Ultrastructure of Spines in Heart Urchins of the Genus *Echinocardium*

Anatoliy L. Drozdov[1, 2], Victoria V. Sharmankina[1]

[1]National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, 17 Palchevsky st., Vladivostok 690059

[2]Far Eastern Federal University, 8 Sukhanov st., Vladivostok 690091
e-mail: anatoliyld[at]mail.ru

Abstract: *We studied and compared the ultrastructure of the skeleton of spines in the sea urchins Echinocardium cordatum (from the Sea of Japan and the Mediterranean Sea) and E. mediterraneum. The spines were identified depending on their position on the test: anterior, anal, latero-dorsal, latero-ventral, subanal, apical spines, and plastron spines. In addition to the high individual plasticity of spines, which is now used as a specific character of heart urchins, we propose a more stable morphological pattern including the features of several types of spines that helps more clearly characterize heart urchins from the Sea of Japan and the Mediterranean Sea.*

Keywords: Echinodermata, sea urchins, *Echinocardium,* skeleton, spine structure

1. Introduction

A sea urchin spine is an external calcareous appendage of the body that participates in a number of life functions such as movement, feeding, and defense. The shape, color, and structural features of spines are the characters commonly used to determine the taxonomic position of sea urchins (Agassiz, Clark, 1907; Mortensen, 1928; Clark, 1912; Dyakonov, 1933; Mortensen, 1935; Jensen, 1974; Baranova, 1957; Bazhin, Stepanov, 2012; Vinnikova, Drozdov, 2011). The general morphology of the spines of echinoids of the order Spatangoida, particularly *E. cordatum*, has been well studied at the light-microscope level and described in a number of works (Mortensen, 1950; 1951; Durham, Melville, 1957; Treatise…, 1966; Smith, 1980). A total of up to 15 types of spines performing various functions are differentiated morphologically depending on the position on the test. Nevertheless, their ultrafine structure remains poorly known.

Our recent studies revealed a set of ultrastructural features of spines that may be helpful in describing sea urchins (Vinnikova, Drozdov, 2011; Drozdov et al., 2016). The differences in the color of spines in cryptic species of the *Echinometra mathaei* complex (Palumbi, Metz, 1991; Palumbi et al, 1997), as well as the existence of two sympatric ecomorphological forms of *Strongylocentrotus intermedius* distinguished only by the color and length of spines (Balakirev et al., 2008) are an interesting phenomenon. This provides a basis to consider the spine, on the one hand, a highly sensitive morphological characteristic that appeared at the early stages of micro-evolutionary transformations and, on the other hand, a stable, highly specialized structure that reflects the historical association of each order of sea urchins with a particular adaptive zone (Solovyev, Markov, 2004).

The sea urchin *E. cordatum* inhabits mainly temperate waters of the Pacific and Atlantic Oceans both in the Northern and Southern Hemisphere. All attempts to divide this widespread species into several separate species based on morphological characters proved to be unsuccessful (Higgins, 1974, 1975; David, Laurin, 1996). A study by Hudson and co-authors (Hudson, Turner, Sewell, 2015) shows that the structural features of the spermatozoa of *E. cordatum* from waters off New Zealand are significantly similar to those in its counterparts from coastal waters of the Sea of Japan, but differ from the ultrastructure of Baltic heart urchin spermatozoa. According to a recent hypothesis, the observed morphological diversity is selectively neutral and reflects the high effective population sizes in the *E. cordatum* complex (Egea et al., 2015).

With the above considerations in mind, it was interesting to investigate and compare the ultrastructure of the skeleton of seven types of spines in the sea urchin *E. cordatum* from the Sea of Japan and the Mediterranean Sea, as well as in *E. mediterraneum*.

We hope that the novel information obtained in this study will contribute to description of the phenomenon of the *E. cordatum* cryptic species complex.

2. Material and Methods

The material of this study was specimens of the sea urchins *E. cordatum* (Pennant, 1777) and *E. mediterraneum* (Forbes, 1844) (the order Spatangoida; the family Loveniidae Lambert, 1905; the genus *Echinocardium* Gray, 1825).

Dry tests with spines (25 specimens) of *E. cordatum* were collected in several localities of the Mediterranean Sea (Sormiou Bay (43°12.587' N; 005°25.429' E), La Vesse Bay (43°20.29' N; 5°15.43' E), Les Lecques Bay (43°10.397' N; 05°41.465' E), and Sanary Bay (43°06.473' N; 05°48.643' E)) and identified by E. Egea and A. Chenuil.

Specimens of *E. cordatum* with a length of 40 mm were collected in Amurskiy Bay of Peter the Great Bay and identified by V.V. Sharmankina.

Dry tests with spines (10 specimens) of *E. mediterraneum* were collected in the Mediterranean Sea (Anse de l'Arène Bay (43°12'24.39' N; 005°32'50.35' E), La Capte Bay (43°03.617' N; 06°09.087' E), and Les Sablettes Bay (43°04.609' N; 05°53.543' E)) and identified by E. Egea and A. Chenuil.

Spines were identified depending on their position on the test (Fig. 1), according to the description by Smith (1980). The ultrastructure of the skeleton of definitive spines was studied using scanning electron microscopy. For this purpose, the spines were washed in running water, dried, mounted onto metal stages, and coated with platinum. Cross-sections through the middle segment of spines were obtained with an IsoMet 1000 precision sectioning cutter (Buehler). To study the spines at different growth stages, they were treated with 20% NaOH solution. The material was examined using an EVO 40 scanning electron microscope (CarlZeiss).
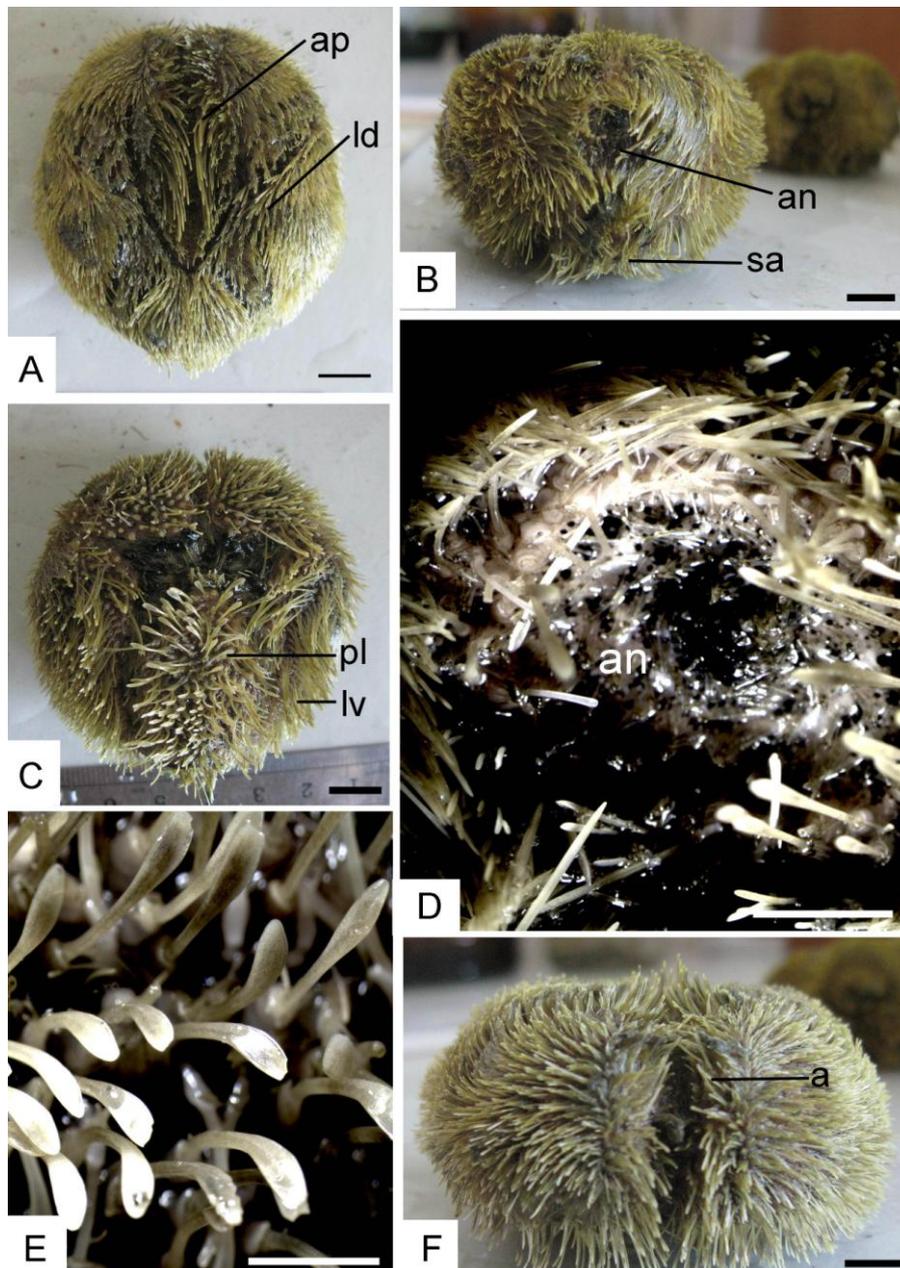

Figure 1: The heart-shaped sea urchin *E. cordatum* (Sea of Japan): (A) general view of the aboral side of test; (B) general view of the posterior side of test; (C) general view of the oral side of test; (D) anal area; (E) spines of the oral side of test (plastron area); (F) general view of the anterior side of test.
Legend: *an*, anal spines; *ap*, apical spines; *lv*, latero-ventral spines; *ld*, latero-dorsal spines; *a*, anterior spines; *pl*, plastron spines; *sa*, subanal spines. Scale bar = 1 cm.

3. Results

Electron microscopic observations revealed the ultrastructure of anterior, anal, latero-dorsal, latero-ventral, subanal, apical spines, as well as plastron spines in the three species of heart urchins.

Anterior spines (Fig. 2A–C)

The wedges of anterior spines in *E. cordatum* from the Sea of Japan and in Mediterranean *E. cordatum* have an elongated trapezoid shape. Clearly triangular wedges of *E. mediterraneum* are located on long and thin trabeculae. The number of wedges in spines of *E. cordatum* from the Sea of Japan is 23; in Mediterranean *E. cordatum* 17; and in *E. mediterraneum*, 12.

Apical spines (Fig. 2D–F)

The apical spines in *E. mediterraneum* and *E. cordatum* from the Sea of Japan have trapezoid wedges; these number 23 in *E. mediterraneum* and 34 in *Echinocardium* sp. The wedges of spines in *E. cordatum* from the Mediterranean Sea are located on thin trabeculae, have a distinct triangular shape, and number 24.

Latero-dorsal spines (Fig. 2G–I)

In cross-section of the spine, the wedges in all the species studied have a trapezoid shape. However, the spines in *E. cordatum* from the Mediterranean Sea and *E. mediterraneum* are massive. There are 14 longitudinal wedges in Mediterranean *E. cordatum*, 21 in *E. cordatum* from the Sea of Japan, and 16 in *E. mediterraneum*,

Latero-ventral spines (Fig. 2J–L)

The wedges in the cross-section of the spine of *E. cordatum* (Sea of Japan) and *E. mediterraneum* are rectangular; in Mediterranean *E. cordatum* they are trapezoid. Spines have 23 wedges in the Mediterranean *E. cordatum*, 32 in *E. cordatum* from the Sea of Japan, and 42 in *E. mediterraneum*.
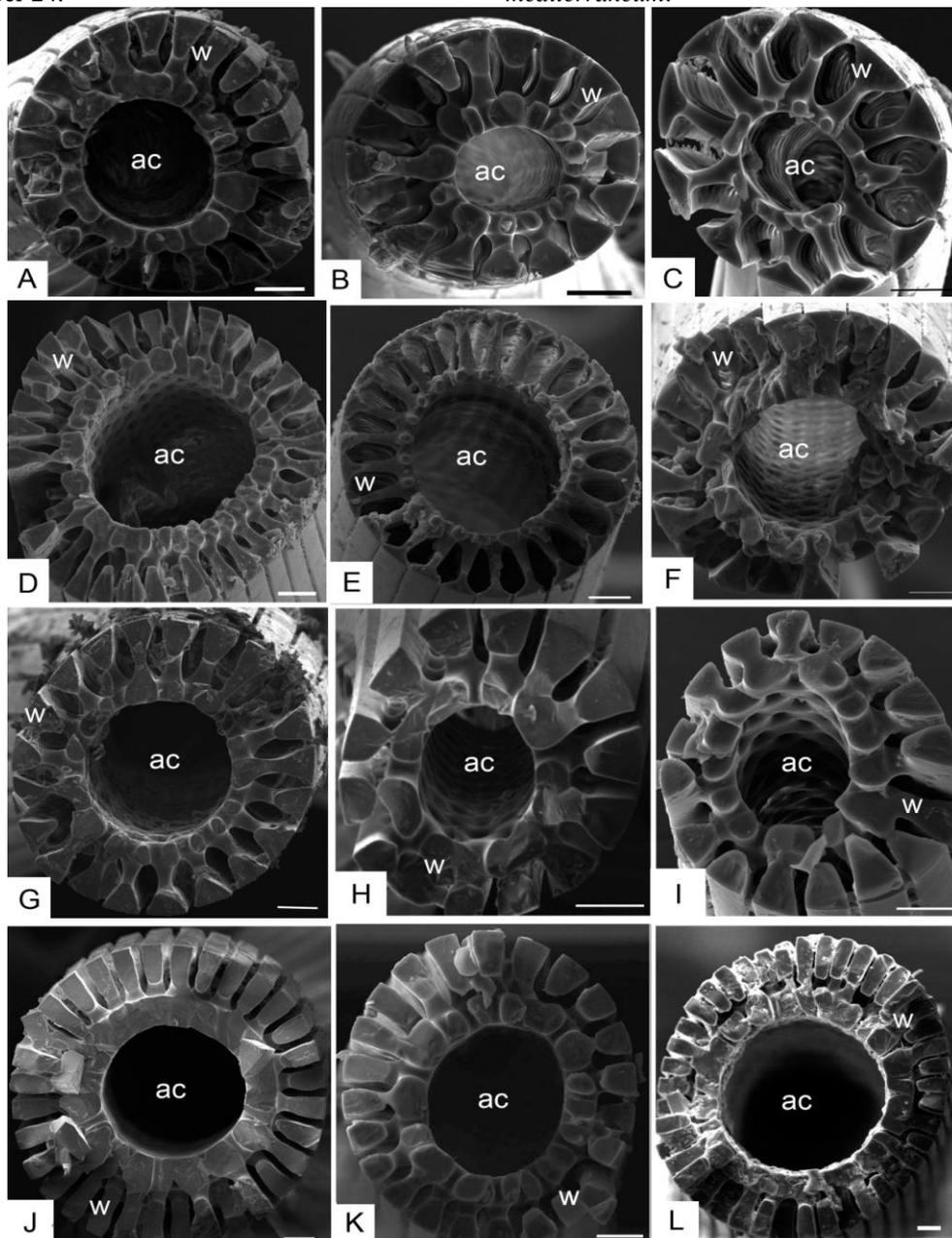

Figure 2: The ultrastructure of spines in *E. cordatum* from the Sea of Japan (A, D, G, J), *E. cordatum* from the Mediterranean Sea (B, E, H, K), and *E. mediterraneum* (C, F, I, L): (A, B, C) cross-section of anterior spine; (D, E, F) cross-section of apical spine; (G, H, I) cross-section of latero-dorsal spine; (J, K, L) cross-section of latero-ventral spine. Legend: *ac*, axial cavity; *w*, longitudinal wedges. Scale bar = 20 μm.

Subanal spines (Fig. 3A–F)

The wedges of subanal spines in the three studied species are covered with transverse frequent ridges. In *E. cordatum* from the Sea of Japan, the wedges are trapezoid; in Mediterranean *E. cordatum* and *E. mediterraneum*, the wedge shape is dactyloid. The longitudinal wedges number 23 in Mediterranean *E. cordatum*, 24 in *E. cordatum* from the Sea of Japan, and 25 in *E. mediterraneum*. The external surface of the wedges is covered with regular longitudinal streaks on which protuberances are often visible.

Anal spines (Fig. 3G–I)

The wedges of anal spines in *E. cordatum* (Sea of Japan) have a trapezoid or rectangular shape and number 23 in the examined specimens. In the axial cavity, there are solitary trabecular outgrowths connecting the opposite sides of the calcareous wall. In Mediterranean *E. cordatum* and in *E. mediterraneum*, the axial cavity is free of trabeculae. The

examined specimens of *E. cordatum* from the Mediterranean Sea have spines with 18 massive, flattened, trapezoid wedges. The wedges surface is smooth. In the spine cross-section of *E. mediterraneum*, there are 23 longitudinal wedges of rectangular shape.

Plastron spines (Fig. 3J–L)

In the cross-section of the same spine, the wedges may vary in shape from trapezoid to rectangular. The rod of the spine is thicker in a segment with trapezoid wedges. The surface of wedges is smooth. Spine wedges in *E. cordatum* (Sea of Japan) are mainly trapezoid, whereas most wedges in the other two examined species are rectangular. There are 31 longitudinal wedges in Mediterranean *E. cordatum*, 28 in *E. cordatum* from the Sea of Japan, and 31 in *E. mediterraneum*.
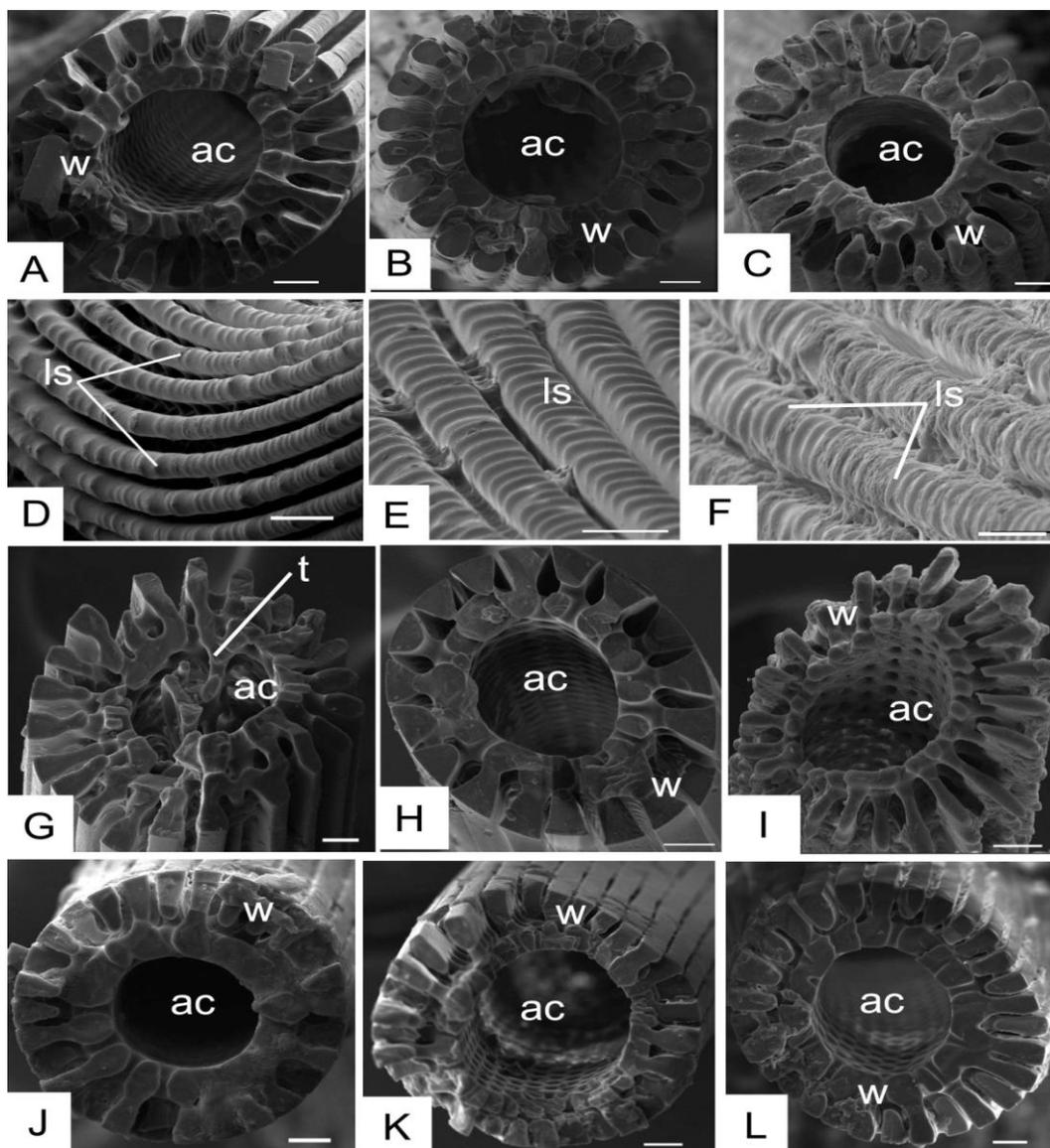

Figure 3: Ultrastructure of spines in *E. cordatum* from the Sea of Japan (A, D, G, J), *E. cordatum* from the Mediterranean Sea (B, E, H, K), and *E. mediterraneum* (C, F, I, L): (A, B, C) cross-section of subanal spine; (D, E, F) surface of subanal spine; (G, H, I) cross-section of anal spine; (J, K, L) cross-section of plastron spine.

Legend: *ac*, axial cavity; *ls*, longitudinal streaks; *t*, trabeculae; *w*, longitudinal wedges. Scale bar = 10 μm.

4. Discussion

Our study of the ultrastructure of 7 types of spines in heart urchins of the genus *Echinocardium* (*E. cordatum* from the Sea of Japan, *E. cordatum* from the Mediterranean Sea, and *E. mediterraneum*) revealed qualitative differences in the structure of their apical, anal, and subanal spines.

The apical spines of *E. cordatum* from the Sea of Japan have trapezoid wedges, tightly adjoining the axial cavity. The wedges of the subanal spines are trapezoid, with acute angles. In the anal spines, the axial cavity is filled with trabeculae, the wedges are trapezoid and rectangular with right-angled margins.

The apical spines of *E. cordatum* from the Mediterranean Sea have triangular wedges located on long trabeculae. The wedges of the subanal spines are trapezoid with rounded angles. The anal spines have trapezoid wedges.

In *E. mediterraneum* from the Mediterranean Sea, the apical spines have trapezoid wedges; the subanal spines have trapezoid wedges with rounded angles; in the anal spines the wedges are rectangular, with rounded margin and varying in length.

The latero-dorsal spines located on the lateral surfaces of sea urchin's test are most similar in structure. The trapezoid shape of the longitudinal wedges is typical for all of them.

The greatest differences were found for the anal and plastron spines. In the anal spines of *E. cordatum* from the Sea of Japan, the wedges may be of both trapezoid and rectangular shape. In the axial cavity, there are solitary trabecular outgrowths that connect the opposite sides of the calcareous wall. The axial cavity of the Mediterranean *E. cordatum* and of *E. mediterraneum* is free of trabeculae. The spine wedges in *E. cordatum* from the Mediterranean Sea are massive, flattened, and trapezoid in shape. In *E. mediterraneum*, the longitudinal wedges in the cross-section of spine have a rectangular shape. In the plastron spines, the shape of the wedge was also found to vary within a spine. Obviously, the wedge shape is related to the functional form of the spine. Thus, the strong curvature of the anal and plastron spines is accompanied by variations in the thickness of the wall of the spine rod, which, in turn, are caused by the elongation of the wedges of the rod and changes in its shape. Apparently, the structure of spines in heart urchins is highly dependent on the environmental factors, as it was shown previously for several different spatangoids (Banno, 2008).

Table: Comparative morphology of heart-shaped sea urchin spines

	E. cordatum (Sea of Japan)	*E. cordatum* (Mediterranean Sea)	*E. mediterraneum* (Mediterranean Sea)
Subanal spine	Wedges trapezoid, with acute angles; axial cavity is not filled by trabeculae	Wedges trapezoid, with rounded angles; axial cavity is not filled by trabeculae	Wedges trapezoid, with rounded angles; axial cavity is not filled by trabeculae
Anal spines	Wedges trapezoid and rectangular; margin of wedge right-angled; axial cavity is filled by trabeculae	Wedges trapezoid in shape; axial cavity is not filled by trabeculae	Wedges rectangular in shape, varying in length; margin of wedge rounded; axial cavity is not filled by trabeculae
Apical spines	Wedges of a trapezoid shape, tightly adjoining the axial cavity	Wedges are triangular in shape, located on long trabeculae; axial cavity is not filled by trabeculae	Wedges trapezoid in shape; axial cavity is not filled by trabeculae

Egea and coauthors suggest that the observed morphological diversity in the *E. cordatum* complex reflects high effective population sizes (Egea et al., 2016). The high individual plasticity of heart urchin spines is commonly used as a specific characteristic in identification of heart sea urchins. Based on the results of this study, we propose a more stable characteristic pertaining to the morphology of several types of spines, which helps more clearly characterize heart urchins from the Sea of Japan and the Mediterranean Sea.

Our data agree with the findings of Petrov and coauthors, who reported genetic differences between the *E. cordatum* population from the North Sea and populations from the Yellow Sea and the Sea of Japan. However, their findings raise some doubt as to whether these sea urchins belong to the same species (Petrov et al., 2016).

5. Acknowledgements

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