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Egg Morphology of Five Species of Sea Urchins from Saipan, CNMI[1]

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Abstract— Using scanning electron microscopy, egg morphology was determined for five sea urchin species collected from Saipan, Commonwealth of the Northern Mariana Islands in June 2014: *Echinometra mathaei*, *Parasalenia poehlii*, *Eucidaris metularia*, *Colobocentrotus mertensii*, and *Mespilia globulus*. Gross morphological observations of these species' eggs generally coincided with previous descriptions. However, due to preservation methods, size discrepancies were present. Eggs collected in 2014 after fixation measured at a smaller diameter than reported in previous studies except for *M. globulus*, which measured between the two previously reported mean diameters. In order to compare measurements with those in previously published studies, additional samples were collected in June 2017 for measurement of fresh unpreserved eggs. Samples of three of these original five species were successfully collected – and all showed shrinkage after fixation and SEM prep. Fresh egg diameter measurements collected in 2017 were comparable to those reported in previous studies, except *M. globulus* which at 149.02 μm was much larger than the previously reported mean diameters of 80 μm and 110.8 μm. Variation in egg microvilli density, size, and shape was observed between all species as viewed using scanning electron microscopy. The present study provides an analysis of the egg surface morphology utilizing scanning electron microscopy of five species of sea urchin collected in Micronesia, three species of which egg surface morphology was previously undescribed. Findings of this study also describe a previously unreported mean diameter of *M. globulus* which could support a latitudinal gradient in egg size of this species.

Introduction

Echinoidea gross egg morphology has been examined in more than 200 species and has revealed that structure is highly conserved (Emlet et al. 1987). General echinoid egg morphology is characterized as clear, round, and covered by vitelline and jelly-like membranes (Harvey 1947, Drozdov & Vinnikova 2010). The cell surface of echinoderm eggs is still incompletely understood. However, available research indicates that the egg is surrounded by a plasma membrane, coated on the outer surface with external peripheral proteins collectively termed the vitelline layer (Kinsey et al. 1980). Scanning electron microscopy demonstrates a dense array of regularly spaced projections of cytoplasmic microvilli in the vitelline layer. The microvilli are interconnected by thin structures presumed to be folds of vitelline layer material used for surface area expansion during fertilization

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(Tegner & Epel 1973). The vitelline layer is what sperm cells attach to during fertilization and must be penetrated in order to fuse with the egg (Glabe & Vacquier 1977).

Little research has been conducted on variation of sea urchin egg surface morphology between species; although, SEM has shown some interspecific differences in urchin eggs, differences in fertilized eggs, and differences after treatment with various chemicals (Eddy & Shapiro 1976, Hagström & Lönning 1976, Tegner & Epel 1976). Tegner and Epel (1976) utilized SEM to describe nine echinoid species eggs and found variations in density, pattern, and spacing of microvilli projections. Despite the differences found between species, the vitelline layers did not show a phylogenetic relationship.

Although there is limited research utilizing SEM in the description of sea urchin egg morphology, egg size variation amongst many species' eggs has been extensively studied using light microscopy. Emlet at al. (1987) describe various factors that have been considered as causes for interspecific variation of urchin egg size. Latitudinal variation is one potential factor. Latitude is positively correlated with egg size among several families of Echinoids. Depth of collection was also determined to negatively correlate with egg size in planktotrophic species of sea urchins. The type of development plays a role, as sea urchin species with planktotrophic larvae produce smaller eggs than species with lecithotrophic larvae. Although no clear relationship between egg size and water temperature has been shown, temperature differences could account for correlation found between latitude and egg size. Finally, the decreasing amount of planktotrophic species also may contribute to increasing egg size at higher latitudes and increased depths.

This study describes morphological features using scanning electron microscopy of preserved female gametes of *Echinometra mathaei*, *Parasalenia poehlii*, *Eucidaris metularia*, *Colobocentrotus mertensii*, and *Mespilia globulus*. Light microscopy was also utilized for morphological descriptions of fresh female gametes of *E. mathaei*, *C.mertensii*, and *M. globulus*. Three of the five species examined in this study, *P. poehlii*, *C. mertensii*, and *M. globulus*, had previously undescribed egg morphology utilizing SEM. Samples were collected from Saipan, Commonwealth of the Northern Mariana Islands in 2014 and 2017.

Materials and Methods

Female sea urchins were collected in June 2014 by snorkel and SCUBA from subtidal habitats on Saipan, Commonwealth of the Northern Mariana Islands (15.18°N 145.75°E) which included three *E. mathaei*, one *P. poehlii*, one *E. metularia*, two *M. globulus*, and two *C. mertensii*. Specimens were placed in buckets of sea water and transported back to the lab where spawning was induced by injection of 0.55 M KCl through the peristomial membrane into the coelomic cavity. The amount of KCl injected ranged from 0.5 ml to 2 ml, depending on the size of species. After injection, sea urchins were lightly agitated and placed into a container of sea water; gametes were collected with a 1 mL Pasteur pipette as they were released from the gonopores into the sea water. Samples were immediately fixed for scanning electron microscopy by storage in 4% glutaraldehyde in 0.1 M sodium cacodylate with 0.35 M sucrose, pH of 7.6. Within three weeks, preserved samples were transported to the University of Hawaii at Manoa, Oahu, Hawaii. There, they were processed for scanning electron microscopy with a protocol similar to one used in Weatherby et al. (1994). Eggs were washed in 0.1 M cacodylate buffer with 0.35 M sucrose for two 15 minute intervals. Each sample was postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for one hour. Dehydration of the sample was performed through a graded ethanol series (30%, 50%, 70%, 85%, 95%) with one change at 30% and 50% and two changes at 70%, 85%, and 95% every three to five minutes. At 85%, samples were transferred to 78 μm pore size microporous specimen capsules lined with Ross Optical Lens Tissue to complete the dehydration. A 100% ethanol dehydration was performed with three changes at ten-minute intervals, the last change in the critical point dryer. Samples were dried in a Tousimis Samdri-795 critical point dryer according to standard protocol. Egg specimens were

mounted on aluminum stubs with double-stick carbon tape, coated with gold/palladium in a Hummer 6.2 sputter coater and examined and measured on a Hitachi S-4800 Field Emission Scanning Electron Microscope at an accelerating voltage of 5 kV. Between 6-20 whole and undamaged eggs were selected for measurement from each female, dependent on the number of eggs released. Eggs were selected by beginning at the top left corner of the stub and selecting the first undamaged egg and then moving to the right to the next field of view and again selecting the first undamaged egg in the field for measurement. When the end of the aluminum stub was reached, the image was shifted an entire field of view and the process was repeated, moving back to the left end of the stub. This process was continued until either the entire stub was viewed or 20 eggs were measured. Due to the oblate spheroid egg shape, two conjugate diameter measurements were taken of each egg in order to find the longest axis; the larger of the two was utilized for comparison. Collected measurements were analyzed using one-way ANOVA with Fisher's exact test for comparison of egg means between species, and an unpaired *t*-test was used for comparison of egg measurements before and after fixation through Minitab 17.

Eggs collected in 2014 were measured after fixation, making comparisons of past recorded egg diameters problematic. Therefore, an attempt was made to collect additional egg samples from the five species previously collected. Urchin specimens from Saipan were again collected using SCUBA and snorkeling methods in June 2017. Three of the five species were successfully obtained: three female *E. mathaei*, nine female *C. mertensii*, and four female *M. globulus*. Spawning was induced as in 2014, and urchin eggs were collected using a 1 mL Pasteur pipette as they were released from the gonopores into the sea water. They were immediately placed on a glass slide and viewed using a Leica DM750 light microscope and measured via the Leica Application Suite X operating system. Between 15-20 whole and undamaged eggs were selected for measurement from each female. Eggs were selected by beginning at the top left corner of the slide and selecting the first undamaged egg and then moving to the right to the next field of view and again selecting the first undamaged egg in the field for measurement. When the end of the slide was reached, the image was shifted down a field of view and then to the left side of the slide and the process was continued until 15-20 eggs were measured. Samples were also preserved and measured via scanning electron microscopy using the same method as in 2014, but with fewer samples from each species measured for comparison.

Results

Samples collected in 2014 from *E. mathaei*, *P. poehlii*, *E. metularia*, *C. mertensii*, and *M. globulus* had oblate spheroid shaped eggs but varied in the general structure of microvilli (Figure 1). Microvilli were found on each species' egg membrane but varied in size, shape, and number. *Echinometra mathaei* microvilli were observed as relatively regularly spaced ovoid projections, each projection approximately 0.2 μm in length (Figure 1b). The microvilli observed on *P. poehlii* were more sporadic in distribution, with longer, irregularly shaped projections laying against the egg surface, each around 0.5 μm in length (Figure 1d). *Eucidaris metularia* eggs had the most distinct membrane appearance, with microvilli in a clustered distribution protruding away from the cell surface giving the membrane a somewhat "furry" appearance. Each projection varied in size and split into multiple extensions making measurement difficult. Smaller round blebs were also present, which may be microvillous projections as well (Figure 1f). The egg cell surfaces from *C. mertensii* appeared most similar to *E. mathaei*, but microvilli were rounder and smaller with projections measuring approximately 0.15 μm. The distribution of microvilli was also more clustered than in *E. mathaei* eggs and folds of vitelline layer material between each projection were more prominent (Figure 1h). *Mespilia globulus* eggs exhibited the most numerous microvilli, but were similar in size and shape to those observed on *E. mathaei*, measuring approximately 0.25 μm in length (Figure 1j).

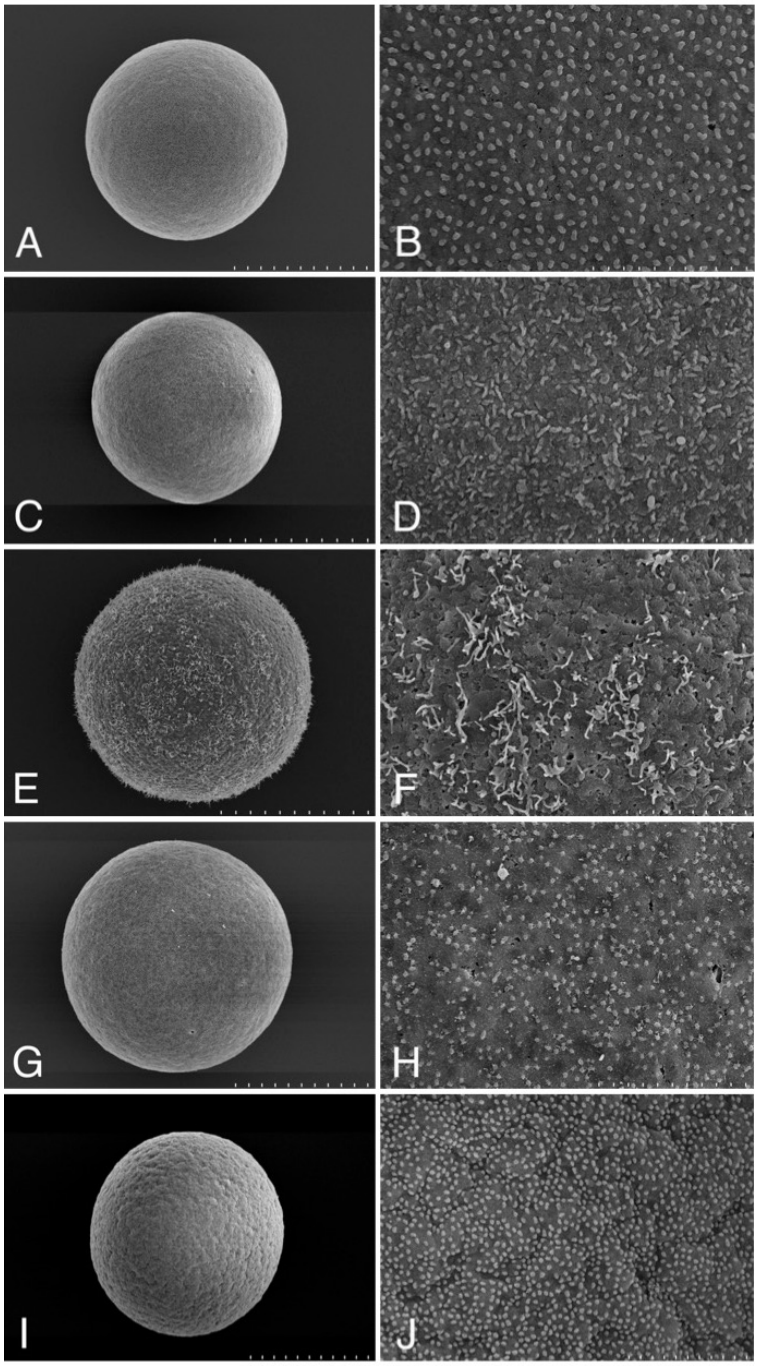

Figure 1. The scanning electron microscope images showing general morphology of eggs along with a close-up image of egg outer membrane surface for each species. (a): *Echinometra mathaei* (scale bar=30.0 µm), (b): close up view of *Echinometra mathaei* (scale bar=4.0 µm), (c): *Parasalenia poehlii* (scale bar=40.0 µm), (d): close up view of *Parasalenia poehlii* (scale bar=5.0 µm), (e): *Eucidaris metularia* (scale bar=20.0 µm), (f): close up view of *Eucidaris metularia* (scale bar=5.0 µm), (g): *Colobocentrotus mertensii* (scale bar=30.0 µm), (h): close up view of *Colobocentrotus mertensii* (scale bar=5.0 µm), (i): *Mespilia globulus* (scale bar=50.0 µm), (j): close up view of *Mespilia globulus* (scale bar=5.0 µm).

In analysis of the fixed samples collected in 2014, ANOVA results showed that species accounted for significant levels of variance in mean egg size. Fisher pairwise comparisons indicate that egg sizes differ significantly between *E. mathaei*, *M. globulus*, and *E. metularia* ($p<0.001$). Larger diameter means of fixed eggs measured in 2014 ranged from 30.75 µm (*E. metularia*) to 88.46 µm (*M. globulus*) (Table 1).

Table1. Conjugate diameter measurements of sea urchin eggs for each collected species in 2014 using scanning electron microscopy; Diameter 1 denotes the larger conjugate diameter and Diameter 2 the smaller.

Species	Diameter 1 (µm)	Diameter 2 (µm)
Eucidaris metularia (n= 6 eggs)	30.75 ± 2.742	30.22 ± 3.055
Echinometra mathaei (n= 36 eggs)	47.47 ± 1.664	45.81 ± 1.528
Parasalenia poehlii (n= 13 eggs)	51.69 ± 1.333	49.26 ± 1.737
Colobocentrotus mertensii (n= 21 eggs)	52.50 ± 1.511	50.99 ± 1.44
Mespilia globulus (n= 19 eggs)	88.46 ± 2.867	84.73 ± 2.487

Egg samples obtained in June 2017 were measured both fresh and post-fixation to determine the extent egg diameter was altered after dehydration and fixation. Measurements showed a significant decrease in egg diameter in all species after fixation for scanning electron microscopy. Fresh *E. mathaei* eggs had a mean egg diameter of 74.77 µm compared to 50.49 µm for fixed eggs. Similarly, fresh *C. mertensii* eggs decreased from a mean diameter of 71.15 µm to 55.37µm after fixation, while *M. globulus* eggs shrank from 149.02 µm fresh to 93.67 µm fixed (Table 2).

Table 2. The larger conjugate diameter of sea urchin eggs for each collected species in 2017 using light microscopy for fresh eggs and scanning electron microscopy for fixed eggs with percent decrease in size observed.

Species	Fresh Diameter (µm)	Fixed Diameter (µm)	Percent Decrease
Echinometra mathaei	74.77 ± 9.232 (n= 51 eggs)	50.49 ± 1.373 (n= 14 eggs)	32.47%
Colobocentrotus mertensii	71.15 ± 4.192 (n= 143 eggs)	55.37 ± 2.317 (n= 45 eggs)	22.17%
Mespilia globulus	149.02 ± 21.418 (n= 75 eggs)	93.67 ± 7.472 (n= 30 eggs)	37.14%

The percent decrease in size between diameters of fresh eggs to glutaraldehyde fixed eggs varied between species. *Echinometra mathaei* showed a decrease in size of 32.47%, *Colobocentrotus mertensii* had a decrease of 22.17%, and *M. globulus* had a decrease of 37.14%.

Measurements taken using SEM in 2017 were compared with samples collected and measured using SEM in June 2014. Comparison utilizing an unpaired *t*-test showed eggs of all three species collected in in 2017 were significantly larger in mean egg size than those collected in 2014 ($p<0.01$) (Table 3).

Table 3. Mean egg size of three sea urchin species measured using SEM in 2014 and 2017. Mean diameters between years were significantly different in all three species ($p<0.01$).

Species	**2014 Mean Diameter (µm)**	**2017 Mean Diameter (µm)**
Echinometra mathaei	47.47 (n= 36 eggs)	50.49 (n= 14 eggs)
Colobocentrotus mertensii	52.50 (n= 21 eggs)	55.37 (n= 45 eggs)
Mespilia globulus	88.46 (n= 19 eggs)	93.67 (n= 30 eggs)

Discussion

Chia et al. (1975) found that variation in sea urchin gamete morphology exists between species, but the general structure remains constant. Consistent with this observation, eggs in this study showed limited variation between species with major differences only observed in size. Differences in microvilli appearance were observed, which may be connected to the selective permeability of specific urchin species' spermatozoa, as glycoproteins of the microvilli are thought to be involved in sperm-egg adhesion (Kinsey et al. 1980) and the ensuing entry of sperm (Chun et al. 2018). In this study, vitelline membrane appearance was most similar between *E. mathaei* and *C. mertensii* both of which belong to family Echinometridae. Similarities in microvilli appearance within a genus was also found in comparing *C. mertensii* egg surface morphology with that of previously described *Colobocentrotus atratus* (Tegner & Epel 1976). The remaining three species, *P. poehlii*, *E. metularia*, and *M. globulus* come from three different families, Parasaleniidae, Cidaridae, and Temnopleuridae respectively, and showed more variable appearances of microvilli. Although similar appearing microvilli were found within one family, it does not appear that the variations in vitelline layers consistently follow a phylogenic relationship. Differences in microvilli have previously been observed between sea urchin eggs of the same genus (Hagström & Lönning 1976), as well as similarities found between species of different families (Tegner & Epel 1976).

Scanning electron microscopy examination of egg surface morphology has been previously described for *E. mathaei* and *E. metularia* (Tegner & Epel 1976). Comparison of morphological findings of these species in this study with those previously described shows microvilli distribution and spacing is similar within both species, however, while the egg surface of *E. metularia* is similar in regards to microvilli distribution, the length of the microvilli projections is unclear when described by Tegner and Epel (1976). This is the first description of egg morphology utilizing SEM for *C. mertensii*, *P. poehlii*, and *M. globulus*.

All samples in this study were collected within a three-week period in June both in 2014 and 2017, which may have contributed to the variance in gametes. It is unknown whether June is within the peak reproductive season for each species, as this is reported to vary with species and location

(Alsaffar & Lone 2000). The induced release of gametes by injection may have resulted in gametes that were not fully developed. However, it could be assumed that response to injection of KCl indicates a readiness to spawn (Lessios 1991 in Mercier & Hamel 2009). Some samples contained eggs that appeared to be degenerating, which may point to collection at the end of the spawning cycle (Zhadan et al. 2015). However, only whole, normal eggs were measured for this study.

The observation of a decrease in mean diameter after fixation confirms shrinkage due to preservation methods. The variation in mean percent shrinkage found between species may be due to differences in amount of fluid present in each egg. Because gametes were collected over a three-week period, egg size could have varied over this period. This may also have contributed to the discrepancies in mean egg diameter in fixed eggs between 2014 and 2017.

Preserved egg diameters of *E. mathaei* and *E. metularia* measured in 2014 were smaller than mean diameters reported when measured fresh in Emlet et al. (1987). Preserved egg diameter of *C. mertensii* also measured at a smaller diameter in the present study compared to when measured fresh in Thet et al. (2004). *Mespilia globulus*, which had two reported means of 80 μm at 34°N (Onoda 1936) and 110.8 μm (Dan 1952 in Harvey 1956), was found to have a preserved mean egg diameter of 88.46 μm in 2014. As shrinkage was found to occur with gamete fixation, this suggested a larger diameter of fresh eggs than previously described. The measurement of fresh eggs from *M. globulus* in 2017 confirmed this, with a mean egg diameter of 149.02 μm; this was much higher than both previously reported egg sizes. The 80 μm *M. globulus* eggs were sampled from a latitude of 34°N (Onoda 1936). In contrast, the 149.02 μm eggs in the present study were observed at 15.18°N. It is unclear at what latitude the 110.8 μm eggs were observed (Dan 1952 in Harvey 1956). If collected from an intermediate latitude, this implies a latitudinal gradient in egg size for this species. As mentioned earlier, Emlet et al. (1987) noted that intraspecific variation of sea urchin gamete size has been observed at different locations, and a higher latitude was determined to positively correlate with egg size in various families. Weak (non-significant) negative correlations of latitude were observed with egg size of multiple species within Temnopleuridae and Clypeasteroida. It should be noted that *M. globulus* is within Temnopleuridae, supporting the possibility of larger egg sizes at lower latitudes. Differences in egg size among latitudes could indicate here-to-for unknown species level differences, but there are no supporting data for this argument.

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References

Alsaffar, A.H. & K.P. Lone. 2000. Reproductive cycles of Diadema setosum and Echinometra mathaei (Echinoidea: Echinodermata) from Kuwait (Northern Arabian Gulf). Bulletin of Marine Science 67: 845-856.

Chia, F., D. Atwood, & B. Crawford. 1975. Comparative morphology of echinoderm sperm and possible phylogenetic implications. American Zoologist 15: 533-565.

Chun, J.T., F. Vasilev, N. Limatola, & L. Santella. 2018. Fertilization in starfish and sea urchin: roles of actin. In Kloc, M. & Kubiak, J.Z. (eds), Marine Organisms as Model Systems in Biology and Medicine, pp. 33-47. Springer International Publishing, Cham, Switzerland.

Drozdov, A.L. & V.V. Vinnikova. 2010. Morphology of gametes in sea urchins from Peter the Great Bay, Sea of Japan. Russian Journal of Developmental Biology 41: 37-45.

Eddy, E.M. & B.M Shapiro. 1976. Changes in the topography of the sea urchin egg after fertilization. The Journal of Cell Biology 71: 35-48.

Emlet, R.B., L.R. McEdward, & R.R. Strathmann. 1987. Echinoderm larval ecology viewed from the egg. In Jangoux, M. & Lawrence, J.M. (eds), Echinoderm Studies, Vol. 2. pp. 55-136. A.A. Balkema, Rotterdam, Netherlands.

Glabe, C.G. & V.D. Vacquier. 1977. Isolation and characterization of the vitelline layer of sea urchin eggs. The Journal of Cell Biology 75: 410-421.

Hagström, B.E. & S. Lönning. 1976. Scanning electron microscope studies of the surface of the sea urchin egg. Protoplasma 87: 281-290.

Harvey, E.B. 1947. Bermuda sea urchins and their eggs. Biological Bulletin 93: 217-218.

Harvey, E.B. 1956. The American Arbacia and Other Sea Urchins. Princeton University Press, London, England. Retrieved from https://archive.org/.

Kinsey, W.H., G.L. Decker, & W.J. Lennarz. 1980. Isolation and partial characterization of the plasma membrane of the sea urchin egg. The Journal of Cell Biology 87: 248-254.

Mercier, A. & J. Hamel. 2009. Advances in Marine Biology Endogenous and Exogenous Control of Gametogenesis and Spawning in Echinoderms. Academic Press, London, England. Retrieved from https://books.google.com/.

Onoda, K. 1936. Notes on the development of some Japanese echinoids with special reference to the structure of the larval body. Japanese Journal of Zoology 6: 637-654.

Tegner, M.J. & D. Epel. 1973. Sea Urchin Sperm-Egg Interactions Studied with the Scanning Electron Microscope. Science 179: 685-688.

Tegner, M.J. & D. Epel. 1976. Scanning Electron Microscope Studies of Sea Urchin Fertilization. Journal of Experimental Zoology 197: 31-57.

Thet, M.M., M. Noguchi, & I. Yazaki. 2004. Larval and Juvenile Development of the Echinometrid Sea Urchin Colobocentrotus mertensii: Emergence of the Peculiar Form of Spines. Zoological Science 21: 265-274.

Weatherby, T.M., K.K. Wong, & P.H. Lenz. 1994. Fine structure of the distal sensor setae on the first antennae of Pleuromamma xiphias Giesbrecht (Copepoda). Journal of Crustacean Biology 14: 670-685.

Zhadan, P.M., M.A Vaschenko, & T.N. Almyashova. 2015. Spawning failure in the sea urchin Strongylocentrotus intermedius in the northwestern Sea of Japan: Potential environmental causes. Journal of Experimental Marine Biology and Ecology 465: 11-23.

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