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DNA metabarcoding focused on difficult-to-culture protists—an effective approach to clarify biological interactions

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SUMMARY

DNA metabarcoding on single organism is a promising approach to clarify the biological interactions (e.g., predator-prey relationships and symbiosis, including parasitism) of difficult-to-culture protists. To evaluate the effectiveness of this method, Radiolaria and Phaeodaria, which are ecologically important protistan groups, were chosen as target taxa. DNA metabarcoding on single organism focused on the V9 region of the 18S rRNA gene revealed potential symbionts, parasites, and food sources of Radiolaria and Phaeodaria. Previously reported hosts and symbionts (parasites) were detected, and newly recognized combinations were also identified. The contained organisms largely differed among Radiolaria and Phaeodaria. In Radiolaria, members of the same order tended to contain similar organisms, and the taxonomic composition of possible symbionts, parasites, and food sources were fixed at the species level. Members of the same phaeodarian family, however, did not contain similar organisms, and body part (i.e., the central capsule or the phaeodium) was the most important factor that divided the taxonomic composition of detected organisms, implying that the selection of appropriate body part is important when trying to ascertain contained organisms, even for unicellular zooplankton. Our results show that DNA metabarcoding on single organism is effective in revealing the biological interactions of difficult-to-culture protists.

ORIGINALITY-SIGNIFICANCE STATEMENT

DNA metabarcoding on single organism is an effective approach to clarify the biological interactions of difficult-to-culture protists. To evaluate the potential of this approach, Radiolaria and Phaeodaria, unicellular zooplankton groups important in marine food web and material cycles, were chosen as target organisms. DNA metabarcoding on single organism successfully revealed potential symbionts, parasites, and food sources in Radiolaria and Phaeodaria, indicating that this approach is effective to reveal the ecological relationships of difficult-to-culture protists. The composition of these detected organisms largely differed among Radiolaria and Phaeodaria, even though they generally have a similar cell size, body structure, and ecological niche. The body part was suggested as the most important factor to divide the taxonomic composition of detected organisms, implying that the selection of an appropriate body part is important when studying contained organisms, even for unicellular zooplankton.

INTRODUCTION

The biological interactions (e.g., competition, predator-prey relationships, and symbiosis, including parasitism) of protists have been widely studied, mainly focusing on “culturable” species in the domain of microbiology or protistology. However, many protists in natural environments cannot be successfully cultured under artificial conditions, and these “difficult-to-culture” protists are reported to play important roles in natural environments (Biard et al., 2016; Ikenoue et al., 2019; Sogawa et al., 2022).

DNA metabarcoding is an effective approach to clarify biological interactions of aquatic organisms, and the taxonomic composition (species diversity) of environmental samples can be

thoroughly clarified by using this technique. For example, DNA metabarcoding has been used to clarify the food sources of crustaceans (Cleary et al., 2012, 2015). However, because multicellular organisms contain numerous cells, a blocking polymerase chain reaction (PCR) with Peptide Nucleic Acid (PNA) must also be performed to reduce the detection of host's DNA (Nakamura et al., 2020a), which creates a bottleneck when trying to analyze numerous species at the same time. Symbionts, parasites, and food sources, however, are more easily detected by DNA metabarcoding focused on unicellular eukaryotes (i.e., protists) because they have a relatively small amount of DNA. In fact, the DNA sequence of difficult-to-culture protists has generally been difficult to clarify because of their small amount of DNA and the high risk of contamination. However, a single-cell DNA analysis method for protists was established, and the DNA sequences of numerous protistan groups have been revealed during the last decade (Decelle et al., 2012a; Pawlowski et al., 2013; Sandin et al., 2019; 2021; Nakamura et al., 2020b; 2021). For these reasons, the combination of single-cell DNA analysis and DNA metabarcoding should be an effective means to clarify the biological interactions of difficult-to-culture protists and other organisms.

Radiolaria and Phaeodaria are difficult-to-culture but ecologically important protists. Radiolaria contain 6 orders and more than 1,100 species (Suzuki & Aita, 2011; Nakamura et al., 2021), while Phaeodaria currently include 18 families and about 300 species (Nakamura & Suzuki, 2015; Nakamura et al., 2015). These two groups are heterotrophic or mixotrophic unicellular zooplankton, most of which have siliceous skeletons. They are thought to be key groups in ecosystems and material cycles in the world ocean because their high abundance and large contribution to material cycles have often been reported in the past decade (Nakamura et al., 2013; Biard & Ohman, 2020; Sogawa et al., 2022). The symbiosis between these two groups and other eukaryotic organisms

has also attracted attention recently. Radiolaria and Phaeodaria are reported to have a symbiotic relationship with crustaceans, which is called the “Rhizarian rider” phenomenon (Nakamura et al., 2019; Saito et al., 2022). Radiolaria are also known for their symbiosis with algae, and their symbiotic algae have been analyzed with different approaches, such as microscopic observation (Anderson, 1983), DNA barcoding (Decelle et al., 2012b), and fluorescence pattern (Zhang et al., 2018). Their symbiosis is thought to be complicated because some Radiolaria can have more than two symbiotic algae (Decelle et al., 2012b). Closely related species have also been reported to have symbiotic algae of totally different origins. For example, *Dictyocoryne profunda* (Radiolaria) has a cyanobacterium (symbiotic alga) (Yuasa et al., 2012), whereas *D*. *truncata* (Radiolaria) possesses a haptophyte (symbiotic alga) (Yuasa et al., 2019). Although a great deal of knowledge has been accumulated during the past 150 years (Table S1), the taxonomic composition of radiolarian symbiotic algae has never been thoroughly clarified. Compared with the case of Radiolaria, knowledge about the symbiosis of Phaeodaria is limited, with less than 10 reports currently available (Table S1).

Radiolaria and Phaeodaria have a similar cell size, body structure, and ecological niche. This study therefore focused on these two groups as the target organisms and to show the first big picture, attempted to explore the interactions between Radiolaria/Phaeodaria and other eukaryotic organisms. DNA metabarcoding on single organism was applied to detect potential symbionts, parasites, and food sources, with the aim of showing a comprehensive big picture of biological interactions of these difficult-to-culture protists.

MATERIALS AND METHODS

Field sampling, microscopy, and treatment

Plankton sampling was conducted in 2012–2019 at 22 stations located in seven marine areas of the Northern Hemisphere (Fig. 1). Radiolaria and Phaeodaria were manually isolated from the bulk plankton samples under a stereomicroscope or inverted microscope (e.g., TMS, Nikon, Japan). The isolated individuals were then photographed with a digital camera (e.g., Nikon 1 V3, Nikon, Japan) attached to the microscopes, and individuals were identified based on their morphological characteristics. The identified specimens were then carefully observed to confirm that no other organisms were attached on their surface. After the observation, the specimens were individually preserved in tubes filled with approximately 2.0 mL of 99.9% ethanol and stored at 4ºC. Among these ethanol-preserved specimens, Orodaria and solitary Collodaria were dissected with a sterilized scalpel under a stereomicroscope, and the central area containing nuclei were isolated. Large Phaeodaria (larger than ca. 400 μm in diameter) were also dissected, and their “central capsule” (the protoplasmic body, including the nuclei) and “phaeodium” (mass of aggregated brown or yellowish particles) were isolated to separately perform further analyses.

After the DNA extraction (described later), some of the specimens, which have solid siliceous skeletons, were observed with a scanning electron microscope (SEM, JSM-6390LV with LaB6 gun, JEOL, Japan). The conditions and parameters were the same as those described in Nakamura et al. (2016).

DNA metabarcoding and cluster analysis

Each isolated specimen (whole cell, central capsule, or phaeodium) was individually put into 100 μL of guanidine-containing extraction buffer (GITC buffer) (Decelle et al., 2012a), and the DNA was extracted according to the method described in Nakamura et al. (2015). Three tubes filled with

ethanol were also analyzed as negative controls in the subsequent experiment. The DNA extraction was conducted in a specialized and sterilized laboratory.

Hitherto reported symbionts, parasites and prey organisms of Radiolaria and Phaeodaria were mainly eukaryotes (Table S1), and to compare with these previous studies, the eukaryote-specific primers were chosen in this study. The V9 hypervariable region of approximately 315 base pairs in the 18S rRNA gene was amplified by PCR following the procedure in Toju (2016). The first fusion primers were designed by combining P5 or P7 adapters, a series of "N" and V9-specific sequences for eukaryotes: 1389F (5'-TTGTACACACCGCCC-3') and 1510R (5'-CCTTCYGCAGGTTCACCTAC-3') (Amaral-Zettler et al., 2009). The structure of primers (for the first and second PCR), The contents of the reaction mixture, and the thermal cycling conditions were the same as in Nakamura et al. (2020a). Three negative controls were also contained in the PCR to check that there was no contamination of eukaryotes. After the second PCR, all of the PCR products were mixed and purified with AMPure XP (Beckman Coulter, U.S.A.). The purified mixture was adjusted to 4 pM before amplicon sequencing using MiSeq (Illumina, U.S.A.). One run of sequencing was performed with MiSeq Reagent kit v3 (600 cycles) (Illumina, U.S.A.), following the recommended protocol and default settings.

The obtained data were analyzed with Claident ver. 0.2.2019.05.10 software (Tanabe & Toju, 2013) according to the Claident manual (Tanabe, 2018). Low-quality sequences, with average quality scores less than 30, were removed, and chimera sequences were also excluded. The sequences were then clustered into OTUs using a minimum identification score of 0.97. The OTU compositions of each specimen are summarized in a matrix, which lists sequences longer than 200 mer with at least 200 reads. After the treatment mentioned above, 0.01–10.31% of the original sequence reads were removed in each sample. The OTUs were taxonomically identified until the

genus or species level by the Basic Local Alignment Search Tool (BLASTN) from the U.S. National Center of Biotechnology Information (https://www.ncbi.nlm.nih.gov/) using the nr database, excluding environmental sample sequences. The taxonomic name of the registered sequence with at least 98% match was assigned to each OTU in most cases. However, some sequences difficult to be identified by BLASTN were (1) further identified by SILVA (Quast et al., 2013) and/or (2) assigned taxonomic names by creating phylogenetic trees containing sequences of related organisms. The classification of phylum- or class-level taxa referred to Adl et al. (2019) and Nakamura et al. (2019). The relative abundance (%) was derived from the ratio of total sequence read and the sequence read of each higher taxon. The raw sequence data were deposited in the DNA Data Bank of Japan database with the accession number DRA010024.

Cluster analyses were based on the taxonomic composition of the detected organisms in each specimen. The read numbers of detected OTUs were collapsed into binary data (0 or 1), and the Euclidean distances within the resulting dataset were calculated by the statistical software College Analysis ver. 6.6 (Fukui & Hosokawa, 2004). We constructed dendrograms based on the higher taxon and habitat by Ward's method (Ward, 1963) to visualize the differences among the layers.

RESULTS

A total of 22 plankton samples were collected over an 8-year period (Fig. 1). From these samples, 28 Radiolaria and 56 Phaeodaria, belonging to almost all orders, were analyzed by the DNA metabarcoding (Figs. 2 and S1, Table S2). In the DNA metabarcoding analyses, the sequences of the hosts (Radiolaria and Phaeodaria) were often detected in most of the specimens (Fig. 3, Table S3). Multiple eukaryotic organisms were detected in most of the radiolarian

specimens, except for specimens Tax4, Kn10b, St2, oth5b, GS14, and Or9, in which only radiolarian sequences were detected. The same taxa tended to be detected in the same Radiolaria, such as Kinetoplastea, *Pelagomonas*, and *Scrippsiella* in *Acanthoplegma krohni* (specimens Ae6 and Ae7), and *Prymnesium* in *Acanthometron pellucidum* (specimens Ae9 and Ae10). Photosynthetic organisms (e.g., Haptophyta, Pelagophyceae, and Dinoflagellata) were frequently detected in the radiolarian orders Acantharia, Taxopodia, Spumellaria, and Collodaria, whereas they were never found in the order Orodaria, in which non-photosynthetic Dinoflagellata and animals (Cnidaria and Chaetognatha) were detected.

Host sequences were also mainly detected in Phaeodaria, followed by other eukaryotic organisms (Fig. 4). However, no or very few hosts of Phaeodaria were detected in the family Astracantha and in the specimens from the phaeodium (specimens with “phd” in their names). Similar to Radiolaria, the same taxa tended to be found in the same Phaeodaria, for example, *Cephaloidophora*/*Thiriotia* in the family Castanellidae and *Dermocystidium* in the family Astracantha. Other eukaryotic organisms were more frequently detected in specimens from the phaeodium than in specimens from the central capsules.

The cluster analysis based on the detected organisms revealed that all specimens could be categorized into two large groups: cluster A including only Phaeodaria and cluster B containing Radiolaria and Phaeodaria (Fig. S2). In cluster B, Phaeodaria appeared in several limited subclusters.

Further analysis on Radiolaria clarified that they could be clustered into three large groups, and this categorization corresponded to radiolarian order-level taxonomy (Fig. S3): cluster C, which contained the orders Acantharia and Taxopodia; cluster D, which included only the order Spumellaria; and cluster E, which is mainly composed of the order Collodaria, although three

specimens belonging to other orders were also present.

Unlike Radiolaria, phaeodarian clusters did not correspond to the order- or family-level taxonomy (Fig. S4). Rather, the difference between body parts (central capsule vs. phaeodium) was highlighted. As a result, Phaeodaria were categorized into two large clusters: cluster F, which chiefly contained the specimens from the phaeodium; and cluster G, which mainly included specimens isolated from the central capsule.

DISCUSSION

1. Radiolaria

The cluster analysis based on the taxonomic composition of organisms detected in the Radiolaria and Phaeodaria specimens suggests that the organisms contained in them largely differ among these two groups (Fig. S2). The high detection of algae (phytoplankton) presumably reflects their symbiosis judging from previous reports concerning the symbiosis of protists (Nowack & Melkonian, 2010; Bjorbækmo et al., 2019). The taxonomic composition of potential symbionts, parasites, and food sources seems to be fixed at the species level, considering that the same species of Radiolaria contained similar organisms (Fig. 3). The cluster analysis focused on Radiolaria also shows that members of the same radiolarian order tend to contain similar other organisms (Fig. S3), suggesting that their biological interactions largely differ among the orders.

The following algae detected in this study have some kind of biological interaction with Radiolaria: Haptophyta, Pelagophyceae, and Dinoflagellata (Fig. 3). The following combinations were recognized for the first time by this study: *Gyrodinium* in *Litholophus* sp. (Acantharia); *Pelagomonas*, *Scrippsiella*, and *Karlodinium* in *Acanthoplegma krohni* (Acantharia); *Pelagomonas*,

Scrippsiella, and *Zooxanthella* in *Sticholonche zanclea* (Taxopodia); and Haptophyta in *Myelastrum trinibrachium* (Spumellaria). The detected organisms may possibly be symbiotic algae judging from the data of previous studies (Table S1), but other analyses, such as observations of substance transportation, are necessary to further clarify details on their symbiosis. The following combinations may be symbiosis with more than two algae, as suggested by (Decelle et al., 2012b): *Pelagomonas* and *Scrippsiella* in *Acanthoplegma krohni* (Acantharia) and *Sticholonche zanclea* (Taxopodia) (Fig. 3). Future studies applying DNA metabarcoding on single organism would further reveal the symbiosis with multiple algae.

Kinetoplastea (Euglenozoa), Apicomplexa, and *Massisteria* (Cercozoa), which were detected in the Radiolaria specimens (Fig. 3), are known to be parasitic to some marine organisms (Gull, 2001; Mylnikov et al., 2015; Seeber & Steinfelder, 2015), and these taxa could be parasites of Radiolaria. This is the first report of parasitism of these three taxa to Radiolaria.

The detection of multicellular organisms (Cnidaria, Chaetognatha, Crustacea, and Chordata, including fishes) should be interpreted carefully because these animals have a large number of cells, and they can be detected more easily than unicellular hosts. It is possible that is that some Radiolaria feed on the carcasses of multicellular animals contained in detritus or marine snow (Nakamura et al., 2017; Ikenoue et al., 2019). Another possibility is that some part of the body of these multicellular animals were contained inside the specimens. Certain large Radiolaria have been reported to be eaten by gelatinous zooplankton, such as Cnidaria and salps (Nakamura et al., 2021), but their fragile bodies are easily damaged during the process of field sampling. They thereby become unrecognizable, but a small amount of their bodies remain inside the radiolarian specimens. This is especially the case in the order Orodaria (Or1 and Or3), which are often fed on by gelatinous zooplankton.

2. Phaeodaria

The cluster analysis focused on Phaeodaria suggested that, unlike the case with Radiolaria, members of the same phaeodarian family do not tend to contain similar organisms (Fig. S4). The body part (i.e., the central capsule or the phaeodium) could be the most important factor dividing the taxonomic composition of detected organisms (Fig. S4), implying that the selection of an appropriate body part is important when determining contained organisms, even for unicellular zooplankton. Previous researchers have suggested that the phaeodium contains undigested prey (Gowing, 1986; 1989), and this idea is partly supported by the results of this study, which revealed that the phaeodium contains numerous small organisms (i.e., possible food sources).

There was a paucity of information about the biological interactions of Phaeodaria (Table S1). Some previous studies thoroughly reviewed the symbiosis of protists, and the biological interactions were well documented for the other culturable cercozoans (e.g., Nowack & Melkonian, 2010; Bjorbækmo et al., 2019). Very little information was, however, available for Phaeodaria, which also belong to Cercozoa. This study succeeded in adding to and updating knowledge on these biological interactions. Previous studies reported that Dinoflagellata are parasitic on Phaeodaria (Cachon-Enjumet, 1961), and this was confirmed by our results. In addition, we found that Apicomplexa, *Massisteria* (Cercozoa), and *Dermocystidium* (Mesomycetozoea) may also be parasites of some Phaeodaria, since these taxa are known as parasites of diverse marine organisms (Gull, 2001; Mylnikov et al., 2015; Seeber & Steinfelder, 2015).

Symbiotic algae have not previously been reported in Phaeodaria, and therefore, the detection of photosymbiotic organisms should be interpreted carefully. Most of these algae may be food

sources, but it is also possible that some of them function as symbiotic algae because some host Phaeodaria were collected in euphotic zones (e.g., *Aulosphaera* sp.1, *Coelanthemum auloceroides*, and *Aulacantha scolymantha*). In addition, the algae detected in these Phaeodaria (e.g., Haptophyta and some autotrophic species of Dinoflagellata) are symbionts of other marine organisms (Bjorbækmo et al., 2019, Takagi et al., 2019; Lee et al., 2022). Considering the Radiolarian results (Fig. 3), Pelagophyceae may also be symbiotic algae of Phaeodaria.

Similar to the case of Radiolaria, multicellular organisms (Chaetognatha, Mollusca, Crustacea, and Chordata, including fishes) were detected in Phaeodaria. These taxa are food sources or possibly contaminants in the plankton sampling process. It is noteworthy that Copepoda were more frequently detected in Phaeodaria than in Radiolaria. This crustacean taxon is one of the most abundant zooplanktons in the world ocean, and consequently, contamination with their body parts during the sampling process is possible. However, some specimens of Phaeodaria and Radiolaria were collected in the same stations (Stas. 101, 102, 103, 104, KJ1 and Ses1) (Table S2), and Copepoda were rarely detected in Radiolaria (Fig. 3). The high detection of Copepoda, therefore, presumably reflects an ecological characteristic of Phaeodaria. It has been suggested that Phaeodaria feed on detritus or marine snow (Gowing, 1989), and the carcasses of Copepoda and other multicellular organisms are often contained in these substances. Copepoda may thus be eaten indirectly by Phaeodaria and presumably be an important food source.

3. DNA metabarcoding of difficult-to-culture protists

The presence of multiple symbionts and parasites is generally difficult to detect, and simultaneous analysis of numerous specimens requires a great deal of time and effort with ordinary

methods. However, by using a combination of single-cell DNA analysis and DNA metabarcoding, we were able to overcome these obstacles. This study succeeded in shedding light on the biological interactions of two groups of difficult-to-culture protists, Radiolaria and Phaeodaria. Moreover, the approach was shown to be effective enough to reveal the ecological relationships of these difficult-to-culture protists.

Future studies should focus on other difficult-to-culture but ecologically important protists such as Ciliophora, Choanoflagellata, and especially Foraminifera. The last group is known as an environmental proxy because of their wide distribution, importance as microfossils, and function as primary producers with symbiotic algae (Takagi et al., 2019). The symbionts of Foraminifera could be clarified more easily than those of Radiolaria and Phaeodaria because the 18S ribosomal RNA sequence of this group is largely different from other eukaryotes, and therefore, the host would not be detected. Indeed, Foraminifera are rarely detected by DNA metabarcoding using eukaryote-specific primers (Sogawa et al., 2022). In addition, more specimens of Radiolaria and Phaeodaria should be examined to further confirm the pattern and specificity of their symbionts, parasites, and food sources.

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AUTHOR CONTRIBUTIONS

Y. N. designed the research; Y. N., H. I., A. T., S. S., A. Y. and K. H. performed the field sampling; Y. N. and E. O.-T. analyzed the data; and Y. N. wrote the paper.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

DATA AVAILABILITY

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Amplicon sequences generated in this study are available through the DNA Data Bank of Japan database with the accession number DRA010024.

SUPPLEMENTARY INFORMATION

Supplementary materials (Figures S1–S4 and Tables S1–S3) are available for this study.

REFERENCES

Adl, S.M., Bass, D., Lane, C.E., Lukeš, J., Schoch, C.L., Smirnov, A. et al. (2019) Revisions to the classification, nomenclature, and diversity of eukaryotes. Journal of Eukaryotic Microbiology, 66, 4–119.

Amaral-Zettler, L.A., McCliment, E.A., Ducklow, H.W. & Huse, S.M. (2009) A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal rna genes. *PLoS One*, 4, e6372.

Anderson, O.R. (1983). *Radiolaria*. New York, U.S.A.: Springer.

Biard, T., Stemmann, L., Picheral, M., Mayot, N., Vandromme, P., Hauss, H. et al. (2016) *In situ* imaging reveals the biomass of giant protists in the global ocean. *Nature*, 532, 504–507.

Biard, T. & Ohman, M. (2020) Vertical niche definition of test-bearing protists (Rhizaria) into the twilight zone revealed by *in situ* imaging. *Limnology and Oceanography*, 65, 2583–2602.

Bjorbækmo, M.F.M., Evenstad, A., Røsæg, L.L., Krabberød, A.K. & Logares, R. (2020) The planktonic protist interactome: where do we stand after a century of research? *The ISME journal*, 14, 544–559.

Cachon-Enjumet, M. (1961) Contribution à l'étude des Radiolaires Phaeodariés. *Archives de Zoologie Expérimentale et Générale*, 100, 151–237.

Cleary, A.C., Durbin, E.G. & Rynearson, T.A. (2012) Krill feeding on sediment in the Gulf of Maine (North Atlantic). *Marine Ecology Progress Series*, 455, 157–172.

Cleary, A.C., Durbin, E.G., Rynearson, T.A. & Bailey, J. (2015) Feeding by *Pseudocalanus*

369 copepods in the Bering Sea: trophic linkages and a potential mechanism of niche partitioning.
370 *Deep Sea Research Part II*, 134, 181–189.
371 Decelle, J., Suzuki, N., Mahé, F., De Vargas, C. & Not, F. (2012a) Molecular phylogeny and
372 morphological evolution of the Acantharia (Radiolaria). *Protist*, 163, 435–450.
373 Decelle, J., Siano, R., Probert, I., Poirier, C. & Not, F. (2012b) Multiple microalgal partners in
374 symbiosis with the acantharian *Acanthochiasma* sp. (Radiolaria). *Symbiosis*, 58, 233–244.
375 Fukui, M. & Hosokawa, M. (2004) Multi-purpose program for social system analysis 8 —canonical
376 correlation analysis, factor analysis, utilities—. *Business Information Studies*, 9, 23–35. (in
377 Japanese)
378 Gowing, M.M. (1986) Trophic biology of phaeodarian radiolarians and flux of living radiolarians in
379 the upper 200 m of the North Pacific central gyre. *Deep-Sea Research*, 33, 655–674.
380 Gowing, M.M. (1989) Abundance and feeding ecology of Antarctic phaeodarian radiolarians.
381 *Marine Biology*, 103, 107–118.
382 Gull, K. (2001) The biology of kinetoplastid parasites: insights and challenges from genomics and
383 post-genomics. *International Journal for Parasitology*, 31, 443–452.
384 Ikenoue, T., Kimoto, K., Okazaki, Y., Sato, M., Honda, M.C., Takahashi, K. et al. (2019) Phaeodaria:
385 An important carrier of particulate organic carbon in the mesopelagic twilight zone of the North
386 Pacific Ocean. *Global Biogeochemical Cycles*, 33, 1146–1160.
387 Lee, L.K., Leaw, C.P., Lee, L.C., Lim, Z.F., Hii, K.S., Chan, A.A., Gu, H. & Lim, P.T. (2022) Molecular
388 diversity and assemblages of coral symbionts (Symbiodiniaceae) in diverse scleractinian coral
389 species. *Marine Environmental Research*, 179, 105706.
390 Mylnikov, A.P., Weber, F., Jürgens, K. & Wylezich, C. (2015) *Massisteria marina* has a sister:
391 *Massisteria voersi* sp. nov., a rare species isolated from coastal waters of the Baltic Sea.

392 *European Journal of Protistology*, 51, 299–310.
393 Nakamura, Y., Imai, I., Yamaguchi, A., Tuji, A. & Suzuki, N. (2013) *Aulographis japonica* sp. nov.
394 (Phaeodaria, Aulacanthida, Aulacanthidae), an abundant zooplankton in the deep sea of the
395 Sea of Japan. *Plankton and Benthos Research*, 8, 107–115.
396 Nakamura, Y. & Suzuki, N. (2015) Chapter 9 Phaeodaria: Diverse marine cercozoans of world-
397 wide distribution. In: *Marine Protists Diversity and Dynamics*. Tokyo, Japan: Springer, pp. 223–
398 249.
399 Nakamura, Y., Imai, I., Yamaguchi, A., Tuji, A., Not, F. & Suzuki, N. (2015) Molecular phylogeny of
400 the widely distributed marine protists, Phaeodaria (Rhizaria, Cercozoa). *Protist*, 166, 363–373.
401 Nakamura, Y., Imai, I., Tuji, A. & Suzuki, N. (2016) A new phaeodarian species discovered from the
402 Japan Sea Proper Water, *Auloscena pleuroclada* sp. nov. (Aulosphaeridae, Phaeosphaerida,
403 Phaeodaria). *Journal of Eukaryotic Microbiology*, 63, 271–274.
404 Nakamura, Y., Somiya, R., Suzuki, N., Hidaka-Umetsu, M., Yamaguchi, A. & Lindsay, D.J. (2017)
405 Optics-based surveys of large unicellular zooplankton: A case study on radiolarians and
406 phaeodarians. *Plankton and Benthos Research*, 12, 95–103.
407 Nakamura, Y., Matsuoka, K., Imai, I., Ishii, K., Kuwata, A., Kawachi, M. et al. (2019) Updated
408 information on plankton groups — the current status of the taxonomy and ecology. *Bulletin of*
409 *the Plankton Society of Japan*, 66, 22–40. (in Japanese with English abstract)
410 Nakamura, Y., Minemizu, R. & Saito, N. (2019) “Rhizarian rider”—symbiosis between
411 *Phronimopsis spinifera* Claus, 1879 (Amphipoda) and *Aulosphaera* sp. (Phaeodaria). *Marine*
412 *Biodiversity*, 49, 2193–2195.
413 Nakamura, Y., Tuji, A., Makino, W., Matsuzaki, S.S., Nagata, N., Nakagawa, M. et al. (2020a)
414 Feeding ecology of a mysid species, *Neomysis awatschensis* — combining approach with

microscopy, stable isotope analysis and DNA metabarcoding. *Plankton and Benthos Research*, 15, 44–54.

Nakamura, Y., Sandin, M.M., Suzuki, N., Tuji, A. & Not, F. (2020b) Phylogenetic revision of the order Entactinaria—Paleozoic relict Radiolaria (Rhizaria, SAR). *Protist*, 171, 125712.

Nakamura, Y., Tuji, A., Kimoto, K., Yamaguchi, A., Hori, R.S. & Suzuki, N. (2021) Ecology, morphology, phylogeny and taxonomic revision of giant radiolarians, Orodaria ord. nov. (Radiolaria; Rhizaria; SAR). *Protist*, 172, 125808.

Nowack, E.C.M. & Melkonian, M. (2010) Endosymbiotic associations within protists. *Philosophical Transactions: Biological Sciences*, 365, 699–712.

Pawlowski, J., Holzmann, M. & Tyszka, J. (2013) New supraordinal classification of Foraminifera: Molecules meet morphology. *Marine Micropaleontology*, 100, 1–10.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F.O. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41: D590–D596.

Saito, N., Kayama, A. & Nakamura, Y. (2022) First record of the maternal care behavior of a “rhizarian rider,” *Phronimopsis spinifera* Claus, 1879 (Amphipoda, Hyperiidea), in association with *Aulosphaera* sp. (Rhizaria, Cercozoa, Phaeodaria, Aulosphaeridae). *Crustacean Research*, 51, 111–113.

Sandin, M.M., Pillet, L., Biard, T., Poirier, C., Bigeard, E., Romac, S. et al. (2019) Time calibrated morpho-molecular classification of Nassellaria (Radiolaria). *Protist*, 170, 187–208.

Sandin, M.M., Biard, T., Romac, S., O'Dogherty, L., Suzuki, N. & Not, F. (2021) A morpho-molecular perspective on the diversity and evolution of Spumellaria (Radiolaria). *Protist*, 172, 125806.

Seeber, F. & Steinfelder, S. (2016) Recent advances in understanding apicomplexan parasites.

F1000 Faculty Review, 5, 1369.

Sogawa, S., Nakamura, Y., Nagai, S., Nishi, N., Hidaka, K., Shimizu, Y. et al. (2022) DNA metabarcoding reveals vertical variation and hidden diversity of Alveolata and Rhizaria communities in the western North Pacific. *Deep-Sea Research Part I*, 183, 103765.

Suzuki, N. & Aita, Y. (2011) Topics on the spatial distributions, standing stocks, and symbiosis of living Radiolaria (Rhizaria, Protoctista). *Bulletin of the Plankton Society of Japan*, 58, 40–48. (in Japanese with English abstract)

Takagi, H., Kimoto, K., Fujiki, T., Saito, H., Schmidt, C., Kucera, M. & Moriya, K. (2019) Characterizing photosymbiosis in modern planktonic foraminifera. *Biogeosciences*, 16, 3377–3396.

Tanabe, A.S. (2018) Metabarcoding and DNA barcoding for Ecologists. URL http://www.fifthdimension.jp. (accessed on 4 May 2022).

Tanabe, A.S. & Toju, H. (2013) Two new computational methods for universal DNA barcoding: a benchmark using barcode sequences of bacteria, archaea, animals, fungi, and land plants. *PLoS One*, 8, e76910.

Toju, H. (2016). *Exploring ecosystems with DNA information — Environmental DNA, Large-scale community analysis, and ecological networks* —. Tokyo, Japan: Kyoritsu Press. (in Japanese)

Ward, J.H. (1963) Hierarchical groupings to optimize an objective function. *Journal of the American Statistical Association*, 58, 236–244.

Yuasa, T., Horiguchi, T., Mayama, S., Matsuoka, A. & Takahashi, O. (2012) Ultrastructural and molecular characterization of cyanobacterial symbionts in *Dictyocoryne profunda* (polycystine radiolaria). *Symbiosis*, 57, 51–55.

Yuasa, T., Kawachi, M., Horiguchi, T. & Takahashi, O. (2019) *Chrysochromulina andersonii* sp. nov.

(Prymnesiophyceae), a new flagellate haptophyte symbiotic with radiolarians. *Phycologia*, 58, 211–224.

Zhang, L., Suzuki, N., Nakamura, Y. & Tuji, A. (2018) Modern shallow water radiolarians with photosynthetic microbiota in the western North Pacific. *Marine Micropaleontology*, 139, 1–27.

FIGURE LEGENDS

Fig. 1. Location of the plankton sampling stations in 2012–2019. Pink dots indicate the sampling stations. The detailed information on each station is shown in Table S2.

Fig. 2. Some specimens of Radiolaria and Phaeodaria collected in this study. a: *Dictyocoryne truncata*, b: *Diplosphaera hexagonalis*, c: *Myelastrum trinibrachium*, d: *Sticholonche zanclea,* e: *Sphaerozoum punctatum*, f: *Acanthoplegma* sp., g: *Castanidium longispinum*, h: *Aulosphaera* sp., i: *Challengeron channeri*, j: *Challengeria naresii*, k: *Atlanticella* sp., l: *Tuscarora tubulosa*.

Fig. 3. Proportion in total sequence reads (%) of Radiolaria (host) and other detected organisms (possible symbionts, parasites and food sources). The first, second and third highest values for each specimen are shown in red, orange and yellow, respectively. Taxa with green circles are photosynthetic autotrophs, which have a potential to be symbiotic algae.
*: 18S rRNA sequences are not registered in NCBI database. **: The proportion of the host.

Fig. 4. Proportion in total sequence reads (%) of Phaeodaria (host) and other detected organisms

484 (possible symbionts, parasites and food sources). The first, second and third highest values for
485 each specimen are shown in red, orange and yellow, respectively. Taxa with green circles are
486 photosynthetic autotrophs, which have a potential to be symbiotic algae.
487 *: 18S rRNA sequences are not registered in NCBI database. **: The proportion of the host.
488
489

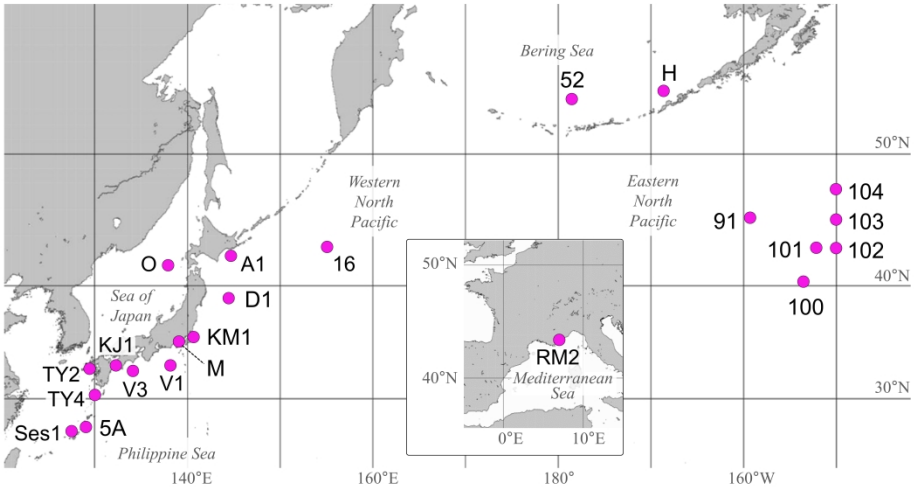

Fig. 1.

Figure 1

254x190mm (600 x 600 DPI)

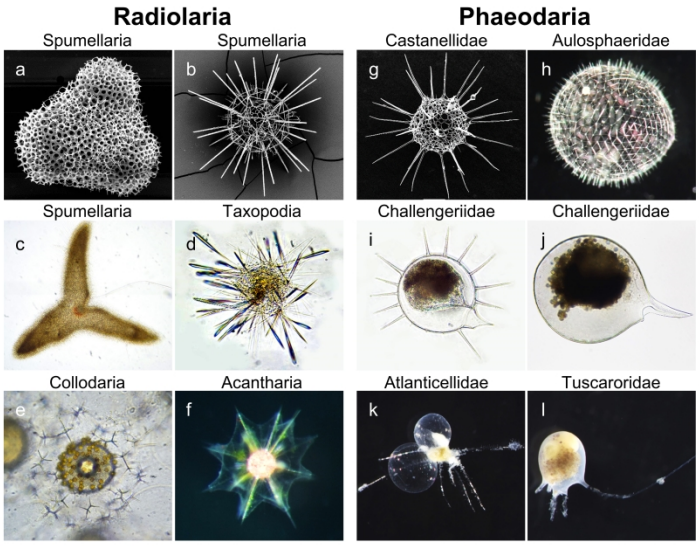

Fig. 2.

Figure 2

190x275mm (600 x 600 DPI)

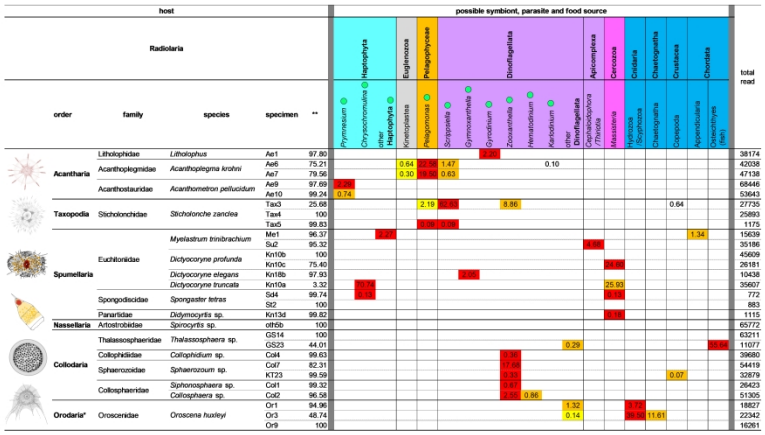

Fig. 3.

Figure 3

275x190mm (600 x 600 DPI)

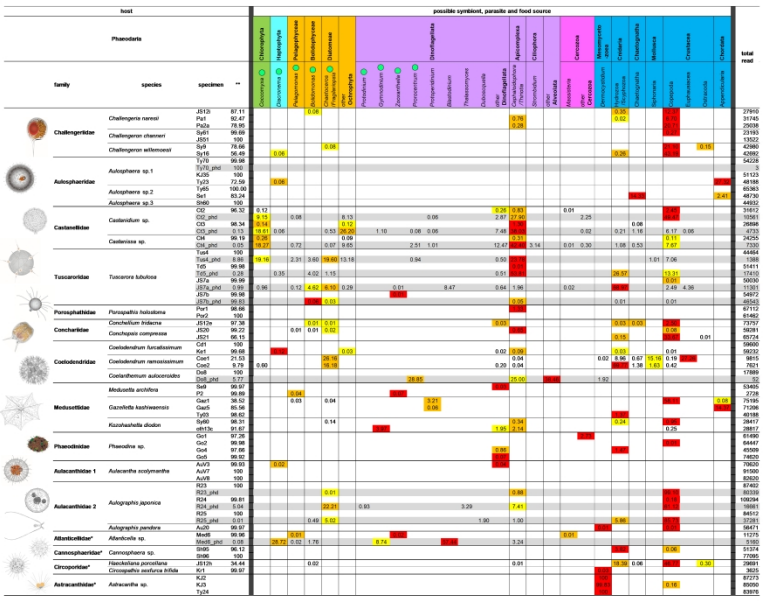

Fig. 4.

Figure 4

275x190mm (600 x 600 DPI)

Radiolaria

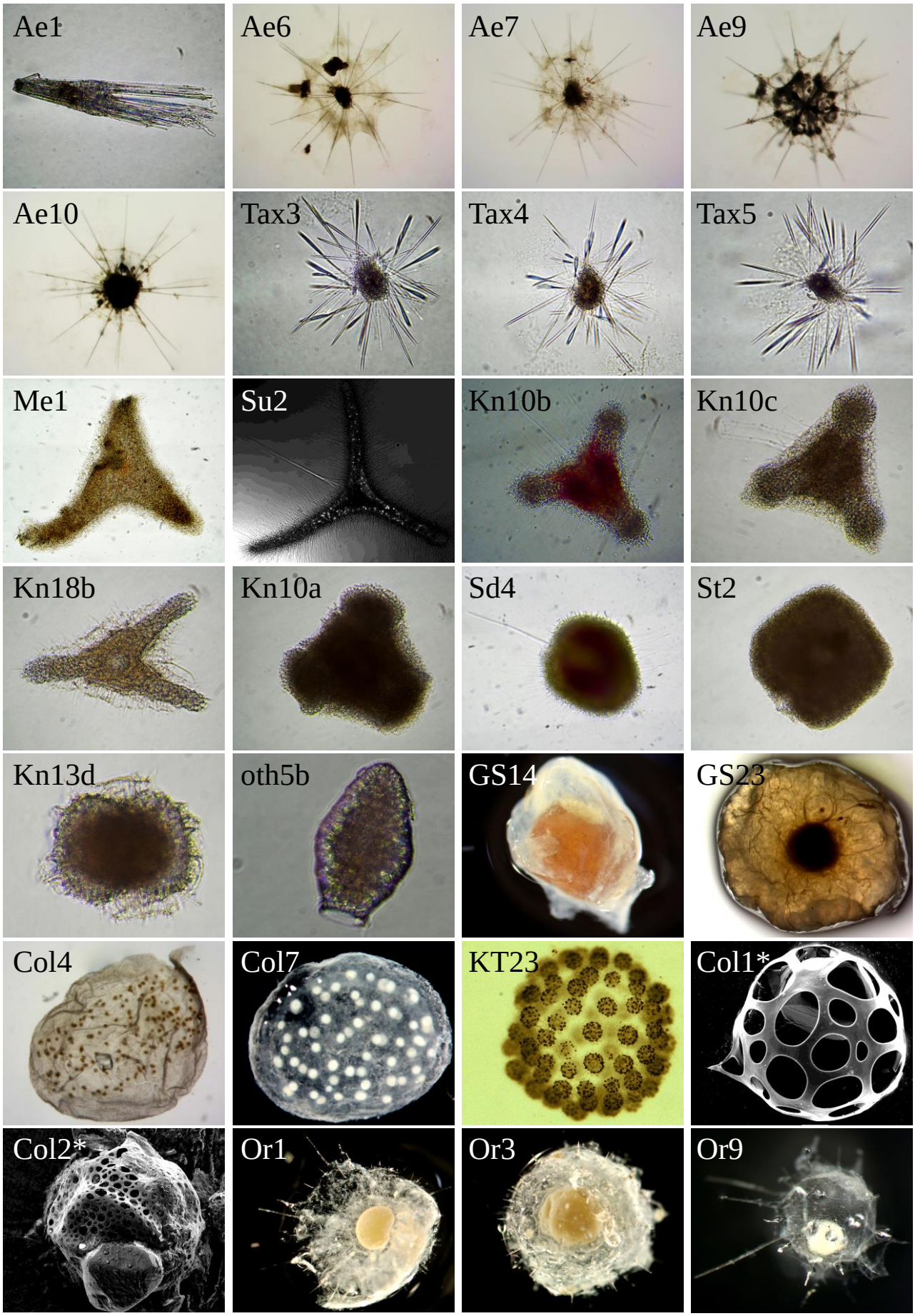

Fig. S1. Images of radiolarian and phaeodarian specimens analyzed in this study. The detailed information on each specimen is shown in Table S2.
*SEM images of the cortical shell of each individual composing a colony.

Phaeodaria

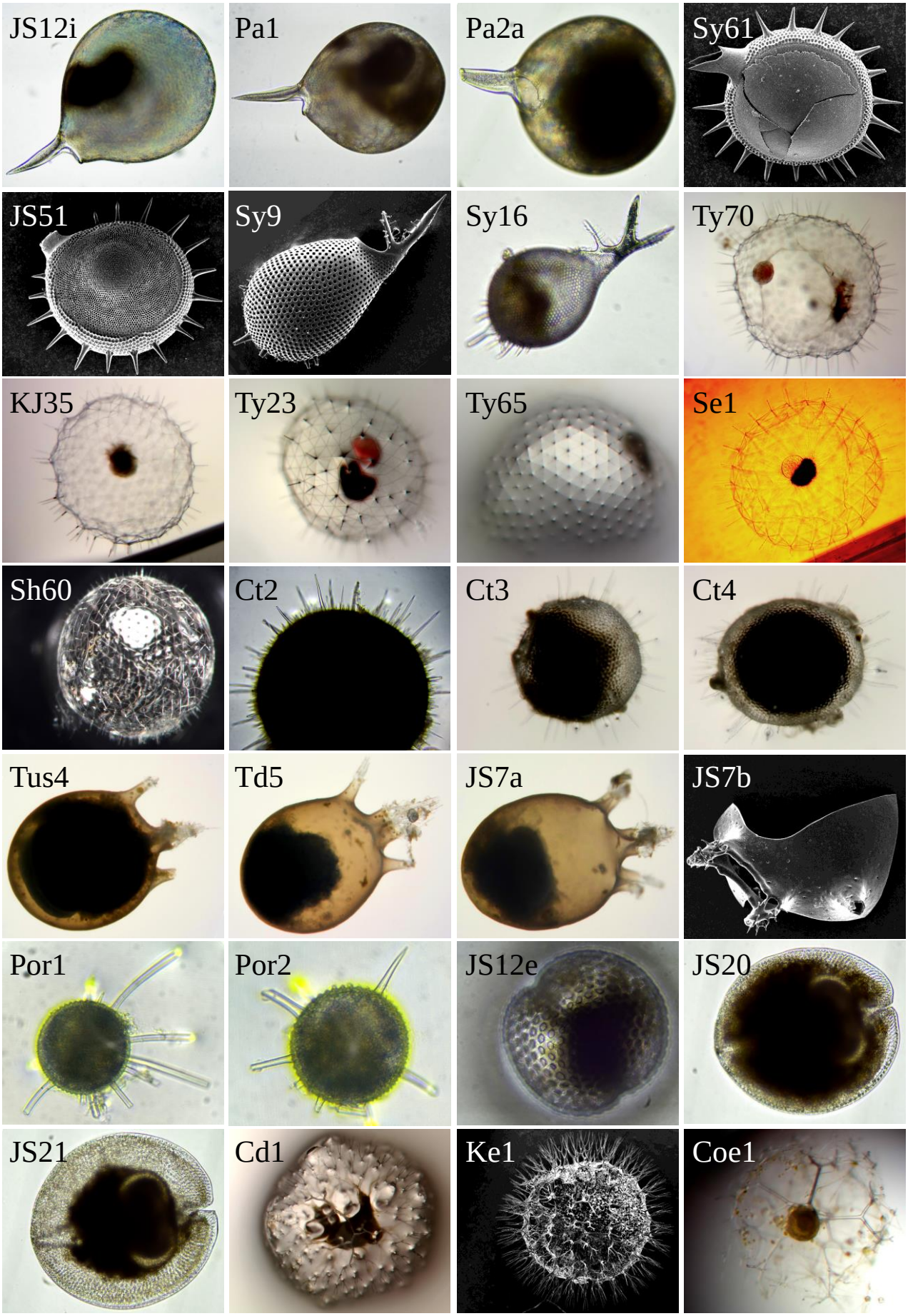

Fig. S1. continued.

Phaeodaria

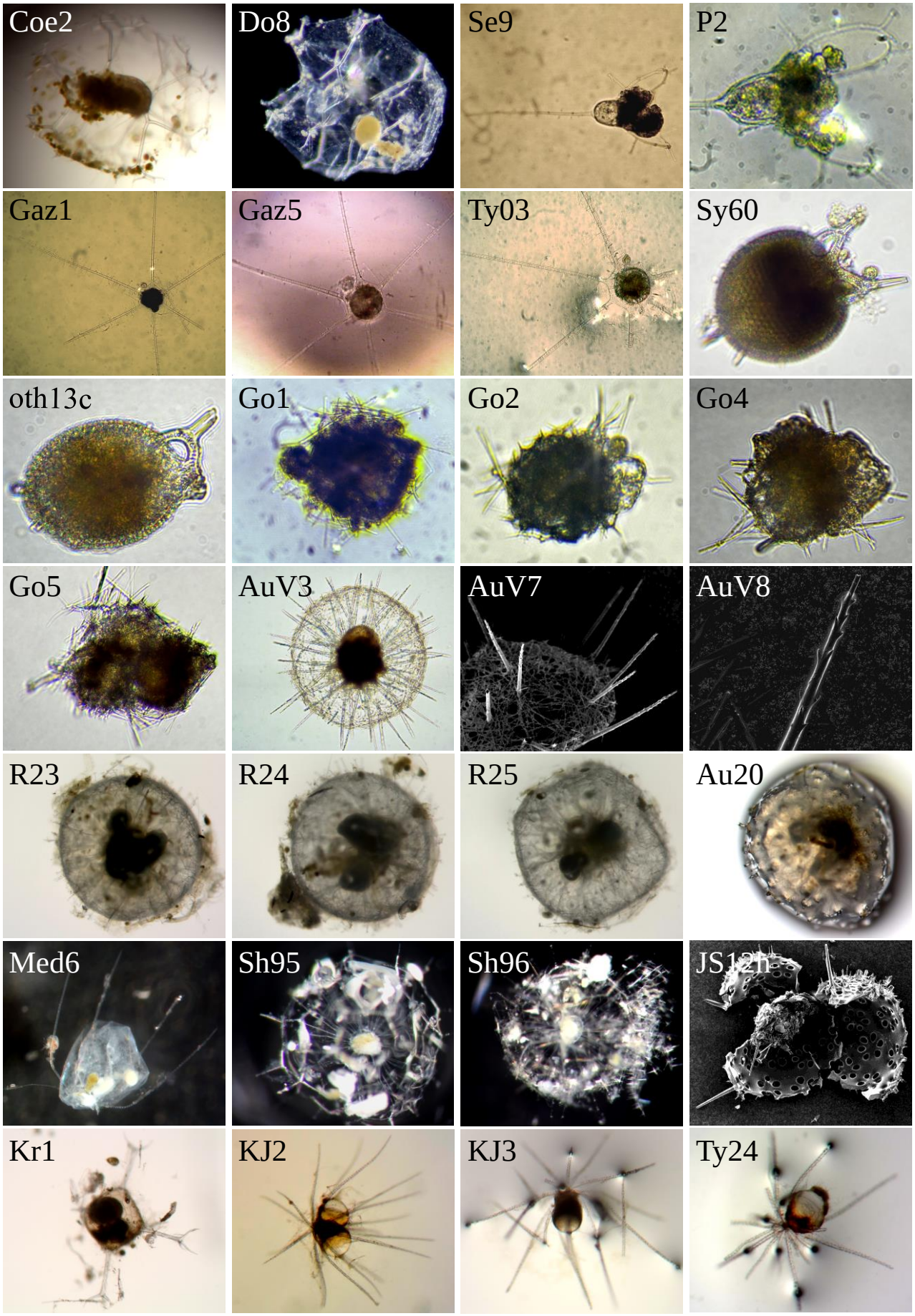

Fig. S1. continued.

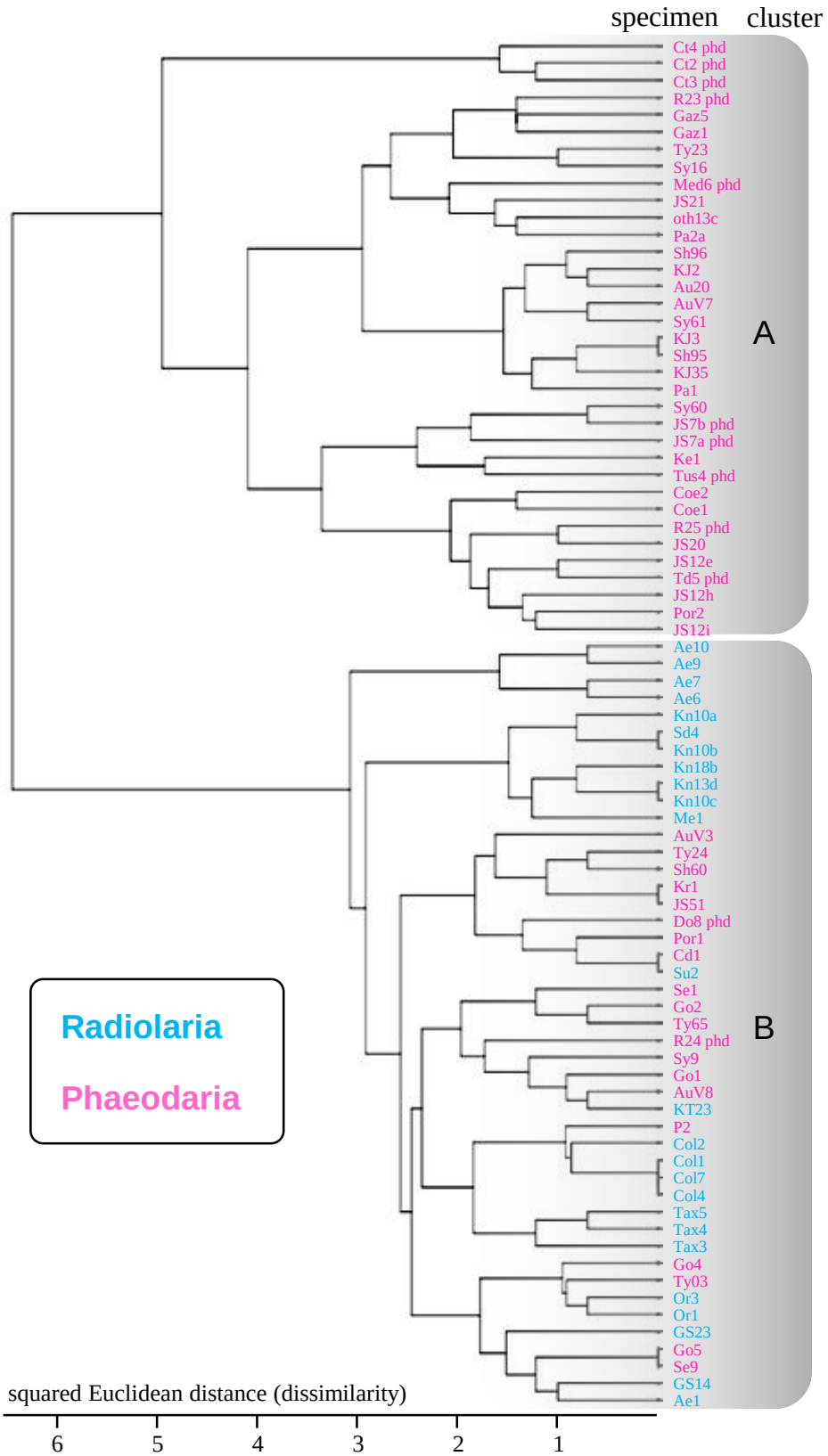

Fig. S2. Dendrogram constructed by the cluster analysis (Ward's method) based on the Euclidean distances calculated from the taxonomic composition of the organisms detected from the radiolarian (blue) and phaeodarian (pink) specimens (Figs. 3–4). In the specimens with “phd”, the DNA was extracted from the “phaeodium” (a mass of brownish particles contained in the phaeodarian body), while the DNA was obtained from the “central capsule” (containing nuclei) in the specimens without “phd”. Note that the specimens analyzed in this study can be categorized into two clusters: A and B.

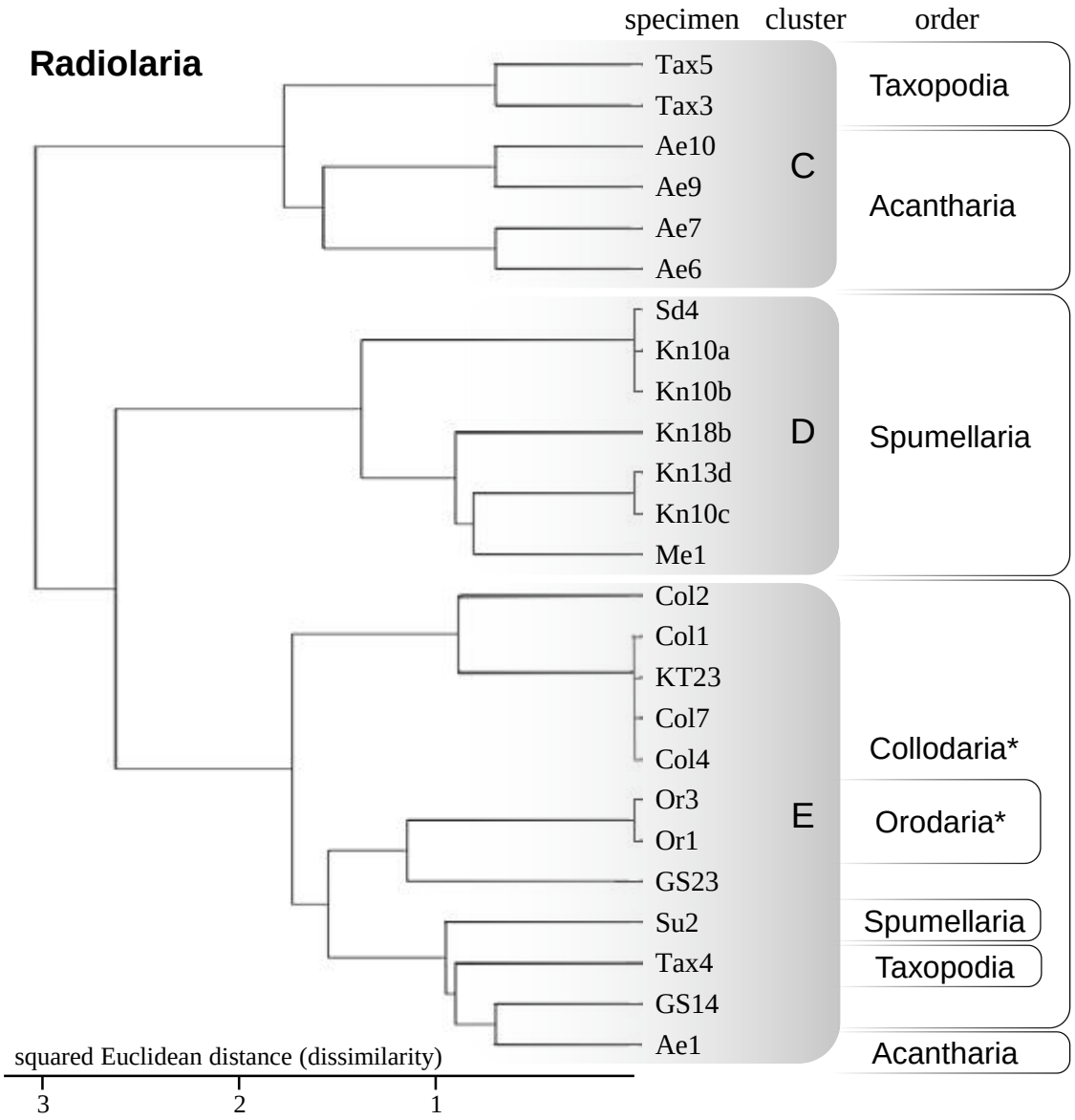

Fig. S3. Dendrogram constructed by the cluster analysis (Ward's method) based on the Euclidean distances calculated from the taxonomic composition of the organisms detected from the radiolarian specimens (Fig. 3). Note that the radiolarian specimens analyzed in this study can be categorized into three clusters: C, D and E. *Collodaria, Orodaria and Nassellaria are closely related from the phylogenetic viewpoint, and therefore, these three orders could be treated as one large lineage (Nakamura et al. 2021).

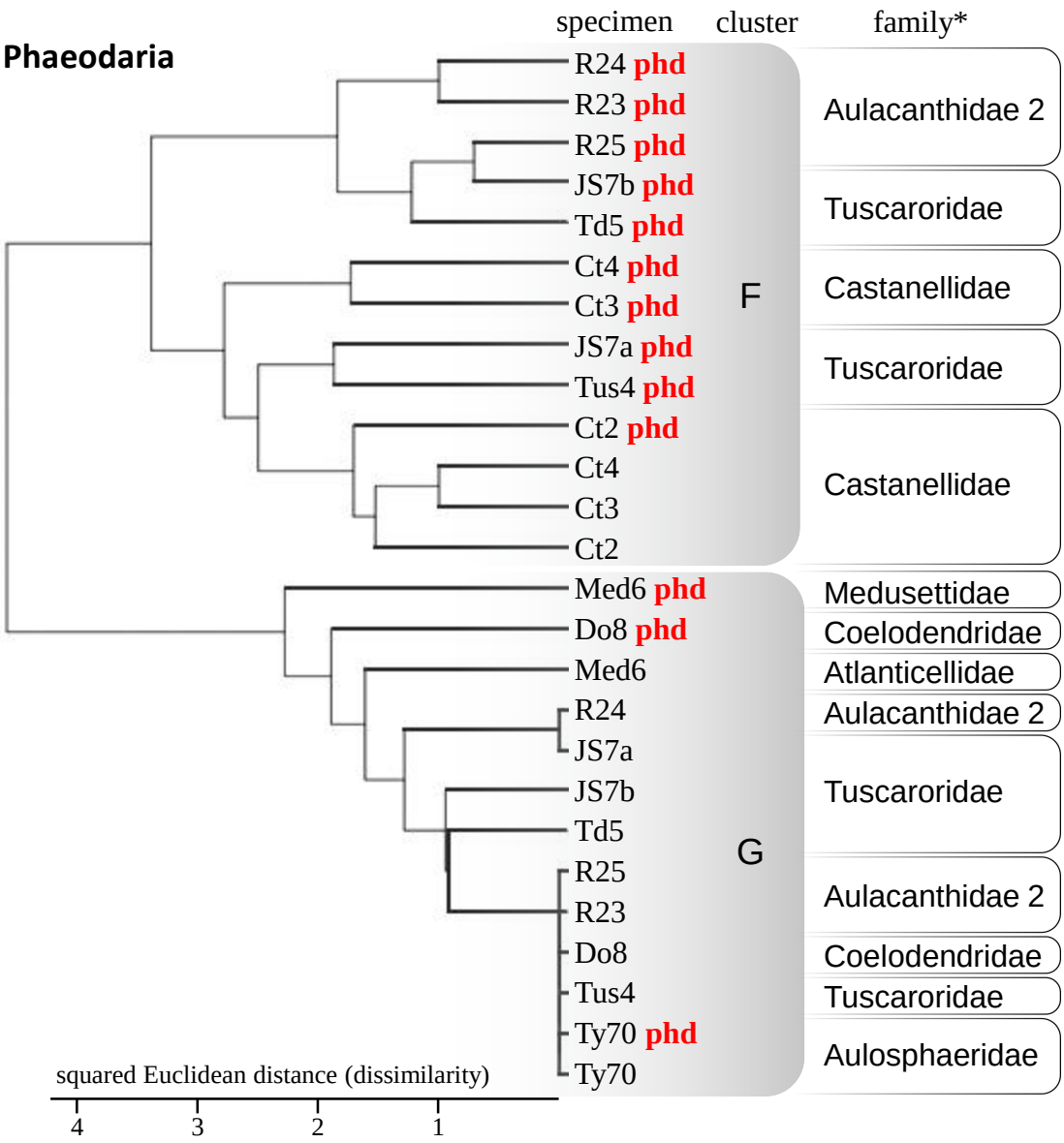

Fig. S4. Dendrogram constructed by the cluster analysis (Ward's method) based on the Euclidean distances calculated from the taxonomic composition of the organisms detected from the phaeodarians specimens (Fig. 4). In the specimens with “phd”, the DNA was extracted from the “phaeodium” (a mass of brownish particles contained in the phaeodarian body), while the DNA was obtained from the “central capsule” (containing nuclei) in the specimens without “phd”.

*: The phaeodarian "orders" in the current classification system do not reflect their phylogeny (Nakamura et al. 2015), and therefore, their order-level classification was ignored in this study. The family-level classification is referred to Nakamura et al. (2015).

TableS1. List of the hitherto-known symbionts and parasites of Radiolaria and Phaeodaria.
1: Biard et al. (2015) was referred to for determining the radiolarian species. 2: Sandin et al. (2021) was referred to for determining the radiolarian species.
*: The phaeodarian orders in the current classification are suggested not to reflect their phylogenetic relationships (Nakamura et al. 2015).

host				symbiont																	parasite																											reference
Rhizaria				**Eubacteria**		**Haptophyta**					**Chlorophyto**			**Dinoflagellata**							**Dinoflagellata**																											
higher taxon	**order**	**superfamily /family**	***genus species***	*Prochlorococcus* sp.	*Cynecococcus* sp.	*Phaeocystis* sp.	*Prymnesiaceae* sp.	*Chrysochromulina*	*Chrysochromulina* sp.	**Haptophyta** sp.	*Prasinophyceae* sp.	*Tetraselmis* sp.	*Pedinomonas*	*Pelagodinium beii* =*Gymnodinium beii*	*Scrippsiella* sp.	*Gymnoxanthella radiolariae*	*Gymnonidium* sp.	*Zooxanthella nutricula* =*Brandtodinium nutriculum*, =*Scrippsiella nutricula*, =*Endodinium nutricula*	*Azadinium* sp.	*Heterocapsa* sp.	*Oodinium acanthometrae*	*Syndinium brandti*	*Syndinium insidiosum*	*Syndinium vernale*	*Syndinium globiforme*	*Syndinium belari* =*Merodinium belari*	*Syndinium mendax*	*Syndinium dolosum*	*Syndinium chattoni*	*Syndinium astutum*	*Syndinium breve*	*Syndinium borgerti*	*Syndinium caryophagum*	*Syndinium* sp.	*Solenodinium fallax*	*Solenodinium leptotaemia*	*Solenodinium densum*	*Solenodinium* sp.	*Atlanticellodinium tregouboffi*	*Amoebophrya acanthometrae*	*Amoebophrya stycholonchae*	*Amoebophrya* sp.	*Duboscquella* sp.	*Dogelodinium ovoides* =*Collinella ovoides*	*Keppenodinium mycetoides* =*Hollandella mycetoides*	*Keppenodinium lobata*	*Keppenodinium piriformis*	
Infraphylum **Radiolaria**	**Acantharia**	Lithopteridae	*Lithoptera muelleri*				+																																									Febvre & Febvre-chevalier (1979)
		Amphilonchidae	*Amphilonche elongata*				+																																									Febvre & Febvre-chevalier (1979)
			Amphilonche sp.			+																												+														Hollande & Enjumet (1955), Cachon (1964), Mars Brisbin et al. (2018)
		Amphibelonidae	*Amphibelone* sp.			+																																										Mars Brisbin et al. (2018)
		Acanthostauridae	*Acanthometron pellucidum* =*Acanthometra pellucida*				+																											+						+				+				Hollande & Enjumet (1955), Cachon (1964), Febvre & Febvre-chevalier (1979)
			Acanthostaurus purpurascens =*Acanthometra purpurascens*																																					+								Koeppen (1894)
			Acanthonia claparedei =*Acanthometra claparedei*																																					+								Borgert (1897)
			Acanthometra serrata																																					+								Koeppen (1894)
			Acanthometra sp.			+															+																			+								Cachon & Cachon (1987), Mars Brisbin et al. (2018)
		Acanthochiasmidae	*Acanthociasma* sp.						+					+	+				+	+																												Decelle et al. (2012b)
		Arthracanthida sp.				+																																										Febvre & Febvre-chevalier (1979), Decelle et al. (2012b,c)
		Symphiacanthida sp.				+																																										Febvre & Febvre-chevalier (1979), Decelle et al. (2012b,c)
	Taxopodia	Sticholonchidae	*Sticholonche zanclea*																															+							+							Fol (1883), Koeppen (1894), Cachon (1964), Cachon & Cachon (1987)
	Spumellaria	Euchitoniidae	*Dictyocoryne truncata*				+	+																																								Anderson & Matsuoka (1992), Yuasa et al. (2019)
			Dictyocoryne profunda		+																																											Yuasa et al. (2012)
			Dictyocoryne elegans													+																																Yuasa et al. (2016)
		Panartidae	*Dydimocyrtis tetrathalamus*															+																														Probert et al. (2014)[2]
		Spongodiscidae	*Spongodrymus* sp.	+	+		+			+		+			+			+																														Anderson et al. (1983b), Gast & Caron (1996), Gast et al. (2000), Gast & Caron (2001), Foster et al. (2006a, b)
			Spongotrocus glacialis														+																															Ishitani et al. (2014)
		Spongosphaeridae	*Spongosphaera* sp.																															+									+		+			Cachon (1964), Hollande (1974), Cachon & Cachon (1987), Suzuki et al. (2009)
		Spongodrymidae	*Plegmosphaera coronata*																																											+		Cachon (1964)
			Plegmosphaera sp.																															+												+		Cachon (1964), Hollande (1974), Cachon & Cachon (1987)
			Plegmosphaeromma sp.															+																														Probert et al. (2014)[2]
		Haliommidae	*Haliomma capillaceum*																																								+					Suzuki et al. (2009)
			Haliomma sp. (*Actinosphaera* sp.)																																+			+					+				+	Hollande & Enjumet (1955), Cachon (1964), Hollande (1974), Cachon & Cachon (1987)
		Hexacromyidae	*Hexacontium gigantheum*																																							+						Kim & Park (2013)
		Hollandosphaeridae	*Hollandosphaera hexagonia*															+																														Probert et al. (2014)[2]
		Cladococcidae	*Cladococcus* sp.																																+			+										Hollande & Enjumet (1955), Hollande (1974)
			Astrosphaeridae sp.								+																																					Anderson (1976)
		Rhizosphaeridae	*Rhizosphaera* sp. (*Arachnorhiza* sp.)																																			+										Hollande & Enjumet (1955), Hollande (1974)
		Zonariidae	*Tetrapyle octacantha*															+																														Probert et al. (2014)[2]
		Spumellaria sp.																+																				+										Cachon & Cachon (1987), Probert et al. (2014)
	Nassellaria	Acanthodesmiidae	*Acanthodesmia vinculata*													+																																Yuasa et al. (2016)
		Lithochytrididae	*Pterocanium praetextum*													+																																Yuasa et al. (2016)
		Eucyrtidiidae	*Eucyrtidium* sp.															+																+														Hollande & Enjumet (1955), Hollande (1974), Cachon & Cachon (1987)
		Nassellaria sp.																+																														Probert et al. (2014)
	Collodaria	Thalassicollidae	*Thalassicolla nucleata*															+																+		+		+										Chatton (1920), Hovasse & Brown (1953), Hollande & Enjumet (1953), Hollande & Carré (1974), Gast & Caron (2001), Gast et al. (2003), Gast (2006)
			Thalassicolla spumida																															+	+													Chatton (1920), Hovasse & Brown (1953), Hollande (1974)
			Thalassicolla pellucida																																		+											Hovasse & Brown (1953), Hollande (1974)
			Thalassicolla sp.												+			+																	+	+	+											Anderson (1976, 1983), Cachon & Cachon (1987), Gast & Caron (1996)
			Thalassolampe margarodes										+																																			Cachon & Caram (1979), Decelle et al. (2015)
			Thalassophysa																															+														Hollande (1974), Cachon & Cachon (1987)
			Thalassicolla caerulea															+																														Probert et al. (2014)[1]
			Thalassicolla melacapsa															+																														Probert et al. (2014)[1]
			Myxosphaera coerulea																									+																						Chatton (1923), Hollande (1974), Cachon & Cachon (1987)
		Sphaerozoidae	*Sphaerozoum acuferum*																												+																		Chatton (1923), Hollande (1974)
			Sphaerozoum armatum															+																														Probert et al. (2014)[1]
			Sphaerozoum brandtii															+																														Probert et al. (2014)[1]
			Sphaerozoum fuscum															+																														Probert et al. (2014)[1]
			Sphaerozoum punctatum															+											+	+	+	+																		Chatton (1923), Hovasse & Brown (1953), Hollande (1974), Probert et al. (2014)[1]
			Sphaerozoum sp.																										+	+	+	+																		Cachon & Cachon (1987)
			Collozoum inerme															+				+		+																								Brandt (1881), Chatton (1923), Hovasse (1923), Hollande (1974)
			Collozoum caudatum												+																																	Gast & Caron (2001)
			Collozoum pelagicum																					+																								Chatton (1923), Hollande (1974)
			Collozoum fulvum																							+	+																					Hollande & Enjumet (1953), Hollande (1974)
			Collozoum sp.															+				+	+	+	+	+																						Hollande & Enjumet (1955), Hollande (1974), Cachon & Cachon (1987), Gast & Caron (1996), Probert et al. (2014)
			Rhaphidozoum acuferum															+																														Probert et al. (2014)[1]
		Collosphaeridae	*Collosphaera tuberosa*															+																														Probert et al. (2014)[1]
			Siphonosphaera abyssi															+																														Probert et al. (2014)[1]
			Collosphaeridae sp.															+																														Probert et al. (2014)[1]
		Collophidiidae	*Collophidium* sp.															+																														Probert et al. (2014)[1]
			Procyttarium prototypus															+																														Probert et al. (2014)[1]
subclass **Phaeodaria**	*	Challengeriidae	*Challengeron* sp.																													+																Cachon-Enjumet (1961)
	*	Coelodendridae	*Coelodendrum ramossissimum*																													+																Cachon-Enjumet (1961)
			Coelodendrum sp.																															+														Hollande & Enjumet (1955), Hollande (1974), Théodoridès 1989
	*	Medusettidae	*Planktonetta atlantica*																																				+									Cachon & Cachon (1987)
	*	Phaeodinidae	*Phaeodina valdiviae*																													+																Cachon-Enjumet (1961)
	*	Phaeosphaeridae	*Phaeosphaera nucleoelongata*																														+															Cachon-Enjumet (1961)
			Phaeosphaera pigmaeae																														+															Cachon-Enjumet (1961)
	*	Aulacanthidae 1	*Aulacantha scolymantha*																													+																Hollande et al. (1953), Hovasse & Brown (1953), Cachon-Enjumet (1961), Hollande (1974), Cachon & Cachon (1987)

Table S2. Detailed information of radiolarian and phaeodarian specimens examined in this study. Note that the phaeodarian specimens with "phd" were dessected, and their "central capsules" and "phaeodium" were separately analyzed.

classification				specimen name	sampling				
higher taxon	order	family	*genus species*		season	area	station	depth (m)	gear
infraphylum **Radiolaria**	**Acantharia**	Litholophidae	*Litholophus*	**Ae1**	July, 2019	PhS (off Manazuru)	M	0–5	hand net
		Acanthoplegmidae	*Acanthoplegma krohni*	**Ae6**	July, 2019	PhS (off Manazuru)	M	0–5	hand net
				Ae7	July, 2019	PhS (off Manazuru)	M	0–5	hand net
		Acanthostauridae	*Acanthometron pellucidum*	**Ae9**	July, 2019	PhS (off Manazuru)	M	0–5	hand net
				Ae10	July, 2019	PhS (off Manazuru)	M	0–5	hand net
	Taxopodia	Sticholonchidae	*Sticholonche zanclea*	**Tax3**	July, 2019	PhS (off Manazuru)	M	0–5	hand net
				Tax4	July, 2019	PhS (off Manazuru)	M	0–5	hand net
				Tax5	July, 2019	PhS (off Manazuru)	M	0–5	hand net
	Spumellaria	Euchitoniidae	*Myelastrum trinibrachium*	**Me1**	Sep., 2017	WNP (off Kominato)	KM1	0–5	hand net
				Su2	Nov., 2016	PhS (off Kashiwajima)	KJ1	0–5	hand net
			Dictyocoryne profunda	**Kn10b**	July, 2018	WNP (off Kominato)	KM1	0–5	hand net
				Kn10c	July, 2018	WNP (off Kominato)	KM1	0–5	hand net
			Dictyocoryne elegans	**Kn18b**	July, 2018	WNP (off Kominato)	KM1	0–5	hand net
			Dictyocoryne truncata	**Kn10a**	July, 2018	WNP (off Kominato)	KM1	0–5	hand net
		Spongodiscidae	*Spongaster tetras*	**Sd4**	Sep., 2017	WNP (off Kominato)	KM1	0–5	hand net
				St2	Mar., 2017	PhS (off Kashiwajima)	KJ1	0–5	hand net
		Panartidae	*Didymocyrtis* sp.	**Kn13d**	July, 2018	WNP (off Kominato)	KM1	0–5	hand net
	Nassellaria	Artostrobiidae	*Spirocyrtis* sp.	**oth5b**	July, 2012	ENP	104	1000-1500	VMPS
	Collodaria	Thalassosphaeridae	*Thalassosphaera* sp.	**GS14**	July, 2012	ENP	91	0-1000	80cm ring net
				GS23	July, 2012	ENP	101	0-1000	80cm ring net
		Collophidiidae	*Collophidium serpentinum*	**Col4**	Dec., 2014	ECS (off Sesoko)	Ses1	0–8	hand net
		Sphaerozoidae	*Sphaerozoum strigulosum*	**Col7**	Dec., 2014	ECS (off Sesoko)	Ses1	0–8	hand net
			Sphaerozoum trigenimum	**KT23**	Sep., 2019	PhS (off Manazuru)	M	0–5	hand net
		Collosphaeridae	*Odontosphaera macropore*	**Col1**	Dec., 2014	ECS (off Sesoko)	Ses1	0–8	hand net
			Collosphaera tuberosa	**Col2**	Dec., 2014	ECS (off Sesoko)	Ses1	0–8	hand net
	Orodaria*	Oroscenidae	*Oroscena huxleyi*	**Or1**	July, 2012	ENP	103	0–1000	vertical net
				Or3	July, 2012	ENP	102	0–1000	vertical net
				Or9	May, 2016	PhS	5A	1630–2045	ORI net
subclass **Phaeodaria**	*	**Challengeriidae**	*Challengeria naresii*	**JS12i**	May, 2012	WNP	16	200–1000	Gamaguchi net
				Pa1	Aug., 2017	BS (off Aleutian Islands)	H	1500–2001	VMPS
				Pa2a	Aug., 2017	BS (off Aleutian Islands)	H	1500–2001	VMPS
			Challengeron channeri	**Sy61**	Feb., 2018	PhS	V3	100–200	closing NORPAC net
				JS51	Aug., 2013	WNP (off Sanriku)	D1	1500–2000	VMPS
			Challengeron willemoesii	**Sy9**	Feb., 2018	PhS	V1	200–500	closing NORPAC net
				Sy16	Feb., 2018	PhS	V1	200–500	closing NORPAC net
	*	**Aulosphaeridae**	*Aulosphaera* sp.1	**Ty70 (phd)**	May, 2015	ECS	TY4	0–30	vertical net
				KJ35	July, 2015	PhS (off Kashiwajima)	KJ1	0–5	hand net
			Aulosphaera sp.2	**Ty23**	May, 2015	ECS	TY4	0–30	vertical net
				Ty65	May, 2015	ECS	TY4	0–30	vertical net
				Se1	Dec., 2014	ECS (off Sesoko)	Ses1	0–8	hand net
			Aulosphaera sp.3	**Sh60**	Nov., 2018	WNP (off Kushiro)	A1	0–500	80cm ring net
	*	**Castanellidae**	*Castanidium* sp.	**Ct2 (phd)**	July, 2012	ENP	102	250–500	VMPS
				Ct3 (phd)	July, 2012	ENP	102	250–500	VMPS
			Castanissa sp.	**Ct4 (phd)**	July, 2012	ENP	102	250–500	VMPS
	*	**Tuscaroridae**	*Tuscarora tubulosa*	**Tus4 (phd)**	July, 2012	ENP	104	1000–1500	VMPS
				Td5 (phd)	May, 2012	WNP	16	200–1000	Gamaguchi net
				JS7a (phd)	May, 2012	WNP	16	200–1000	Gamaguchi net
				JS7b (phd)	May, 2012	WNP	16	200–1000	Gamaguchi net
	*	**Porospathidae**	*Porospathis holostoma*	**Por1**	Aug., 2013	WNP	D1	150-250	VMPS
				Por2	Aug., 2013	WNP	D1	1500-2000	VMPS
	*	**Conchariidae**	*Conchellium tridacna*	**JS12e**	May, 2012	WNP	16	200–1000	Gamaguchi net
			Conchopsis compressa	**JS20**	May, 2012	WNP	16	200–1000	Gamaguchi net
				JS21	May, 2012	WNP	16	200–1000	Gamaguchi net
	*	**Coelodendridae**	*Coelodendrum furcatissimum*	**Cd1**	July, 2012	ENP	102	0–1000	80cm ring net
				Ke1	July, 2012	ENP	100	0–1000	80cm ring net
			Coelodendrum ramosissimum	**Coe1**	July, 2012	ENP	103	0–1000	80cm ring net
				Coe2	July, 2012	ENP	103	0–1000	80cm ring net
			Coelanthemum auloceroides	**Do8 (phd)**	Sep., 2018	MS	RM2	0–5	vertical net
	*	**Medusettidae**	*Medusetta arcifera*	**Se9**	Dec., 2014	ECS (off Sesoko)	Ses1	0–8	hand net
				P2	Nov., 2012	MS (off Villefranche-sur-Mer)	RM2	0–50	vertical net
			Gazelletta kashiwaensis	**Gaz1**	Dec., 2015	PhS (off Kashiwajima)	KJ1	0–5	hand net
				Gaz5	Dec., 2015	PhS (off Kashiwajima)	KJ1	0–5	hand net
				Ty03	May, 2015	ECS	TY2	0–30	vertical net
			Kozohashetta diodon	**Sy60**	Feb., 2018	PhS	V3	100–200	closing NORPAC net
				oth13c	July, 2012	ENP	102	500–750	VMPS
	*	**Phaeodinidae**	*Phaeodina* sp.	**Go1**	Nov., 2012	MS (off Villefranche-sur-Mer)	RM2	0–50	vertical net
				Go2	Nov., 2012	MS (off Villefranche-sur-Mer)	RM2	0–50	vertical net
				Go4	Nov., 2012	MS (off Villefranche-sur-Mer)	RM2	0–50	vertical net
				Go5	Nov., 2012	MS (off Villefranche-sur-Mer)	RM2	0–50	vertical net
	*	**Aulacanthidae 1** clade I**	*Aulacantha scolymantha*	**AuV3**	Nov., 2012	MS (off Villefranche-sur-Mer)	RM2	0–50	vertical net
				AuV7	Nov., 2012	MS (off Villefranche-sur-Mer)	RM2	0–50	vertical net
				AuV8	Nov., 2012	MS (off Villefranche-sur-Mer)	RM2	0–50	vertical net
	*	**Aulacanthidae 2** clade K**	*Aulographis japonica*	**R23 (phd)**	Apr., 2014	SJ	O	250–750	Gamaguchi net
				R24 (phd)	Apr., 2014	SJ	O	250–750	Gamaguchi net
				R25 (phd)	Apr., 2014	SJ	O	250–750	Gamaguchi net
			Aulographis pandora	**Au20**	July, 2012	ENP	101	0–1000	80cm ring net
	*	**Atlanticellidae**	*Atlanticella* sp.	**Med6 (phd)**	July, 2012	ENP	52	1500–2000	VMPS
	*	**Cannosphaeridae**	*Cannosphaera* sp.	**Sh95**	Nov., 2018	WNP (off Kushiro)	A1	0–500	80cm ring net
				Sh96	Nov., 2018	WNP (off Kushiro)	A1	0–500	80cm ring net
	*	**Circoporidae**	*Haeckeliana porcellana*	**JS12h**	May, 2012	WNP	16	200–1000	Gamaguchi net
			Circospathis sexfurca trifida	**Kr1**	July, 2012	ENP	102	1500–2000	VMPS
	*	**Astracanthidae**	*Astracantha* sp.	**KJ2**	July, 2015	PhS (off Kashiwajima)	KJ1	0–5	hand net
				KJ3	July, 2015	PhS (off Kashiwajima)	KJ1	0–5	hand net
				Ty24	May, 2015	ECS	TY4	0–30	vertical net

*: The phaeodarian "orders" in the current classification system do not reflect their phylogeny (Nakamura et al. 2015), and therefore, their order-level classification was ignored in this study.
**: The phaeodarian clades phylogenetically different from each other (Nakamura et al. 2015).
Abbreviations.
PhS: Philippine Sea. ECS: East China Sea. SJ: Sea of Japan. BS: Bering Sea. ENP: Eastern North Pacific. MS: Mediterranean Sea. WNP: Western North Pacific. ORI net: Ocean Research Institute net. VMPS: Vertical Multiple Plankton Sampler

Table S3. Proportion in total sequence reads (%) of the host (Radiolaria and Phaeodaria) and other detected organisms (possible symbionts, parasites and food sources).
*: The phaeodarian "orders" in the current classification system do not reflect their phylogeny (Nakamura et al. 2015), and therefore, their order-level classification was ignored in this study.
**: The phaeodarian clades phylogenetically different from each other (Nakamura et al. 2015).
†: 18S rRNA sequences are not registered in NCBI database.