8 m depth (AR 5–8) (31 colonies), from Arethoussa between 9 and 13 m depth (AR 9–13) (39 colonies), from Phidonissi between 5 and 10 m depth (PH 5–10) (45 colonies) and from Phidonissi between 11 and 13 m depth (PH 11–13) (48 colonies). The relative hydrodynamic exposure of the two sampling sites was investigated using the method described by Kaandorp (1986). The above parameter was estimated quantitatively by relating the exposure to water movement to the erosion of gypsum blocks placed in the same depth. The erosion value was defined as the weight loss of the gypsum blocks during a lunar day. After the application of this method, it was demonstrated that Arethoussa site was significantly more exposed than Phidonissi.

A sample, 1 cm long, was taken randomly from the top, middle and base of the gorgonian colonies from each subpopulation. These samples were dissolved in a solution of sodium hypochlorite 3%. Two forms of sclerites, spindles (Fig. 2) and clubs (Fig. 3), were isolated with the usage of a pipette Paster and afterwards they were fixed for microscopic observation. The measurements of microfeatures were realized in Musée d'Histoire Naturelle in Paris (France). For the sclerite measurements we have acquired the digital image using a video camera connected to a microscope. Analysis and measurements of the acquired image were realized using the programs Adobe and Optilab. The usage of informatics help, for the sclerite measurements, is much more precise than the usage of a micrometric scale adapted to microscope. For each one of the sclerite type from the three different parts of the colonies and from the different sites studied, the following microfeatures were measured or calculated:

1. Projection, which is defined as the surface area of the sclerites projection in pixels, given in surface unities (μm^2) (Fig. 4, dark area)
2. Length, which is defined as the difference $X_{max} - X_{min}$ in the horizontal axis X (Fig. 4).
3. Width, which is defined as the difference $Y_{max} - Y_{min}$ in the vertical axis Y (Fig. 4).
4. Elongation factor, which is defined as the ratio between the longest segment of the sclerites and the mean value of the perpendicular segments.

Fig. 1 Map of Arethoussa and Phidonissi sites, where sampling of *Eunicella singularis* took place
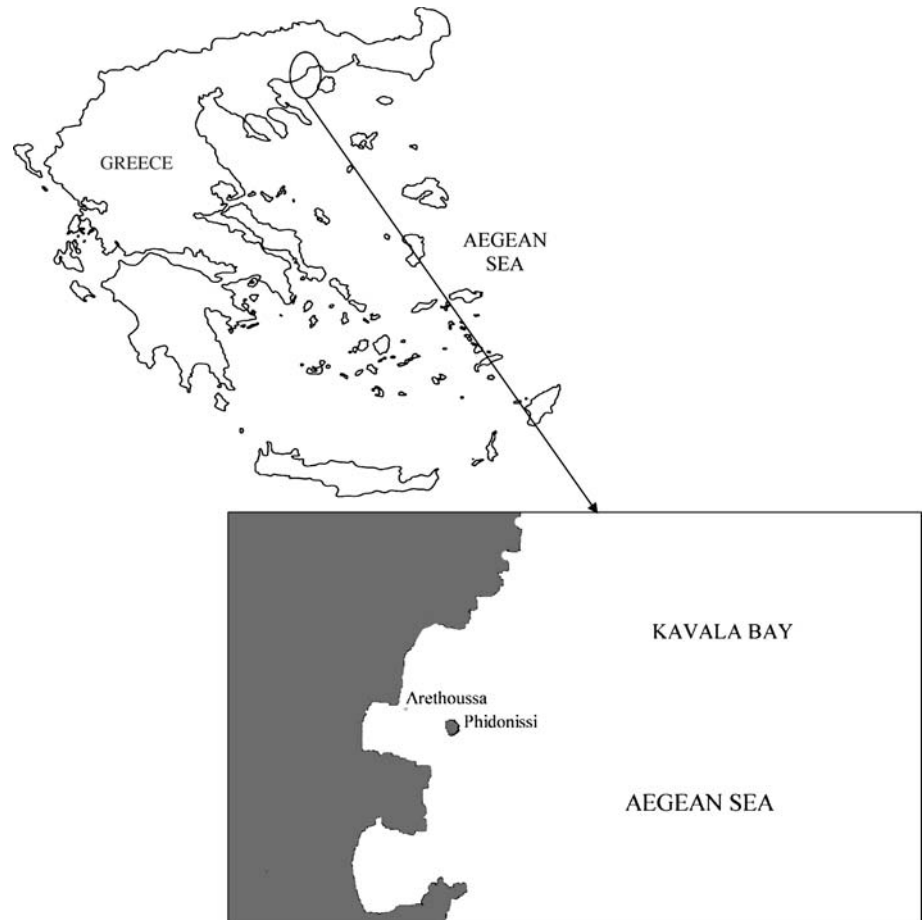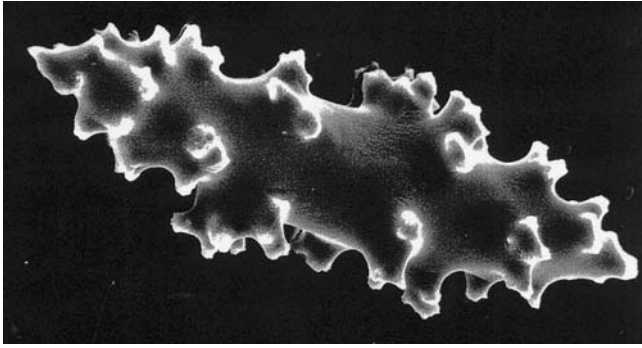
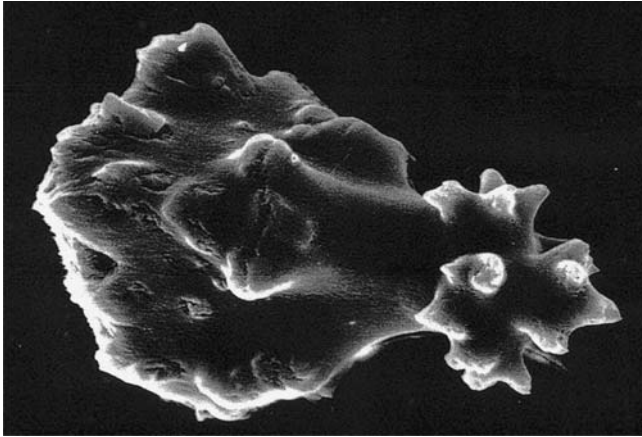**Fig. 2** Spindle form of sclerites in *E. singularis* gorgonian

Fig. 3 Club form of sclerites in *E. singularis* gorgonian
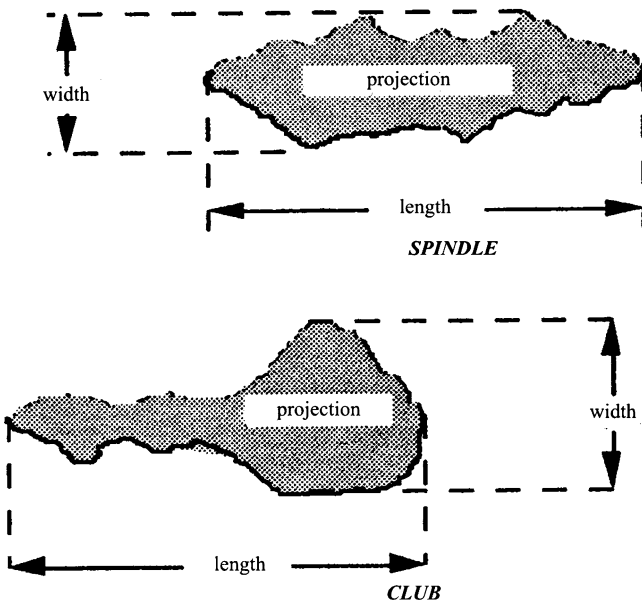

Fig. 4 Projection (*dark area*), length and width of the two sclerites forms (*spindles* and *clubs*) of *E. singularis* gorgonian

5. Compactness factor, which is defined as the ratio of the surface area of the sclerites projection and the smaller rectangle that can include this area.

6. $L \times W$, which is defined as the product of length and width.
7. L/W, which is defined as the ratio of length and width.

Results

The mean values ($\pm$ SD) of *E. singularis* microfeautures are presented in Table 1. Spindles from the top of the colonies from site AR 5–8 exhibited significant higher values (ANOVA, $df = 3$, $f = 11.6$, $P < 0.05$), as regards the projection, than spindles from the same colony position from the other three sites. Important differences were observed as well, concerning the clubs. The mean projection values were higher at the top of the colonies from site AR 9–13 (ANOVA, $df = 3$, $f = 4,7$, $P < 0.05$) and remarkably lower for the basal clubs from the same site (ANOVA, $df = 3$, $f = 4$, $P < 0.05$).

A general observation, concerning the length of the spindles, remarked that the mean values were increased in the middle of the colonies (ANOVA, $df = 3$, $f = 3.5$, $P < 0.05$). An exception of this observation was noticed in the spindles from the top of the colonies from the shallow exposed site (AR 5–8). The mean length values of the clubs for colonies from the exposed sites (AR 5–8 and AR 9–13) decreased from top to the base.

The width of the spindles and clubs varied as well. Significantly higher mean width values (ANOVA, $df = 3$, $f = 9.5$, $P < 0.05$) were observed in the spindles from exposed sites colonies (AR 5–8 and AR 9–13). However, the clubs from the four study sites did not exhibit important differences (ANOVA, $df = 3$, $f = 1.7$, $P > 0.05$).

Elongation factor mean values of spindles were lower at the top (ANOVA, $df = 3$, $f = 3.3$, $P < 0.05$) and at the middle (ANOVA, $df = 3$, $f = 6$, $P < 0.05$) for the colonies from the shallow exposed site (AR 5–8), when compared to those from the other sites. The highest elongation mean values of spindles were observed in the middle part of the deep colonies (AR 9–13 and PH 11–13) (ANOVA, $df = 3$, $f = 6$, $P < 0.05$). The highest mean elongation factor value was observed at the clubs of the middle part of the less exposed shallow colonies (PH 5–10) and the lowest elongation factor mean value was observed at the clubs of the base of the less exposed deep colonies (PH 11–13).

Another microfeature measured during the present study is the compactness factor. The mean values of compactness factor concerning spindles were increased in the apical position from the shallow exposed colonies (AR 5–8) (ANOVA, $df = 3$, $f = 9.1$, $P < 0.05$) comparing with those from the other sites. The mean compactness values of the clubs did not exhibit significant differences (ANOVA, $df = 3$, $f = 2.1$, $P > 0.05$).

Except the above measured factors, two more were calculated. The product length $\times$ width is considered

Table 1 Microfeatures of spindle and club sclerites of *Eunicella singularis* gorgonian (mean ± SD)

Sites	Part of colony	Projection (μm²)	Length (μm)	Width (μm)	Elongation factor	Compactness factor	L × W (μm²)	L/W
Spindles								
AR 5–8	Top (n = 50)	3,639.728 ± 1,014.692	148.328 ± 28.081	45.349 ± 5.206	6.190 ± 1.091	0.535 ± 0.045	6,818.407 ± 1,893.311	3.268 ± 0.503
	Middle (n = 50)	3,077.938 ± 866.350	135.170 ± 22.577	42.330 ± 5.991	6.108 ± 0.961	0.531 ± 0.040	5,804.412 ± 1,618.490	3.120 ± 0.451
	Base (n = 50)	2,147.018 ± 804.631	134.171 ± 26.946	43.268 ± 5.568	5.862 ± 1.042	0.541 ± 0.046	5,885.085 ± 1,735.027	3.108 ± 0.493
AR 9–13	Top (n = 50)	3,140.768 ± 809.139	134.8242 ± 23.137	41.188 ± 5.548	5.903 ± 0.978	0.563 ± 0.051	5,629.563 ± 1538 ± 045	3.285 ± 0.467
	Middle (n = 50)	3,355.054 ± 834.221	147.696 ± 21.262	43.146 ± 6.048	6.713 ± 1.167	0.523 ± 0.046	6,416.251 ± 499.602	3.470 ± 0.611
	Base (n = 50)	2,985.136 ± 801.880	131.070 ± 23.202	42.758 ± 6.064	5.901 ± 0.818	0.528 ± 0.038	5,692.653 ± 1,568.640	3.078 ± 0.416
PH 5–10	Top (n = 50)	2,597.582 ± 794.701	127.867 ± 26.163	39.902 ± 5.130	6.486 ± 1.334	0.504 ± 0.049	5,183.252 ± 1,614.766	3.207 ± 0.516
	Middle (n = 50)	3,308.150 ± 867.766	146.023 ± 22.451	43.942 ± 6.911	6.631 ± 1.118	0.512 ± 0.041	6,490.848 ± 1,730.815	3.361 ± 0.492
	Base (n = 50)	2,996.614 ± 802.362	138.822 ± 24.594	42.248 ± 5.659	6.598 ± 1.080	0.507 ± 0.051	5,937.500 ± 1,647.596	3.303 ± 0.498
PH 11–13	Top (n = 50)	3,103.148 ± 893.385	133.094 ± 25.979	43.391 ± 6.176	5.860 ± 1.102	0.535 ± 0.057	5,878.114 ± 1,848.635	3.070 ± 0.414
	Middle (n = 50)	3,256.998 ± 947.870	149.450 ± 29.531	42.452 ± 5.868	7.017 ± 1.118	0.507 ± 0.042	6,448.274 ± 1862.210	3.523 ± 0.534
	Base (n = 50)	3,069.366 ± 579.314	128.540 ± 20.374	43.840 ± 4.098	5.489 ± 1.174	0.547 ± 0.049	5,661.503 ± 1,205.826	2.946 ± 0.484
Clubs								
AR 5–8	Top (n = 50)	1,844.776 ± 392.685	76.276 ± 8.460	36.434 ± 4.731	3.220 ± 0.296	0.658 ± 0.038	2,805.605 ± 596.409	2.107±0.191
	Middle (n = 50)	1,768.633 ± 430.675	74.705 ± 10.241	32.210 ± 5.289	3.247 ± 0.480	0.648 ± 0.048	2,743.472 ± 691.389	2.086±0.247
	Base (n = 50)	1,667.281 ± 299.337	69.870 ± 6.261	35.471 ± 4.405	2.975 ± 0.282	0.665 ± 0.038	2,514.688 ± 481.759	1.970±0.183
AR 9–13	Top (n = 50)	2,043.940 ± 329.884	80.437 ± 6.988	38.189 ± 4.007	3.207 ± 0.321	0.664 ± 0.032	3,048.287 ± 500.305	2.121±0.214
	Middle (n = 50)	1,896.503 ± 303.801	76.786 ± 7.583	36.944 ± 3.997	3.158 ± 0.414	0.667 ± 0.036	2,846.847 ± 458.441	2.097±0.266
	Base (n = 50)	1,653.312 ± 322.458	71.482 ± 5.947	34.680 ± 5.197	3.161 ± 0.400	0.665 ± 0.040	2,495.524 ± 515.953	2.0940.269
PH 5–10	Top (n = 50)	1,912.172 ± 286.585	74.236 ± 5.690	38.638 ± 4.471	2.921 ± 0.265	0.666 ± 0.250	2,880.389 ± 462.502	1.9380.198
	Middle (n = 50)	1,938.844 ± 358.901	79.764 ± 6.636	37.434 ± 5.222	3.346 ± 0.374	0.647 ± 0.038	3,007.089 ± 599.467	2.155±0.223
	Base (n = 50)	1,784.612 ± 640.065	75.582 ± 7.736	35.782 ± 4.801	3.266 ± 0.429	0.658 ± 0.033	2,719.939 ± 537.830	2.136±0.265
PH 11–13	Top (n = 50)	1,801.182 ± 360.281	76.888 ± 7.544	35.108 ± 4.740	3.344 ± 0.325	0.664 ± 0.042	2,726.576 ± 590.339	2.207±0.191
	Middle (n = 50)	1,721.919 ± 375.391	72.644 ± 9.103	35.312 ± 4.902	3.131 ± 0.413	0.667 ± 0.033	2,592.365 ± 598.447	2.075±0.240
	Base (n = 50)	1,831.628 ± 274.149	73.032 ± 5.294±	36.883 ± 3.636	2.953 ± 0.273	0.678 ± 0.032	2,702.668 ± 392.274	1.993±0.181

also as a microfeature, which confirms the other obtained data. The spindles of the colonies from the shallow exposed site (AR 5–8) showed significantly higher mean values of L × W than the ones of the other colonies (ANOVA, $df = 3$, $f = 8$, $P < 0.05$). The clubs from the base of colonies from the shallow exposed site (AR 5–8) exhibited mean values of L × W remarkably lower than the others.

The ratio length/width showed important differences as well. The spindles from the middle of the colonies collected from the shallow exposed site exhibited low mean values (ANOVA, $df = 3$, $f = 3.4$, $P < 0.05$). The minimum values of the ratio L/W for the clubs sclerites were observed in the apical part of the colonies from the shallow sheltered colonies (ANOVA, $df = 3$, $f = 16$, $P < 0.05$).

Discussion

The macrostructure of the gorgonian axis, as known, is based on the sclerites microstructure (Lewis and Wallis 1991). Several types of sclerites can be observed in the gorgonian mesoglea and each one of them is typical of the gorgonian species. As observed subsequently (Weinberg 1976; Velimirov 1976) two potential forms of sclerites are involved in the architecture of the gorgonian of the genus *Eunicella*: the spindles and the clubs. The current study confirms this observation for *E. singularis* colonies from the East Mediterranean Sea and includes separate examination of the two sclerites types. Using their form and their position in the gorgonian axis as the criterion, several hypotheses have been postulated concerning their mechanic role (Muzik and Wainwright 1977; Wainwright et al. 1982). The spindle sclerites were found in a greater concentration at the external part of the gorgonian axis and their longitudinal axes were parallel to the gorgonian axes (Bayer et al. 1983). Their most probable function is the colonies defense and the support of the polyps (Lewis and Wallis 1991; West 1997) . The observations of the current study, concerning *E. singularis* spindle sclerites confirmed the hypothesis for their possible role. The spindle sclerites size (length, width) increased in the case of the apical position of the colonies from the most exposed site (AR 5–8). In addition, the compactness factor of the spindle sclerites from the apical part exhibited higher values in the samples from the exposed site than from the sheltered site. An explanation for these observations concerns the protecting role of the spindle sclerites. When the water current velocity increases the gorgonian colonies are consequently exposed to potential forces. The most effective adaptation is an increase of the flexion capacity of the colonies. However, the important flexion of the gorgonian colonies demands bigger spindle sclerites, in order to assure the colonies covering. In the case of the species, *E. cavolinii*, the size of the spindle sclerites is alike in exposed and sheltered colonies.

The data obtained from the biometry of the clubs support also the hypothesis for their mechanic role (Lewis and Wallis 1991). According to this hypothesis the clubs rotation is involved in colony torsion. The clubs sclerites of *E. singularis* are significantly smaller (length, width) at the middle part of the exposed colonies. The smaller clubs size increases the torsion capacity of the gorgonian colonies. This micro morphological adaptation is much more interesting, when related to the capacity of the gorgonian to resist to the high water current velocity. Similar results have been obtained in the study for *E. cavolinii* (Velimirov 1976). The size of the clubs sclerites diminishes at the stem of the exposed colonies and consequently increases the flexion capacity of the colonies.

The data obtained under the frame of the current survey enrich the macroscopic studies of gorgonian axis (Leversee 1984; Velimirov 1976) and demonstrate the importance of the microscopic architectural of sclerites in *E. singularis*. The functional role of sclerites in adaptation mechanisms is described: spindle sclerites are involved in the recovering of the gorgonian axis and clubs sclerites have an important role in the increase of the flexion and torsion capacity of the gorgonian colonies.

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