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Distribution Patterns of the Radiolarian Nuclei and Symbionts Using DAPI-Fluorescence

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Abstract This study is the first report on the successful application of the DAPI (4′,6-diamidino-2-phenylindole) staining technique to 22 families of five of the six radiolarian orders (Acantharia, Collodaria, Nassellaria, Spumellaria, and Taxopodia, excluding Entactinaria). A total of six Acantharian species, three Collodarian species, three Spumellarian species, 18 Nassellarian species, and one Taxopod species emitted blue light under epifluorescence microscopy after DAPI staining. These results and existing data indicated that Acantharia and two collodarian families (Collosphaeridae and Sphaerozoidae) are multi-nucleated, whereas Nassellaria, Spumellaria, Taxopodia, and Thalassosphaeridae form a single-nucleus group. The shape, location, and number of nuclei in Radiolaria were variable not only at the family level but also at lower taxonomic levels. Even within species such as *Spirocyrtis scalaris*, nuclei can move from the cephalis to intracapsulum lobes below the cephalis.

Key words: cytology, DAPI, nucleus, Radiolaria.

Introduction

Radiolarians are planktonic marine unicellular protoctists that appeared during the Cambrian period and currently consist of six orders: Acantharia, Collodaria, Entactinaria, Nassellaria, Spumellaria, and Taxopodia (De Wever *et al.*, 2001; Kunitomo *et al.*, 2006). Siliceous test-bearing radiolarians belonging to Collodaria, Entactinaria, Nassellaria, and Spumellaria are traditionally grouped as polycystine radiolarians or simply polycystines. Polycystine radiolarians are generally considered single-nucleated Protoctista (De Wever *et al.*, 2001), but carmine-dying methods have indicated that several species of Acantharia and Collodaria contain many nuclei in a singl cell (Hertwig, 1879; Brandt, 1885; Schewiakoff, 1926; Febvre, 1989). These studies put into question their taxonomic relationships of Radiolaria with respect to the evolution of the variability in nuclear traits. Haeckel (1887) described the nuclear characteristics of several radiolarian groups, such as uni- versus multi-nuclear, central versus eccentric positions, homogenous

versus allogenous composition, shape, and differences in ontogenetic growth. Thus, the Entactinaria, Nassellaria, and Spumellaria each have single nuclei except during the reproductive stage. However, these century-old descriptions of nuclear traits require verification using modern techniques.

The number of nuclei in radiolarian cells is thought to remain constant throughout the life cycle, except during the reproductive stage, and the cytoplasmic trait of whether radiolarians are single- or multi-nucleated is presumed to be an order-level characteristic. To determine the veracity of these assumptions, we examined the nuclear status of about 60 species of radiolarians. Cells were dyed with the fluorogenic compound DAPI (4′,6-diamidino-2-phenylindole), as the position and number of nuclei within a cell are easily distinguished with this compound. DAPI, one of the most common fluorescent compounds for the recognition of nuclei, passes through the intact cell membrane of both live and fixed cells and binds with double-stranded DNA. The stained DNA is excited as blue emission (461 nm at an emission maximum wavelength) with ultraviolet (358 nm at a maximum absorption wavelength) under epifluorescence microscopy. Despite the wide application of DAPI, only a few studies have applied it to radiolarians (*e.g.*, Takahashi *et al.*, 2003). We dyed radiolarian species that varied in developmental stages from young to mature.

Materials and Methods

Samples were collected from the surface water of the Pacific Ocean near the Nansei Islands, south of mainland Japan, during the No. 2009-02 cruise of the *TRV Toyoshio-Maru* of Hiroshima University from 18 to 28 May, 2009 (hereafter, Toyoshio sample) and from the surface water off of Sesoko Island near the Okinawa Island, the Ryukyus, on 1 December 2008 (hereafter, Sesoko sample). Both the Toyoshio and Sesoko samples were collected during 5-min tows using a 38–43-μm mesh plankton net. The Toyoshio samples were fixed in 2% formaldehyde and kept at 5°C for 2 weeks, whereas the Sesoko samples were preserved in 2% glutaraldehyde after substitution of 100% methanol for 6 months at −10°C.

The fixed radiolarian samples were distributed onto glass slides, dyed with 1 μg/ml DAPI for 5–10 min and examined under an epifluorescence microscope (Olympus AX80 Provis) at the Department of Botany, National Museum of Nature and Science, Tokyo. Images were captured using a single-lens reflex camera (Canon EOS KISS), after which deconvolution processing using PopImaging ver. 4.0 for Windows® (Digital Being Kids, http://www.dbkids.co.jp/) was applied to the images.

Results

Acantharia

Six Acantharian taxa from the Sesoko sample harbored numerous small nuclei. *Acanthostaurus henseni* (Popofsky) (Phyllacanthoidea, Phyllostauridae) had several very small, oblong to round nuclei scattered throughout the intracapsulum (Table 1). *Phyllostaurus cuspidatus* (Haeckel) (Phyllacanthoidea, Phyllostauridae) (Fig. 1E, F) and *Xiphacantha* sp. A (Phyllacanthoidea, Stauracanthidae) (Fig. 1G, H) had spherical intracapsulum, in which small spherical nuclei were marginally distributed. The nuclei of *Heteracon* (?) sp. (Chaunacanthoidea, Stauroconidae) (Fig. 1C, D) were apparently distributed in a similar manner to *P. cuspidatus*. *Diploconus fasces* Haeckel (Sphaenacanthoidea, Diploconidae) (Fig. 1A, B) had a spherical to ellipsoidal central shell with two characteristic reversed corn (cornets) that contained nuclei at both ends. These nuclei were easily distinguished from algal symbionts, as the latter emitted a red autofluorescence. The nuclei of *D. fasces* were large compared to those of other Acantharians.

Collodaria

We examined three species of Collodarians (Table 1). *Collozoum huxleyi* Müller (Collosphaeridae) (Fig. 1I-K) exhibited a single layer

Table 1. Summary results of DAPI application to Radiolaria

Order	Superfamily	Family	Taxon name	Results on DAPI patterns	Fig.
Acantharia	Pyllacanthoidea	Phyllostauridae	*Acanthostaurus henseni* (Popofsky)	numerous along the four longer spines	
			Phyllostaurus cuspidatus (Haeckel)	ca. 90 nuclei visible on the half hemisphere of central capsule	1E, 1F
		Stauracanthidae	*Xiphacantha* sp. A	ca. 16 nuclei visible on the half hemisphere of central capsule	1G, 1H
	Sphaenacanthoidea	Diploconidae	*Diploconus fasces* Haeckel	24 nuclei visible in both cornets	1A, 1B
	Chaunacanthoidea	Stauroconidae	*Heteracon* (?) sp.	ca. 60 nuclei visible in the intracapsulum	1C, 1D
Collodaria		Collosphaeridae	*Collosphaera huxleyi* Müller	ca. 100 nuclei vislble on the half hemisphere of central capsule	1I, 1K
			Disolenia zanguebarica (Ehrenberg)	ca. 10–15 nuclei in each central capsule	1L, 1M
		Sphaerozoidae	*Sphaerozoum haeckeli* Brandt	ca. 4–5 nuclei in the each central capsule	1N, 1O
Spumellaria	Actinommoidea	Astrosphaeridae	*Arachnosphaera myriacantha* Haeckel	ambigous	
		Ethmosphaeridae	*Cenosphaera sphaerica* (Hollande et Enjumet)	spherical in shape in the peripherical part inside the intracapsulum	3E, 3F
		Spongosphaeridae	*Spongosphaera streptacantha* Haeckel	ambigous	
		Hexastylidae	*Hexalonchetta* sp. A (young cell)	heteropolar distribution	2A, 2B
			Hexalonchetta sp. B (young cell)	spherical	2C, 2D
		Rhizosphaeridae	*Haliommilla capillaceum* Haeckel	not emitted	3K, 3L
	Pylonioidea	Pyloniidae	*Tetrapyle octacantha* Müller (young cell)	ambigous	3O, 3P
			Pyloniidae gen. et sp. indet.	spherical in the center	
	Spongodiscoidea	Euchitoniidae	*Spongaster tetras tetras* Ehrenberg	ambigous	3M, 3N
Nassellaria	Eucyrtidioidea	Artostrobiidae	*Spirocyrtis scalaris* Haeckel (young cell)	spherical in cephalis	2E, 2F
		Pterocorythidae	*Theocorythium trachelium* (Ehrenberg)	ambigous	
			Pterocorys sp. (young cell)	spherical in cephalis	2G, 2H
		Stichocapsidae	*Lithopera bacca* Ehrenberg (young cell)	spherical in cephalis	2I, 2J
			Pterocanium praetextum (Ehrenberg)	cephalis and intracapsulum lobe ?	
	Acanthodesmoidea	Stephaniidae	*Zygocircus productus* Hertwig	not emitted	3I, 3J
	Plagiacanthoidea	Lophophaenidae	*Acanthocorys castanoides* Tan et Tchang	ambigous	
			Botryopera testudus (Popofsky)	lobe-like	
			Lophophaena buetschli (Haeckel)	lobe-like in cephalis	
			Lophophaena hispida (Ehrenberg)	spherical in cephalis	
			Lophophaena variabilis (Popofsky)	lobe-like in cephalis	
			Peromelissa phalacra (Haeckel)	spherical in cephalis	
			Plectacantha oikiskos Jørgensen	lower half in the intracapsulum	2K, 2L
			Plectacantha sp. A	lower half in the intracapsulum	2M, 2N
			Psilomelissa thoracites (Haeckel)	lobe-like in cephalis	
		Plagiacanthidae	*Plagiacantha abietina* Hertwig	periperiphy in the intracapsulum	3A, 3B
			Pseudocubus obeliscus (Haeckel)	lobe-like	3C, 3D
		Sethoperidae	*Clathrocanium coarctatum* Ehrenberg	reversed conical shape in the cephalis	2O, 2P
		Sethophormidae	*Tetraphormis dodecaster* Haeckel	spherical in cephalis	
Taxopodia		Sticholonchidae	*Sticholonche zancelea* Hertwig	elliptical in the ventral portion	3G, 3H

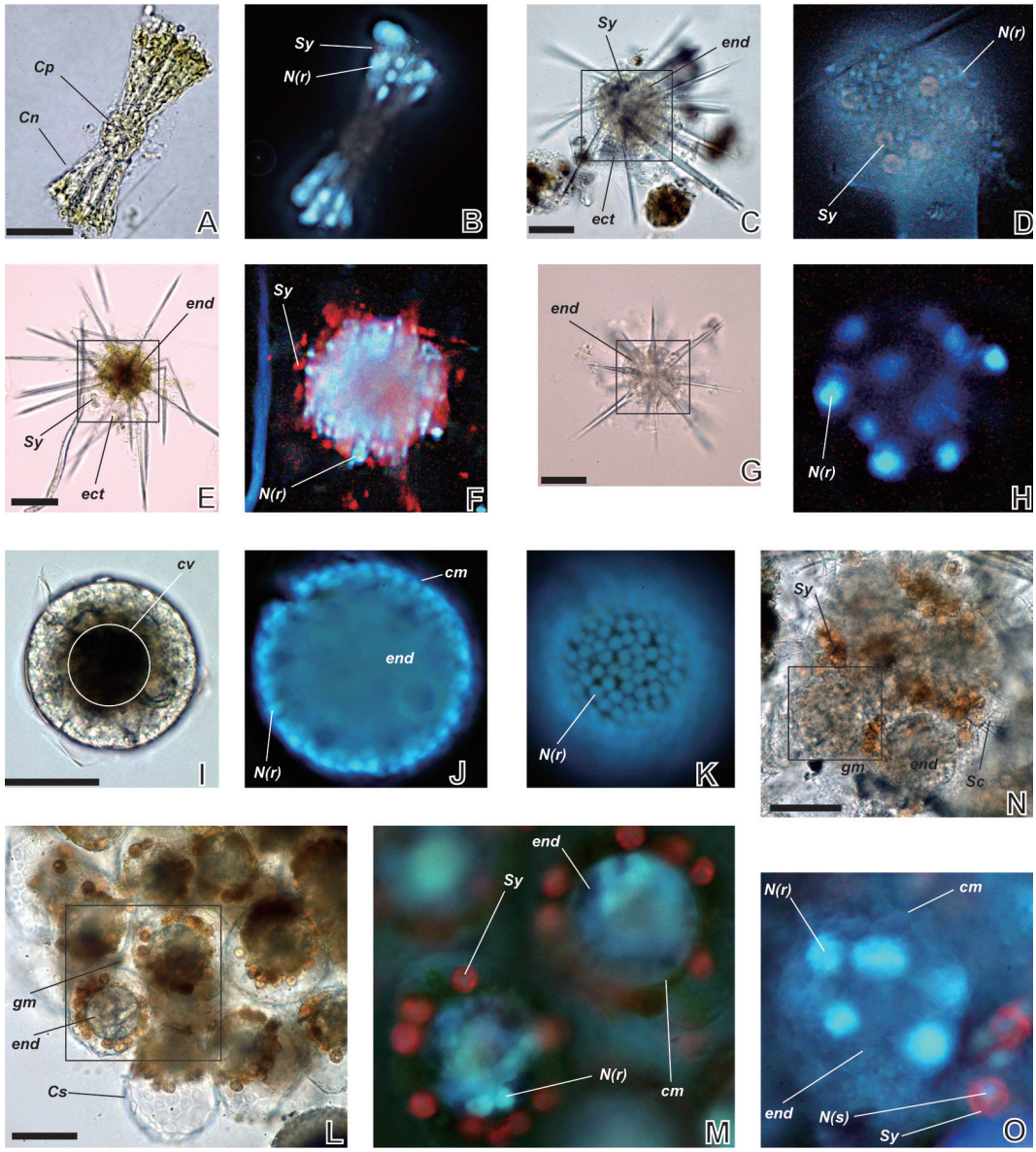

Fig. 1. Acantharia and Collodaria. A, B: *Diploconus fasces* (Sesoko sample); C, D: *Heteracon* (?) sp. (Sesoko sample); E, F: *Phyllostaurus cuspidatus* (Sesoko sample); G, H: *Xiphacantha* sp. A (Sesoko sample); I–K: *Collosphaera huxleyi* (Toyoshio sample); L, M: *Disolenia zanguebarica* (Toyoshio sample); N, O: *Sphaerozoum haeckeli* (Toyoshio sample). Figure 1D, F, H, M, and O show enlarged views of the boxes in Fig. 1C, E, G, L, and N, respectively. Figures with light backgrounds, including Fig. 1N are transmission light microscopy photographs, whereas those with black backgrounds represent epifluorescence microscopy with DAPI. Scale bars=50 μm. Abbreviations: *cm*=capsular membrane, *Cn*=cornet, *Cp*=central portion, *Cs*=cortical shell, *cv*=*central vacuole, ect*=ectoplasm, *end*=endoplasm, *gm*=gelatin matrix, *N*(*r*)=nucleus of radiolarians, *N*(*s*)=nucleus of symbionts, *Sc*=isolated spicules, *Sy*=symbionts.

of small nuclei just beneath the capsular membrane. These nuclei were distributed equidistant from one another. The diameter of each nucleus (6 μm in diameter) was slightly larger than the diameter of the pores (4.9 μm in diameter) on the cortical shell, and each nucleus was distributed under one pore. An aggregated distribution was detected in the nuclei (9.5 μm in diameter) of *Disolenia zanguebarica* (Ehrenberg) (Fig. 1L, M), but fewer nuclei were present in each cell compared to *C. huxleyi*. Moreover, the pore distributions of the cortical shell did not correspond to the distribution of nuclei. We also stained *Collosphaera tuberosa* Haeckel with propidium iodide (PI), a fluorescent compound, and several spheres in the periphery of the intracapsulum emitted bright red light, indicating multi-nucleation.

Sphaerozoum haeckeli Brandt (Sphaerozoidae) (Fig. 1N, O) contained many loosely scattered tiny siliceous spicules throughout the cell. The colony examined in our study was composed of six *S. haeckeli* cells and numerous algal symbionts. Each cell harbored five nuclei, each of which was large (10 μm in diameter) compared to the nuclei of *C. huxleyi*.

Entactinaria and Spumellaria

We stained nine species of these groups, but only three successfully fluoresced (Table 1). Young cells of *Hexalonchetta* sp. A exhibited blue luminescence along the periphery and in the upper lateral portion between the microsphere and macrosphere (Fig. 2A, B), whereas *Hexalonchetta* sp. B emitted spherical blue light outside of the microsphere (Fig. 2C, D). In *Cenosphaera sphaerica* (Hollande et Enjumet), the periphery of the inside of the central capsule emitted blue light, but we could not determine whether the nuclear area was multi-nucleated (Fig. 3E, F).

The remaining six species did not clearly emit blue light. Both *Haliommilla capillaceum* Haeckel (Fig. 3K, L) and *Tetrapyle octacantha* Müller (Fig. 3O, P) emitted weak blue light throughout the entire portion of the central capsule, but the emissions were not distinguishable from blue autoluminescence. *Spongaster tetras tetras* Ehrenberg (Fig. 3M, N) also appeared ambiguously blue, even after splitting the specimen into pieces.

Nassellaria

All of the Nassellarian species that emitted blue light using DAPI were confirmed to be single-nucleated (Table 1). One spherical to elliptical nucleus was observed in *Clathrocanium coarctatum* Ehrenberg (Fig. 2O, P), *Lophophaena buetschlii* (Haeckel), *Lophophaena hispida* (Ehrenberg), *Lophophaena variabilis* (Popofsky), *Peromelissa phalacra* Haeckel, *Psilomelissa thoracites* (Haeckel), and *Tetraphormis dodecaster* Haeckel. The nuclei of *Plagiacantha abietina* Hertwig (Fig. 3A, B), *Plectacantha oikiskos* Jørgensen (Fig. 2K, L), and *Plectacantha* sp. A (Fig. 2M, N) were irregular in form within the cephalis. *Acanthocorys castanoides* Tan et Tchang, *Pseudocubus obeliscus* (Haeckel) (Fig. 3C, D), and *Trisulcus testudus* Petrushevskaya each contained one undulated nucleus. Thus, all of the plagiacanthoid species harbored a single nucleus inside the cephalis, the shape of which could be classified into three types: spherical to elliptical, peripheral, or undulated.

Several species belonging to the superfamily Eucyrtidioidea (*sensu* Petrushevskaya, 1971) were also examined using DAPI. Of these, *Lithopera bacca* Ehrenberg (young cell; Fig. 2I, J), *Spirocyrtis scalaris* Haeckel (young cell; Fig. 2E, F), *Theocorythium trachelium* (Ehrenberg) (not quite mature cell), *Pterocanium charybdeum charybdeum* (Müller) (mature cell), and *Pterocorys* sp. (young cell; Fig. 2G, H) emitted blue light to some extent. *L. bacca*, *S. scalaris*, and *Pterocorys* sp. emitted blue light inside the cephalis (4-7 μm in diameter). Two cells of the *Zygocircus productus* Hertwig group (Fig. 3I, J) were also examined using both DAPI and PI. PI generated relatively better emission than DAPI, but the presence of a nucleus was difficult to verify using either stain in the species.

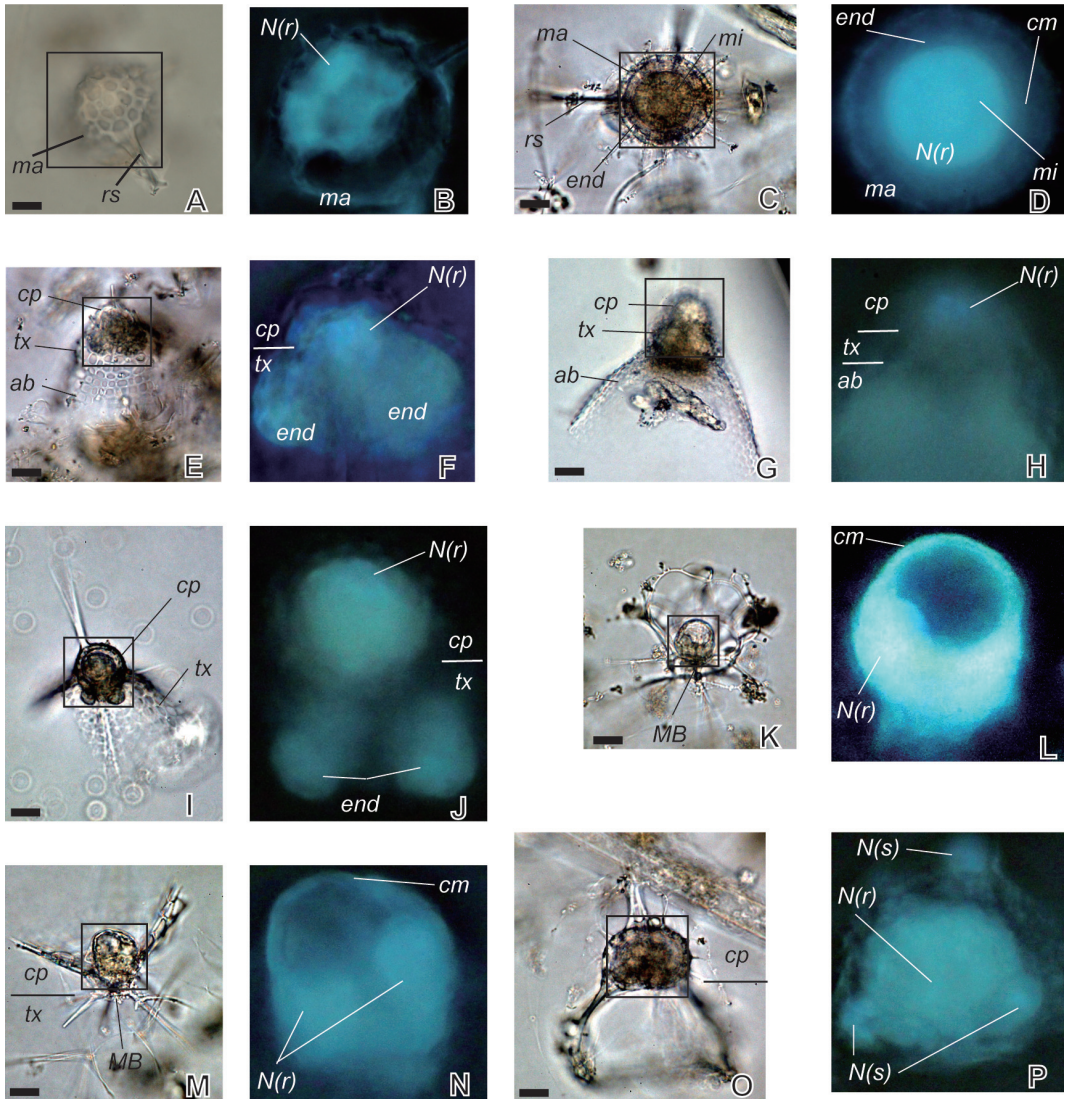

Fig. 2. Spumellaria and Nassellaria. A, B: Young *Hexalonchetta* sp. A (Sesoko sample); C, D: Young *Hexalonchetta* sp. B (Sesoko sample); E, F: Young *Spirocyrtis scalaris* (Sesoko sample); G, H: Young *Pterocorys* sp. (Sesoko sample); I, J: Young *Lithopera bacca* (Sesoko sample); K, L: *Plectacantha oikiskos*; M, N. *Plectacantha* sp. A; O, P: *Clathrocanium coarctatum*. Figure 2B, D, F, H, J, L, N, and P are enlarged views of the boxes in Fig. 2A, C, E, G, I, K, M, and O, respectively. Figures with light background are transmission light microscopy photographs, whereas those with black backgrounds represent those by epifluorescence microscopy using DAPI. Scale bars=10 μm. Abbreviations: *ab*=abdomen, *cp*=cephalis, *ma*=macrosphere, ***MB***=median bar, *mi*=microsphere, *nc*=nuclear capsule, *rs*=radial spine, *tx*=thorax. For all other abbreviations, see Fig. 1.

Taxopodia

The one species examined, *Sticholonche zanclea* Hertwig (Table 1), exhibited very little blue luminescence within the nuclear capsule (Fig. 3G, H); instead, the ventral side of the cell emitted substantially more light.

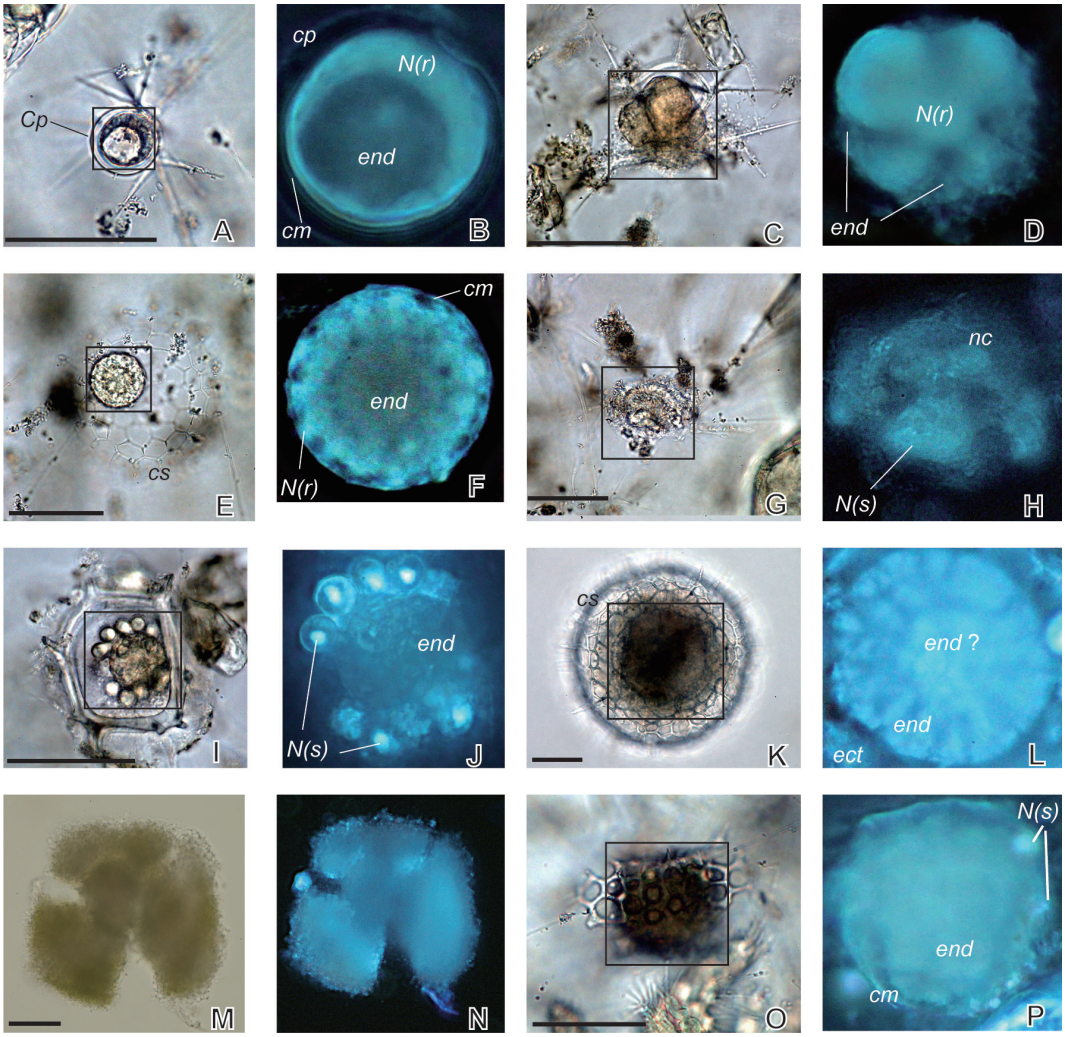

Fig. 3. Nassellaria, Taxopodia, and non-emitting Spumellaria. A, B: *Plagiacantha abientina* (Sesoko sample); C, D: *Pseudocubus obeliscus* (Sesoko sample); E, F: *Cenosphaera sphaerica*; G, H: *Sticholonche zanclea* (Sesoko sample); I, J: *Zygocircus productus* (Sesoko sample); K, L: *Haliommilla capillaceum* (Toyoshio sample); M, N: Smashed cell of *Spongaster tetras tetras* (Toyoshio sample); O, P: *Tetrapyle octacantha* (Sesoko sample). Figure 3B, D, F, H, J, L, N, and P show enlarged views of the boxes in Fig. 3A, C, E, G, I, K, M, and O, respectively. Figures with light backgrounds are transmission light microscope views, whereas those with black backgrounds represent epifluorescence microscopy using DAPI. Scale bars = 50 μm. For all abbreviations, see Figs. 1 and 2.

Discussion

Technical issues

The most serious problem encountered with DAPI was that nearly all polycystine radiolarians, except for Collodaria, emitted blue light only very weakly or did not emit. In addition, the capsular membrane is thick and chitinous, likely inhibiting the infiltration of DAPI into the intracapsulum. We attempted to expose the intracapsulum within the capsular membrane by strongly pushing the cell with a ballpoint pen, but the capsular membrane never burst. We also tried to cut the capsular membrane with the edge of a cover glass (0.15–0.19 mm thick), but the membrane

adhered to the cover glass and the kerf closed soon after cutting. Chemical treatment with bleach and an interfacial active agent was not successful in penetrating the capsular membrane. We were, however, able to expose the intracapsular protoplasm by simply mashing the cell with a ballpoint pen, but the tissues in the intracapsulum were crushed as well. Clearly, a new technique or other fluorescent compounds are needed for further cytological studies of Radiolaria.

Acantharia

Acantharia was recognized as a multi-nucleated Protoctista as early as the 1870s (Hertwig, 1879), but the nuclei of Acantharia and algal symbionts were indistinguishable in the illustrations of very old studies (Hertwig, 1879; Schewiakoff, 1926). Although Schewiakoff (1926) described the presence of many algal symbionts in most Acantharian species, only Haptophyceae (Pymnesiophyceae) could be identified in the extracapsulum of *Acanthometra pellucida* Müller (Acanthometridae), *Amphilonche elongata* (Müller) (Acanthometridae), and *Lithoptera muelleri* Haeckel (Lithopteridae) by transmission sectioned light and transmission electron microscopy (TEM) (Febvre and Febvre-Chevalier, 1979). The taxonomy of the Acantharian symbionts examined here has not yet been identified, but the red autofluorescence of *Phyllostaurus cuspidus* (Fig. 1F), for example, points to the presence of algal symbionts, as chlorophyll *a* emits bright red light. This unique autofluorescence of chlorophyll *a* allowed us to be the first to distinguish between the Acantharian nucleus and algal symbionts under epifluorescence microscopy.

Collodaria

Our DAPI results indicated multiple nuclei in *Collosphaera huxleyi*, *Disolenia zanguebarica*, and *Sphaerozoum haeckeli*. Under PI staining, *C. tuberosa* also appeared to be multi-nucleated (data not shown). We did not examine whether the number of nuclei in these species was variable across ontogenetic stages.

The multi-nucleation of Collodaria was initially reported in the late 19th century (Brandt, 1885). Dark spheres (<20 μm in diameter) beneath the capsular membrane (size of intracapsulum approximately 100 μm in diameter) of *C. huxleyi* were first noticed by Cienkowski (1871), but he erroneously assigned the large central vacuole (vacuole size approximately 60 μm in diameter) to be a nucleus. The multi-nuclear habit of collodarians has already been recognized in *Polysolenia spinosa* (Haeckel) (=*Acrosphaera spinosa* in the original paper) (Collosphaeridae), *C. huxleyi*, *Sphaerozoum fuscum* Meyen (=*Sphaerozoum punctatum*), and *Rhaphidozoum acuferum* (Müller) (=*Sphaerozoum acuferum*) (Sphaerozoidae) (Brandt, 1885). In contrast, *Thalassicolla nucleata* Huxley (Thalassicollidae) has a single, large nucleus in the center of the intracapsulum (Huth, 1913). Observations of tissue sections under TEM revealed that *Collozoum* species possess multiple nuclei (Anderson, 1976c; Swanberg and Anderson, 1981; Anderson *et al.*, 1999), whereas *T. nuculeata* was confirmed as having a single nucleus (Anderson, 1976a). Although multiple nuclei have been observed in the mitotic phase of *S. fuscum* (=*S. punctatum* in the original paper) (Anderson, 1976b), mitotic nuclei are easily distinguishable from trophont nuclei, because the intracapsulum fills with very small nuclei during mitosis. Thus, the multi-nuclear habit of Collosphaeridae and Sphaerozoidae observed here is part of the trophont stage.

Collosphaera, Disolenia, and *Polysolenia* (=*Acrosphaera*) belong to the family Collosphaeridae; *Collozoum*, *Sphaerozoum*, and *Rhaphidozoum* belong to Sphaerozoidae; and *Thalassicolla* belongs to Thalassicollidae, suggesting a trophont multi-nuclear mode in Collosphaeridae and Sphaerozoidae and a trophont mono-nuclear mode in Thalassicollidae. The molecular cladograms presented in Yuasa *et al.* (2005) and Kunitomo *et al.* (2006) showed three clades in Collodaria: a *Thalassicolla* species clade, a *Collozoum-Sphaerozoum-Rhaphidozoum* clade, and an *Acrosphaera-Collosphaera-*

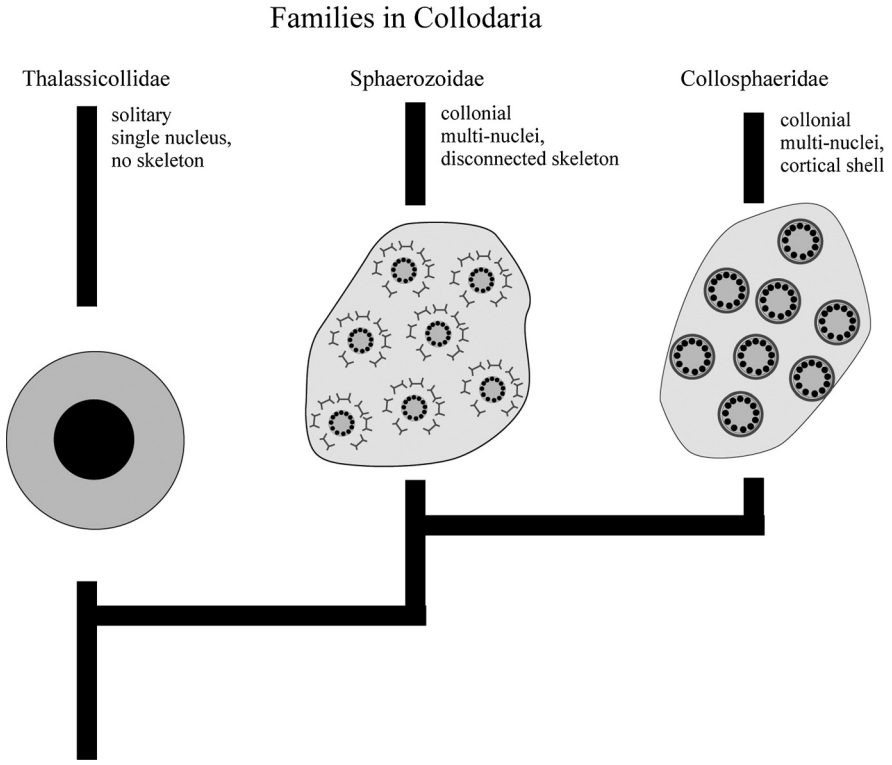

Fig. 4. Schematic illustration of hypothesized evolution in Collodaria. Schematic dendrogram adapted from the molecular phylogeny in Yuasa *et al.* (2005) and Kunitomo *et al.* (2006).

Siphonosphaera clade. These three clades correspond to the families Thalassicollidae, Sphaerozoidae, and Collosphaeridae, respectively. The molecular cladograms also describe a close phylogenetic relationship between Collosphaeridae and Sphaerozoidae, with both families evolving from Thalassicollidae. This phylogeny suggests that a trophont multi-nuclear mode emerged during the evolution of Collosphaeridae and Sphaerozoidae from the single-nucleated Thalassicollidae (Fig. 4).

Nassellaria

Here, Plagiacanthoidea was classified as having three forms of nuclei. *Clathrocanium*, *Lophophaena*, *Peromelissa*, *Psilomelissa*, and *Tetraphormis* had one spherical nucleus; *Plagiacantha* and *Plectacantha* each had a peripheral nucleus; and *Acanthocorys*, *Botryopera*, and *Pseudocubus* contained an undulated nucleus. The peripheral nucleus form is illustrated in Figs. 2L, 2N, and 3B. As shown in Fig. 2L and N, elliptical cytoplasm consisting of endoplasm and the nucleus was located above the median bar (***MB***, a cephalic internal spicular system), and the lower half of the elliptical cytoplasm contained the light-emitting portions of the nucleus. In the upper view of the cephalis (Fig. 3B), the marginal part just inside of the capsular membrane emitted blue light.

Phylogenetic relationships based on the shape of the nucleus in Plagiacanthoidea need careful discussion because the taxonomy of this group at both the family and genus levels has not yet been settled. Petrushevskaya (1971) thoroughly revised the classification of Plagiacanthoidea through a detailed examination of the internal structures of the cephalis, and subsequent studies established several genera based on cephalic structure (Nishimura, 1990; Sugiyama, 1993;

Funakawa, 1994, 1995). They clearly defined the classification at the genus level, but their criteria require examination of fine cephalic structure, which are not easily observable under light microscopy. Due to these uncertainties, phylogenetic relationships in Plagiacanthoidea are not fully understood. Molecular studies based on ribosomal DNA sequences support a single clade among *Lophophaena cylindrica* (Cleve) (=*Lithomelissa* sp. 2003 in the original paper), *Psilomelissa thoracites* (=*Lithomelissa* sp. 8012), and *Pseudocubus obeliscus* (Kunitomo *et al.*, 2006). *Lophophaena* and *Psilomelissa* belong to Lophophaenidae, whereas *Pseudocubus* belongs to Plagiacanthidae. The three types of nuclear forms described above overlap across families. For example, families exhibiting a spherical nucleus include Lophophaenidae (*Lophophaena*, *Peromelissa*, *Psilomelissa*), whereas *Plectacantha* and *Botryopera* (both Lophophaenidae as well) harbor a peripheral nucleus and an undulated nucleus, respectively. If these genera are monophyletic, the shape of the nucleus appears to have developed independently.

Blue fluorescence was observed in the cephalis of *Lithopera bacca* and *Spirocyrtis scalaris*, suggesting the presence of the nucleus in the cephalis. In contrast to our results for the latter species, the nucleus of the *S. scalaris* cell occurred below the cephalis in TEM images (Sugiyama and Anderson, 1997). Our cell was perhaps too young and contained so little intracapsulum protoplasm that the nucleus was not yet located below the cephalis. These results suggest that the nucleus can change position at different ontogenetic stages.

Entactinaria and Spumellaria

According to Hertwig (1879) and Hollande and Enjumet (1960), species of Entactinaria and Spumellaria contain single nuclei at the center of the siliceous skeleton. Hertwig (1879), however, did not present the entire image of each specimen; thus, the species identities were impossible to determine from his illustrations. Hollande and Enjumet (1960) documented that the nucleus is not always located at the center of the protoplasm and the sectioned nucleus appeared in sector form in their periaxoplastid group. Our results indicated that *Hexalonchetta* sp. A (Fig. 2A, B) had a dome-shaped nucleus because the sector form of the nucleus in thin section could only be derived from a dome shape. In contrast, a taxonomically similar species, *Hexalonchetta* sp. B (Fig. 2C, D), exhibited a spherical nucleus in the center of the test. Our observations demonstrate a variety of nuclei shapes at the species level, rather than at the family level or higher. Hollande and Enjumet (1960) emphasized the relationship between the nucleus and the axoplast (the bundle of tubulins inside the intracapsulum) as a family or higher taxonomic-level classification; however, our results somewhat contradict this concept.

The shape of the nucleus can apparently change at not only the species level but also within a species. Hollande and Enjumet (1960) clearly showed a nearly spherical nucleus in *Cenosphaera sphaerica*, but we observed a peripheral nucleus just beneath the capsular membrane in the same species (Fig. 3F).

Taxopodia

Cachon and Cachon (1978) described the nucleus of *Sticholonche zanclea* as occurring inside the nuclear capsule on the dorsal side of the cell. However, we observed bright blue light in the ventral portion of the intracapsulum, outside the nuclear capsule. *S. zanclea* is often infected by a parasitic dinoflagellate, *Amoebophrya stycholonchae* Koeppen, which always remains in the ventral portion of the host (Fol, 1883; Borgert, 1898; Drebes, 1984). Thus, the emitting ventral portion of the examined specimen was likely the nucleus of the parasite.

Types of nuclei

Based on the present and previous data, the shapes of the radiolarian nucleus can be categorized into 11 types (Table 2, Fig. 5). According to Haeckel (1887, p. 8–16), the typical shape of the nucleus is primarily spherical but can be classified into five types: ellipsoidal (Nassellaria, Lith-

Table 2. Nucleus types recognized in Radiolaria and their corresponding definitions

Nucleus type	Abbreviated explanation	Characteristic features	Typical example	Fig.
Multi-nculeus type				
	scattered multiple nuclei	nuclei are scattered throughout intracapsulum	*Diplocomus*, *Phyllostaurus*, *Xiphacantha* (Acantharia); *Disolenia* (Collodaria)	5A
	peripheral multiple nuclei	nuclei are arraged in the exterior portion in the central capsule	*Collosphaera*, *Sphaerozoum* (Collodaria); *Cenosphaera sphaerica* (Spumellaria)	5B
Single-nucleus type				
	spherical single nucleus	nucleus is positioned in the center of the central capsule	*Hexalonchetta* sp. A (Spumellaria)	5C
	dome-shaped nucleus	nucleus is dome-shaped so that it is seen fan-shaped lateral profile while spherical from the bottom or upper view	*Hexalonchetta* sp. B (Spumellaria)	5D
	peripheral single nucleus	nucleus is eccentrically positioned in intracapsulum	periaxoplastid Spumellaria and Entactinaria	5E
	spherical single nucleus in the cephalis	In Nassellaria, the nucleus is centered in the intracapsulum above ***MB***	*Lithopera* (Nassellaria)	5F
	peripheral single nucleus in the cephalis	In Nassellaria, the nucleus is eccentrically positioned in the intracapsulum above ***MB***	*Pterocorys* (Nassellaria)	5G
	undulated single nucleus	In Nassellaria, the nucleus is irregularly shaped just above ***MB***	*Plectacantha*, *Plagiacantha* (Nassellaria)	5H
	lobe-like single nucleus above and below the ***MB***	In Nassellaria, the nuclues is located on ***MB***, but is slightly sliced through ***MB***	*Eucyrtidium*, *Spirocyrtis*, *Clathrocanium* (Nassellaria)	5I
	lobate nucleus	In Nassellaria, the nucleus is mainly located on ***MB***, and the lower part of the nucelus is hanged from ***MB***	*Pseudocubus* (Nassellaria)	5J
	nucleus outside of the cephalis	In Nassellaria, the nucleus is not positioned inside the cephalis	*Spirocyrtis* (Nassellaria)	5K

Abbreviation: ***MB*** = median bar, a rod of the cephalic internal spicular system.

eloidea, and Pylonioidea in Spumellaria), discoidal (Spongodiscoidea in Spumellaria), stellate (Thalassicollidae), amoeboid (irregular forms of Spumellaria and Acantharia), and lobate (cyrtid Nassellaria). We cannot specify type of nuclei between our results and Haeckel's scheme because we did not examined the radiolarians regarded in Haeckel (1887).

Conclusions

1. DAPI application was successful for Acantharia, Collodaria, plagiacanthoid Nassellaria, and several young cells of Nassellaria and Spumellaria. In contrast, mature cells of Nassellaria and Spumellaria did not emit blue light.
2. The presence of multiple nuclei was confirmed in Acantharia and two Collodarian families (Collosphaeridae and Sphaerozoidae), and whereas the nuclei in Acantharia were scattered throughout the intracapsulum, those in Collosphaeridae and Sphaerozoidae were located just beneath the capsular membrane. Nassellaria, Spumellaria, Taxopodia, and Thalassosphaeridae in Collodaria possessed a single nucleus.
3. Based on molecular analyses of Collodarian families (Yuasa *et al.*, 2005; Kunitomo *et al.*, 2006), the evolutionary progression of multiple nuclei in Collodaria was as follows: a single nucleus in Thalassosphaeridae progressed to multiple nuclei in Sphaerozoidae followed by multiple nuclei in Collosphaeridae.
4. In the Plagiacanthoidea of Nassellaria, *Clathrocanium*, *Lophophaena*, *Peromelissa*, *Psilomelissa*, and *Tetraphormis* each exhibited one spherical nucleus; *Plagiacantha* and *Plectacantha* had a peripheral nucleus; and *Acanthocorys*, *Botryopera*, and *Pseudocubus* contained an undulated nucleus. In Eucyrtidioidea, *Spirocyrtis scalaris* and *Lithopera bacca* each harbored a spherical nucleus in the cephalis.
5. In Spumellaria, *Hexalonchetta* exhibited a

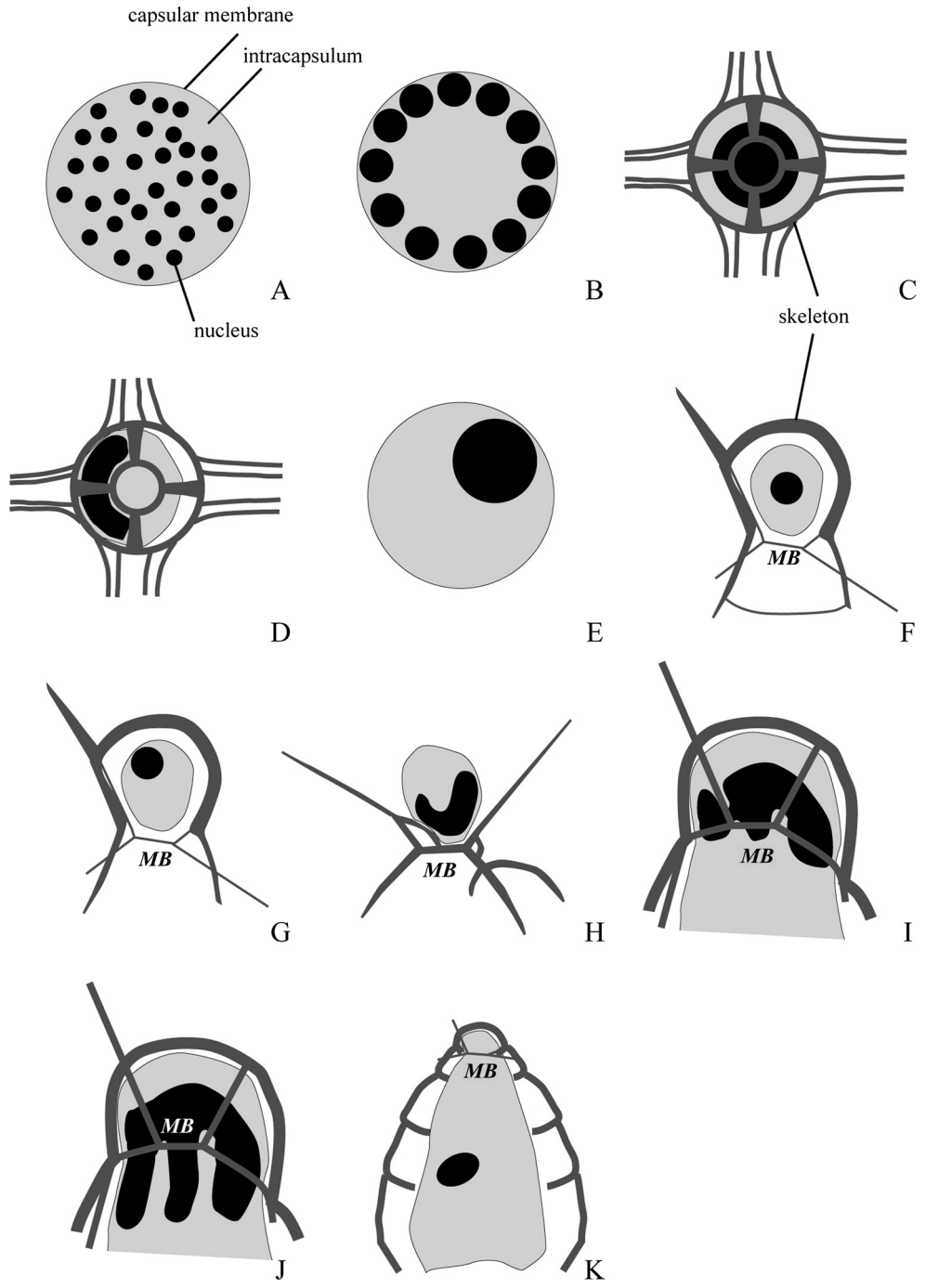

Fig. 5. Nucleus types in Radiolaria (no scale): (A) scattered multiple nuclei, (B) peripheral multiple nuclei, (C) spherical single nucleus, (D) dome-shaped single nucleus, (E) peripheral single nucleus, (F) spherical single nucleus in the cephalis, (G) peripheral single nucleus in the cephalis, (H) undulated single nucleus, (I) lobe-like single nucleus above and below the ***MB***, (J) lobate nucleus, (K) nucleus outside of the cephalis. Abbreviations: ***MB***＝median bar, a rod of the cephalic internal spicular system.

dome-shaped or spherical nucleus, and *Cenosphaera sphaerica* had a peripheral or spherical nucleus, suggesting that the shape of the nucleus is variable within these species of Spumellaria.

6. The shape and position of the nucleus have historically been considered family-level or higher taxonomic characteristics of spherical radiolarians (Hollande and Enjumet, 1960). In fact, nucleus shape was utilized as such in the well-known comprehensive book, *Illustrated Guide to the Protozoa* (Cachon and Cachon, 1985). However, our results clearly indicated that the shape, position, and number of nuclei cannot reliably be used to distinguish higher taxonomy.

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