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SEM observations of the fracture morphology of Acantharea (Radiolaria) from the East China Sea, Okinawa, Japan

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Abstract

Scanning electron microscopy (SEM) and X-ray energy dispersive spectroscopy (EDS) observations were performed to investigate the fracture morphology of acantharian species (*Dorataspis micropora* Haeckel, *Hexalaspis heliodiscus* Haeckel, *Amphilonche elongata* (Müller), and *Lonchostaurus rhombicus* (Haeckel)) from northwestern Okinawa, with the aim of determining their resistance to dissolution in seawater. SEM observations revealed the accumulation of microcrystals (slender idiomorphic prisms) on the surface of the acantharian skeletons and EDS data indicated the existence within the skeletons of strontium, sulfur, and oxygen. Accordingly, the acantharian skeleton is made of celestite ($SrSO_4$) microcrystals. These properties mean that the skeleton is readily dissolved in seawater and easily broken into micro-fragments. Although the acantharian skeletons were not porous (as in the case of phaeodarians), they generally broke apart easily and dissolved completely in seawater. This finding suggests that the dissolution of the acantharian skeletons is determined solely by the characteristics of the constituent $SrSO_4$ rather than the construction of the skeleton.

Key words: acantharians, Radiolaria, skeleton, SEM, EDS, $SrSO_4$, celestite

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Introduction

Radiolaria are holoplanktonic protists that are widely distributed in tropical, subtropical, and even polar marine environments, occurring throughout the water column from the surface to the greatest depths (Casey, 1971). Radiolaria have roughly spherical cells and thread-like pseudopodia extending radially over the endoskeleton, thereby facilitating a floating existence (e.g., Anderson, 1983).

Currently, many researchers use the term “Radiolaria” as a conventional name that encompasses the Acantharea, Polycystinea, and Phaeodarea. In a modern taxonomic system, Levine et al. (1980) did not recognize the “Radiolaria” as a taxonomic group and excluded the term from their system, apparently because of marked differences that exist among species within individual classes. For example, within

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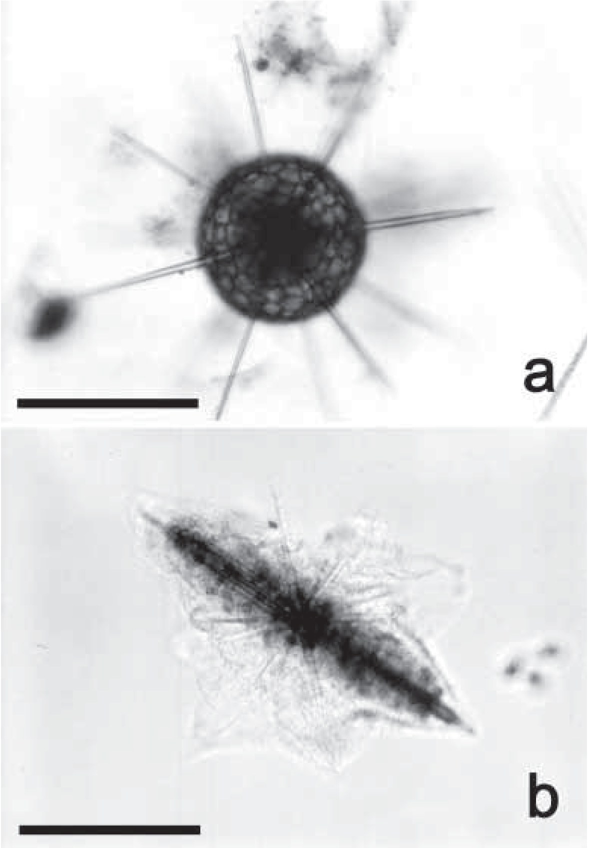

Fig. 1. Light micrographs (LM) of acantharians. Scale bars indicate 100 μm. 1a: *Stauraspis* sp., 1b: *Amphibelone hydrotomica* (Haeckel).

the Actinopoda, the chemical composition of the skeletons varies from strontium sulfate ($SrSO_4$) in the Class Acantharea to opaline silica (SiO_2) in the Class Polycystinea and opaline silica with organic matter in the Class Phaeodarea.

Recent molecular phylogenetic works based on small subunit (SSU) rDNA sequences have provided evidence for the monophyletic origin of the Acantharea and the Polycystinea as Rhizaria, excluding the cercozoan Phaeodarea (e.g., Adl et al., 2005). Hence, the phylogenetic relationships among Radiolaria remain a subject of ongoing debate.

Acantharea was first described by Müller (1858) as a living specimen of “Acanthometren” in the Mediterranean Sea. They have a delicate, generally spherical or sometimes flattened shell, consisting of 10 diametral or 20 radial spines that cross in the center of the cytoplasm (Fig. 1). The spicular arrangement defines a regular pattern that has been called “Müller’s law” (Müller, 1858). As mentioned above, the skeleton is composed of strontium sulfate (for a review, see Anderson, 1983). On the basis of the spicule arrangement, the organization of the cell body, and other criteria, the Acantharea comprises about 50 genera grouped into 20 families and 4 orders: Holocanthida, Symphyacanthida, Chaunacanthida, and Arthracanthida (Febvre et al., 2000).

Acantharians are ubiquitous throughout the water column in various marine environments, yet their skeletons are generally absent in marine sediments and are much more poorly preserved than those of polycystines (e.g., Massera Bottazzi, 1978), reflecting their fragile structure and contrasting chemical composition of the skeleton relative to the polycystines and phaeodarians. Accordingly, acantharians are thought to be an important component of the biogeochemical cycle of strontium in the ocean (e.g., Bernstein et al., 1992).

In the present study, we report on scanning electron microscopy (SEM) and X-ray energy dispersive spectroscopy (EDS) observations of the fracture morphology of some acantharian species from northwestern Okinawa and discuss their resistance to dissolution in seawater.

Materials

Acantharian samples were collected on May 12 and 14, and October 6 and 7 in 2008 from surface seawater (up to 3 m depth) using a plankton net (60 cm circle opening with 37 μm mesh net) at Site 990528 (26°37’N, 127°49’E) in the eastern East China Sea, approximately 5 km northwest of Okinawa Island, southernmost Japan (Fig. 2). The

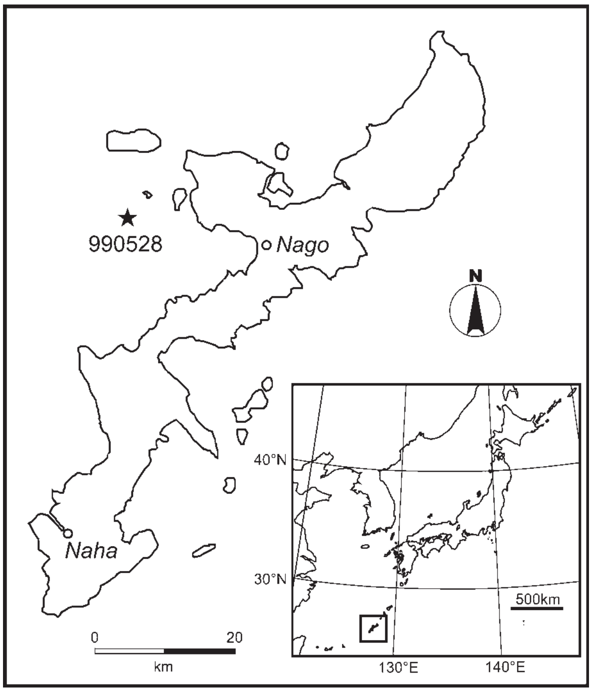

Fig. 2. Map showing the location of the Site 990528, northwestern Okinawa Island, modified from Takahashi et al. (2003).

samples were placed in jars diluted with seawater from the same site, and immediately brought to the laboratory of the Tropical Biosphere Research Center of the University of Ryukyus. The radiolarians were then separated using an inverted microscope and a binocular stereomicroscope; they were isolated, identified to species, and transferred to culture dishes containing filtered seawater. All acantharian specimens were then transferred into 0.5-ml microtubes containing 99.5% ethanol and stored at room temperature until observation by SEM and EDS.

We identified and observed the following four acantharian species: *Dorataspis micropora* Haeckel, *Hexalaspis heliodiscus* Haeckel, *Amphilonche elongata* (Müller), and *Lonchostaurus rhombicus* (Haeckel).

Dorataspis micropora Haeckel (Order Arthracanthida; Family Dorataspidae) (Fig. 3) has a spherical shell of about 200 µm in diameter. The spicules are sword-shaped with blunt tips. There exist one or two sutural pores on the surface that are about five times smaller than the main aspinal pores.

Hexalaspis heliodiscus Haeckel (Order Arthracanthida; Family Hexalaspidae) (Fig. 4) has a thick-walled lenticular or discoidal shell of about 100 µm in diameter. The shell surface is covered with subcircular, funnel-shaped pits with secondary spicules. The six main spicules are generally equal in shape and size, although two main ones may be longer and thicker than the other four. The secondary spicules, being much shorter and thinner than the main spicules, generally break off and dissolve relatively readily.

Amphilonche elongata (Müller) (Order Arthracanthida; Family Acanthometridae) (Fig. 5) has two large main spicules of almost equal length. They are distally quadrangular with very sharp tips. The secondary spicules have a sword-like shape, and are 3-7 times shorter than the main spicules.

Lonchostaurus rhombicus (Haeckel) (Order Arthracanthida; Family Phyllostauridae) (Fig. 6) has two large and two small equatorial spicules, and sixteen secondary spicules. Their tips are blunt and distally quadrangular. The larger equatorial spicules are about 150 µm in length, which are longer but narrower than the small equatorial ones. The distal ends of the equatorial spicules are tapered.

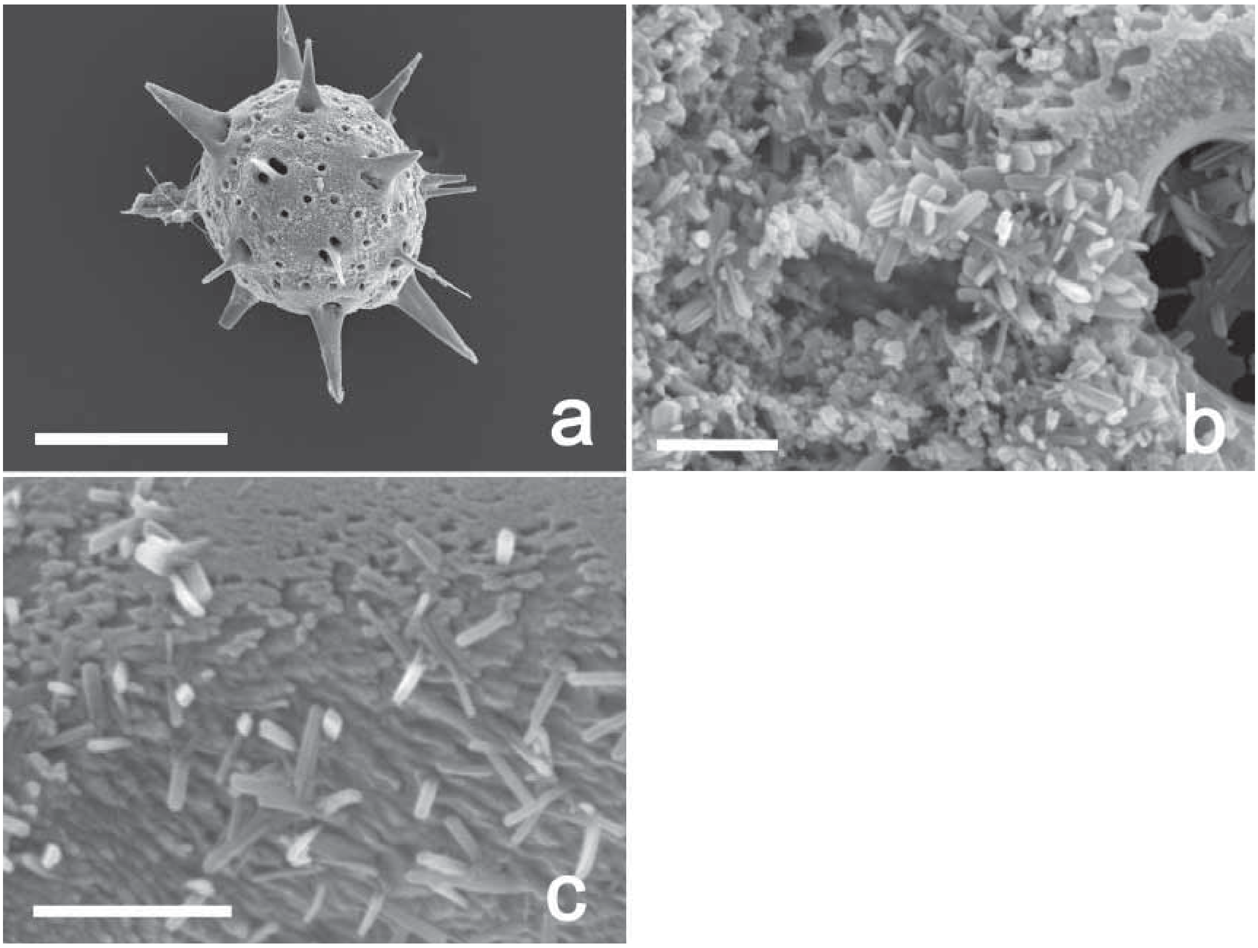

Fig. 3. *Dorataspis micropora* Haeckel. 3a: Scale bar is 100 µm. 3b: Enlargement of the pore frame. Scale bar is 4 µm. 3c: Enlargement of the surface of the shell. Scale bar is 4 µm.

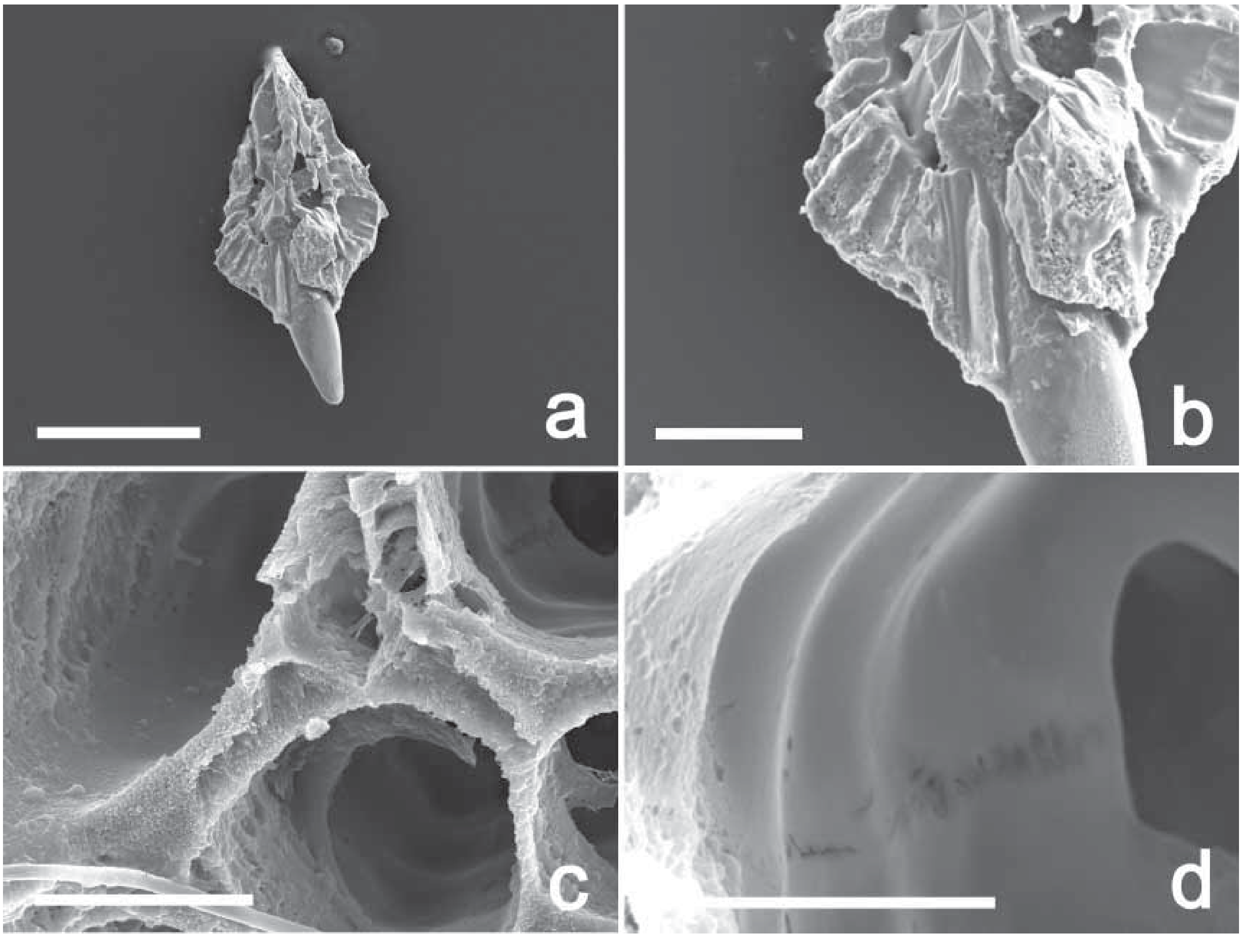

Fig. 4. *Hexalaspis heliodiscus* Haeckel. 4a: A specimen purposely broken at the axis. Scale bar is 100 μm. 4b: Enlargement of the shell wall. Scale bar is 20 μm. 4c: Enlargement of the tubular pore frame. Scale bar is 10 μm. 4d: Enlargement of the interior of the pore wall. Scale bar is 5 μm.

Methods

Each specimen was broken or cut into several pieces in 99.5% ethanol on a glass slide using a razor blade, and then rinsed twice in 99.5% ethanol to remove any organic matter on or within the skeleton. The specimens were then mounted, gold-coated, and analyzed by SEM and EDS. EDS was used for qualitative chemical analyses of the acantharian skeletons. Both SEM and EDS analyses were performed on a 5800LV SEM (JEOL) equipped with a Voyager X-ray spectrometer (Norman Inst.), operated at 15 kV with an analysis time of 100 second.

Results and Discussion

SEM observations of *Dorataspis micropora* revealed an accumulation of microcrystals (slender idiomorphic prisms) on the surface of the skeleton and that skeletal dissolution had already begun by the time of analysis (Figs 3b and 3c). The crystal form resembled the celestite described by Hollande and Martoja (1974) and our EDS data (Fig. 7) indicated the existence of strontium, sulfur, and oxygen within the skeleton of *D. micropora*.

In separate experiments, specimens of *D. micropora* completely dissolved within several hours of being placed back in seawater; however, skeletal dissolution did not occur in seawater with high concentrations of strontium (data not shown). These findings suggest that acantharian skeletons have a fundamentally different mineral composition and structure from those of polycystines, as described in previous studies (e.g., Bernstein et al., 1999). The microcrystals that make up the skeleton of *D. micropora*, as observed in the present study, are celestite ($SrSO_4$). Because of this construction and composition, the skeleton is highly soluble in seawater and readily breaks into micro-fragments.

The fractures of the broken pieces of three acantharian species were observed by SEM and EDS. An important

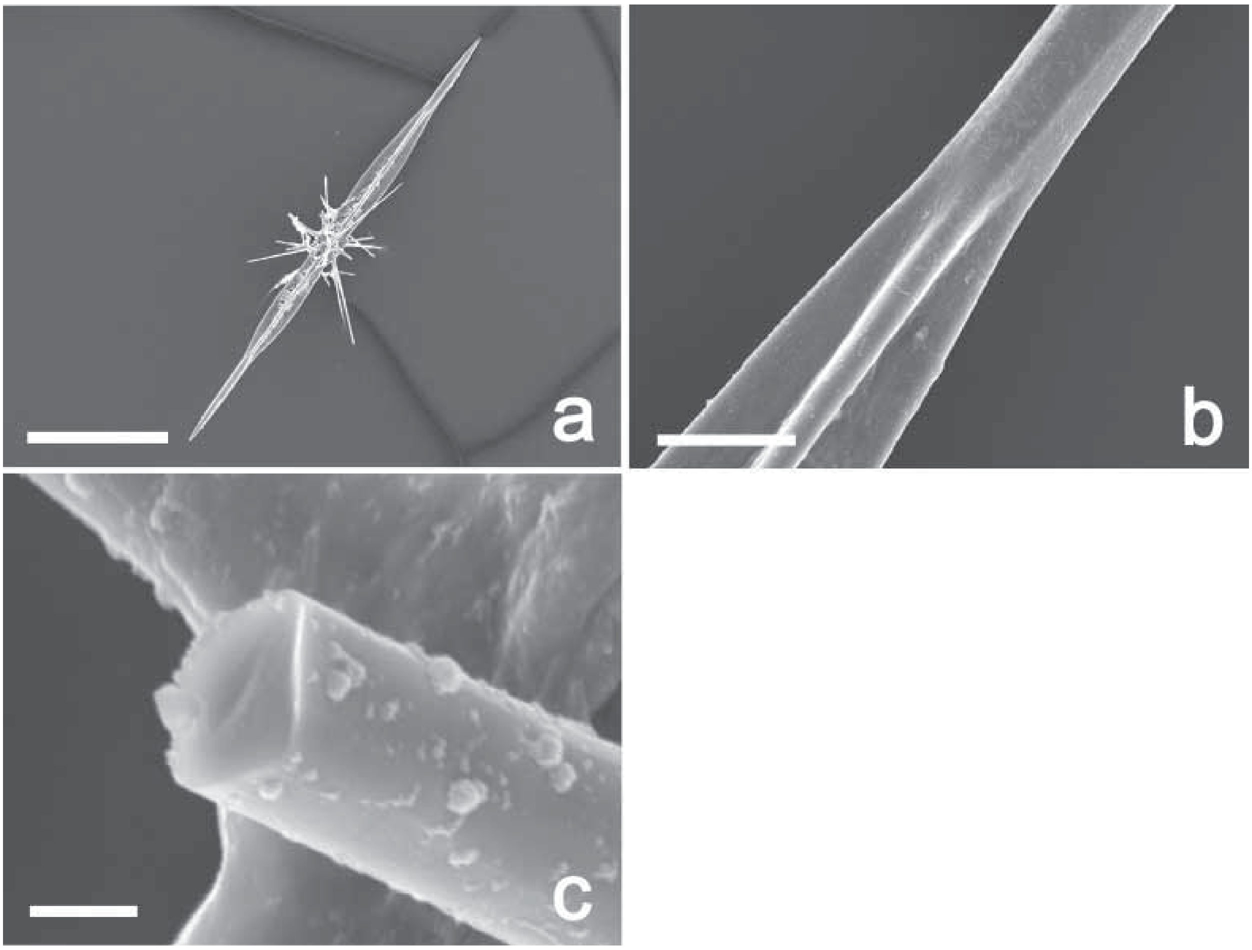

Fig. 5. *Amphilonche elongata* (Müller). 5a: Scale bar is 100 μm. 5b: Enlargement of the large main spicule. Scale bar is 20 μm. 5c: Enlargement of the fracture of the secondary spicule. Scale bar is 4 μm.

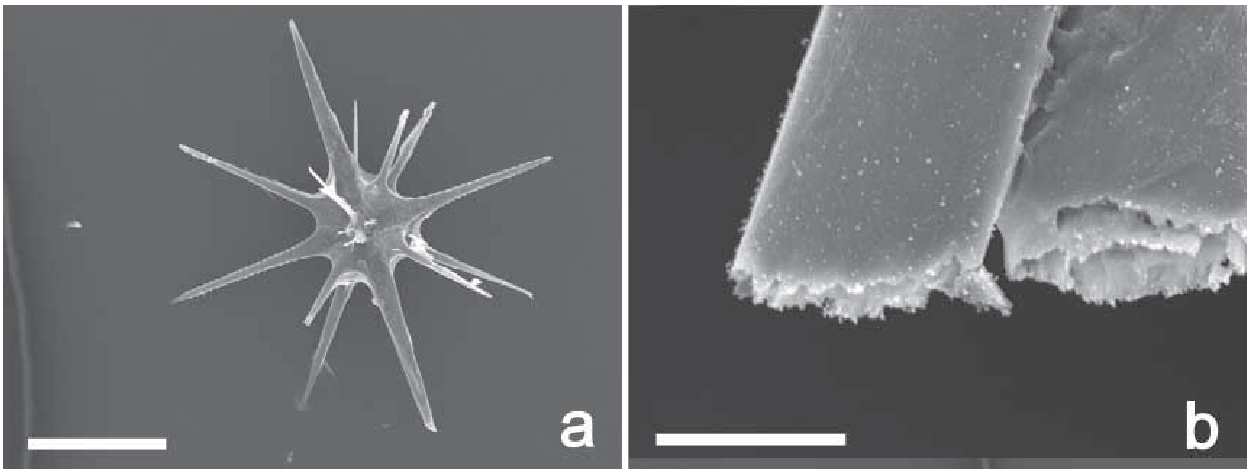

Fig. 6. *Lonchostaurus rhombicus* (Haeckel). 6a: Scale bar is 100 μm. 6b: Enlargement of the fracture of the small equatorial spicule. Scale bar is 10 μm.

finding was a progressive change in the morphology of the acantharian skeletons with ongoing dissolution. In all cases, the skeletons became fragile and disassembled with increasing dissolution. However, it was not clear that all of the acantharians observed in the present study have intricately porous skeletons, compared with those of solid polycystine skeletons. For example, *Amphilonche elongata* (Fig. 5) and *Lonchostaurus rhombicus* (Fig. 6) have fragile, thin and/or slender secondary spicules. We did not observe porous features in the structures of these species; the fractures

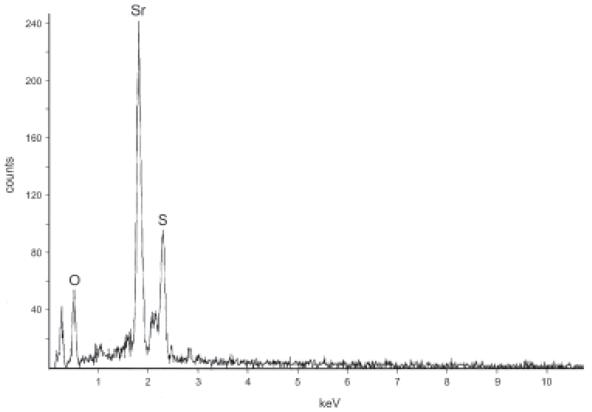

Fig. 7. X-ray energy dispersive spectroscopy (EDS) spectrum of *Dorataspis micropora* Haeckel. Spectrum obtained with X-ray spectrometer examined at 15 kV for 100 live second. Data indicates the existence of strontium, sulfur, and oxygen within the skeleton.

appeared dense and solid. The skeletons of phaeodarian radiolaria consist of an admixture of silica and organic matter, and their porous nature is considered to explain their poor preservation (Takahashi et al., 1983). In the present study, against our expectation, we found that acantharian skeletons are not porous in contrast to phaeodarians.

Hexalaspis heliodiscus has a thicker shell than that of any of the other acantharians, and the six main spicules are quite massive than those of other acantharian species (Figs 4a and 4b); nevertheless, these massive spicules also generally break off (although relatively slow) and dissolve completely in seawater. This finding suggests that the texture of the acantharian skeleton is unlikely to exert a significant control on its dissolution. Instead, the dissolution of acantharian skeletons is possibly determined solely by the characteristic of the constituent $SrSO_4$ rather than the nature of skeleton construction.

Concluding remarks

The acantharian species are outwardly similar in appearance to the Polycystinea, but have a skeleton of $SrSO_4$. Amaral Zettler et al. (1997) proposed that the similar appearance of the Acantharea and Polycystinea resulted from convergent evolution; however, the Polycystinea, in addition to the acantharians, has also the ability to use $SrSO_4$ in constructing its skeleton. The vegetative adults of colonial spumellarians are known to contain a crystal of $SrSO_4$ in their central capsules (Hollande and Martoja, 1974), and the biflagellated swarmers of some kinds of spumellarian species also contain crystals of $SrSO_4$ in the membrane-bound vesicles (Anderson, 1976).

Recent molecular studies (Amaral Zettler et al., 1997; López-García et al., 2002; Nikolaev et al., 2004; Takahashi et al., 2004; Yuasa et al., 2005, 2006; Kunitomo et al., 2006) based on small-subunit ribosomal DNA (SSU rDNA) sequences have resolved some of these taxonomic conflicts that existed among the Radiolaria. These studies suggested that the Polycystinea and Acantharea form a monophyletic group; that is, they share a common evolutionary ancestor. However, it is unknown exactly when the ability to fix $SrSO_4$ evolved in any of these clades or what role, if any, it may have played in their diversification. To resolve these uncertainties, further information is required, particularly fossil evidence and molecular data for both the acantharians and polycystines.

Acknowledgements

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References

Adl, S. M., Simpson, A. G. B., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R. Bowser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C. E., Lewis, L. A., Lodge, J., Lynn, D. H., Mann, D. G., Mccourt, R. M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S. E., Nerad, T. A., Shearer, C. A., Smirnov, A. V., Spiegel, F. W., and Taylor, M. F. J. R., 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. J. Eukaryot. Microbiol. 52, 399–451.

Amaral Zettler, L., Sogin, M. L., Caron, D. A., 1997. Phylogenetic relationships between the Acantharea and the Polycystinea: a molecular perspective on Haeckel's Radiolaria. Proc. Natl. Acsd. Sci. USA 94, 11411–11416.

Anderson, O. R., 1983. Radiolaria. Springer-Verlag, New York, 355 pp.

Anderson, O. R., 1976. Fine structure of a collodarian radiolarian (*Sphaerozoum punctatum* Müller 1858) and cytoplasmic changes during reproduction. Marine Micropaleontology 1, 287–297.

Bernstein, R. E., Kling, S. A., and Boltovskoy, D., 1999. Acantharia. In: Boltovskoy, D. (Ed.), South Atlantic Zooplankton. Backhuys Publishers, Leiden, pp. 75–147.

Bernstein, R. E., Byrne, R. H., Betzer, P. R., and Greco, A. M., 1992. Morphologies and transformations of celestite in seawater: the role of acantharians in strontium and barium geochemistry. Geochimica et Cosmochimica Acta 56, 3273–3279.

Casey, R. E., 1971. Distribution of polycystine Radiolaria in the oceans in relation to physical and chemical conditions. In: Funnell, B. M. and Riedel, W. R. (Eds.), The Micropalaeontology of Oceans. Cambridge University Press, Cambridge, pp. 151–159.

Febvre, C., Febvre, J., and Michaels, A., 2000. Acantharia. In: Lee, J. J., Leedale, G. F., and Bradbury, P. (Eds.), The Illustrated Guide to the Protozoa. Second Edition. Lawrence, pp. 783–803.

Hollande, A. and Martoja, R., 1974. Identification du cristalloide des isospores de Radiolaires à un cristal de celestite ($SrSO_4$). Détermination de la constitution du cristalloide par voie cytochimique et à l'aide de la microsonde électronique et du microanalyseur à émission ionique secondaire. Protistologica 10, 603–609.

Kunitomo, Y., Sarashina, I., Iijima, M., Endo, K., and Sashida, K., 2006. Molecular phylogeny of acantharian and polycystine radiolarians based on ribosomal DNA sequences, and some comparisons with data from the fossil record. European Journal of Protistology 42, 143–153.

Levine, N. D., Corliss, J. O., Cox, F. E. G., Deroux, G., Grain, J., Honigberg, B. M., Leedale, G. F., Loeblich, A. R., Lom, J., Lynn, D. H., Merinfeld, D., Page, F. C., Poljansky, G., Sprague, V., Vavra, J., and Wallace, F. G., 1980. A newly revised classification of the Protozoa. J. Protozool. 27, 37–58.

López-García, P., Rodríguez-Valera, F., Moreira, D., 2002. Toward the monophyly of Haeckel's Radiolaria: 18S rRNA environmental data support the sisterhood of Polycystinea and Acantharea. Mol. Biol. Evol. 19, 118–121.

Massera Bottazzi, E., 1978. Systematic-ecological aspects of Radiolaria with special reference to Acantharia. Boll. Zool. 45, 133-144.

Müller, H., 1858. Über die Thalassicollen, Polycystinen und Acanthometren des Mittelmeeres. Kgl. Akad. Wiss. Abh., Berlin, 1–62.

Nikolaev, I. S., Berney, C., Fahrni, F. J., Bolivar, I., Polet, S., Mylnikov, P. A., Aleshin, V. V., Petrov, B. N., Pawlowski, J., 2004. The twilight of Heliozoa and rise of Rhizaria, an emerging supergroup of amoeboid eukaryotes. Proc. Natl. Acad. Sci. USA 101, 8066-8071.

Takahashi, K., Hurd, D. C., and Honjo, S., 1983. Phaeodarian skeletons: their role in silica transport to the deep sea. Science 222, 617-618.

Takahashi, O., Mayama, S., and Matsuoka, A., 2003. Host-symbiont associations of polycystine Radiolaria: epifluorescence microscopic observation of living Radiolaria. Marine Micropaleontology 49, 187–194.

Takahashi, O., Yuasa, T., Honda, D., and Mayama, S., 2004. Molecular phylogeny of the solitary shell-bearing Polycystinea (Radiolaria). Revue de Micropaléontology 47, 111–118.

Yuasa, T., Takahashi, O., Honda, D., Mayama, S., 2005. Phylogenetic analyses of the polycystine Radiolaria based on the 18S rDNA sequences of the Spumellarida and the Nassellarida. European Journal of Protistology 41, 287–298.

Yuasa, T., Takahashi, O., Dolven, J. K., Mayama, S., Matsuoka, A., Honda, D., Bjørklund, K. R., 2006. Phylogenetic position of the small solitary phaeodarians (Radiolaria) based on 18S rDNA sequences by single cell PCR analysis. Marine Micropaleontology 59, 104–114.

沖縄本島北西沿岸から産するアカンサリア（放散虫）の殻断面形態の走査電子顕微鏡観察

新免　浩太郎・高橋　修・湯浅　智子

宇宙地球科学分野

要　旨

沖縄本島北西沿岸表層水中からサンプルしたアカンサリア4種：*Dorataspis micropora* Haeckel，*Hexalaspis heliodiscus* Haeckel，*Amphilonche elongata* (Müller)，および *Lonchostaurus rhombicus* (Haeckel)の殻断面を，走査電子顕微鏡（SEM）とエネルギー分散型X線分光装置（EDS）を用いて観察・分析した。SEM観察では，アカンサリアの殻骨格表面は細長い独特の形態を持つ微小結晶の集合からなることを，またEDSのデータは，ストロンチウム・硫黄・酸素がその殻骨格中に存在することを示した。これらの結果より，沖縄本島北西沿岸産のアカンサリアについてもその殻骨格は，これまで他地域で報告されているように天青石（$SrSO_4$）の微小結晶からなること，またこれらの特性により，アカンサリアの殻骨格は同じく海水に溶けやすいファエオダリアのように多孔質ではないが，容易に崩壊して海水に溶解することがわかる。すなわち，今回の観察結果は，アカンサリアの海水への溶けやすさは，殻骨格の構造よりもむしろ構成している$SrSO_4$の特性によって決定されるものと考える。

キーワード: アカンサリア，放散虫，殻骨格，$SrSO_4$，走査電子顕微鏡