**Aulographis japonica** sp. nov. (Phaeodaria, Aulacanthida, Aulacanctidae), an abundant zooplankton in the deep sea of the Sea of Japan

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**Abstract:** Zooplankton samples from the deep water of the Sea of Japan often contain yellowish semitransparent spheres (1.0–1.5 mm in diameter). We recognized these spheres as a single phaeodarian species (Cercozoa, Rhizaria) and described them as *Aulographis japonica* sp. nov. (family Aulacanthidae) in this paper. This species has a high abundance in the Japan Sea Proper Water (JSPW) and occasionally higher biomass than that of copepods. Molecular analysis based on 18S SSU rDNA revealed that *Aulacantha scolymantha*, which belongs to the same family as *A. japonica*, is closer to *Aulosphaera trigonopa* and *Protocystis* spp., which belong to different orders, than to the present species. The distribution of *A. japonica* is apparently restricted to low temperature water. Its biomass was the highest in the uppermost layer of JSPW, and this phaeodarian species was the second most important zooplankton below 250 m depth in terms of biomass among the total zooplankton groups. This is probably due to its generalist type of feeding. Considering its large biomass, *A. japonica* possibly plays an important role in matter cycles within the Sea of Japan.

**Key words:** *Aulographis japonica*, Cercozoa, new species, Phaeodaria, the Sea of Japan

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**Introduction**

The deep water in the Sea of Japan has a unique hydrography with its own particular fauna. The central area of the Sea of Japan is deeper than 3,000 m, while the four straits that connect it to the surrounding seas are relatively shallow. Through these four straits, surface water can be exchanged with the open sea (Nishimura 1974). By contrast, the deeper water below 300 m depth is characterized by a constant low temperature (ca. 1°C) and salinity (ca. 34), and this stable water mass is called the Japan Sea Proper Water (JSPW) (Uda 1934, Nishimura 1974). The isolated hydrographic properties of JSPW are supposed to contribute to the low reported diversity of zooplankton in this sea area (e.g. Nishimura 1974, Horikoshi 1996). In the JSPW, we found an abundance of a protozoan zooplankton of 1.0–1.5 mm in diameter, the form of which resembles a yellowish fish egg, and this zooplankton was identified as a species of phaeodarian.

The subclass Phaeodaria is a holoplanktonic marine protistan group, belonging to the phylum Cercozoa of the infrakingdom Rhizaria (Nakayama 2011) and was formerly considered to be a group within the Radiolaria (Suzuki & Aita 2011). They are mainly distributed in the deep sea worldwide and in cold surface waters in the Polar Regions (Reshetnyak 1955). The cell size of Phaeodaria ranges from several tens of micrometers to a few millimeters, and Phaeodaria are recognizable by the presence of a delicate opal skeleton that contains a double-layered spherical protoplasmatic body named the central capsule, and a mass of brown aggregated particles called the phaeodium. In our sampling cruise in the eastern margin of the Sea of Japan, west of the Japanese Islands, in June 2011, we found a marked abundance of phaeodarians in the deep sea. Our careful observations of the skeletal and protoplasmatic characteristics of these phaeodarians proved that they belong to a single species. Further morphological examination and
genetic analysis revealed that the species belongs to the genus *Aulographis* but differs from hitherto-known species of the genus. We describe it as a new species, and briefly discuss its ecological roles in the Sea of Japan.

**Materials and Methods**

**Sampling**

Plankton samples were collected using a Vertical Multiples Plankton Sampler (VMPS; mouth opening 0.25 m$^2$, mesh size 60 $\mu$m) at two stations, Sta. SJ1 (41°59′N, 138°21′E) and Sta. SJ2 (38°28′N, 135°30′E), in the Sea of Japan on 15 and 21 June 2011, during a cruise of the T/S *Oshoro-Maru* (Hokkaido University) (Fig. 1). At each station, we sampled 12 layers: 0–25, 25–50, 50–75, 75–100, 100–150, 150–250, 250–500, 500–750, 750–1000, 1000–1500, 1500–2000 and 2000–3000 m depth. After sampling, samples were immediately fixed and preserved in 5% borax-buffered formalin-seawater on board.

For molecular phylogenetic analysis, we also sampled with a *Gamaguchi*-net (mouth opening 60 cm, mesh size 60 $\mu$m; Kawamura 1989) from the 250–750 m layer at Sta. SJ3 (41°52′N, 139°06′E) and at Sta. SJ4 (41°37′N, 139°14′E) on 24 and 25 April 2012, during a cruise of the T/S *Ushio-Maru* (Hokkaido University) (Fig. 1). After sampling, each sample was immediately divided into two aliquots. One of the aliquots was immediately preserved in 5% borax-buffered formalin-seawater, while the other one was used to isolate phaeodarian individuals for genetic analysis. A hundred individuals were extracted from the sample under a stereomicroscope, and then each individual was put into separate wells of a cell culture plate. After incubation at 4°C for 12 hours to cleanse them, they were separately preserved in approximately 2.0 mL of 99.9% ethanol. Water temperature and salinity were measured with a CTD system (SBE911plus, Sea-Bird, U.S.A.).

**Microscopic observation and estimation of biomass**

For an examination of morphological characters, 120 individuals were extracted from the 24 formalin-preserved samples from Sta. SJ1 and Sta. SJ2 under a stereomicroscope, and their morphological variations and differences were examined under an inverted microscope at 50 to 400X magnitudes. Examined phaeodarian individuals were photographed with a digital camera (DIGITAL SIGHT DS-R1i, Nikon, Japan) attached to the microscope.

For detailed examinations of the fine structures, 20 individuals that were selected under an inverted microscope were observed with a SEM (JSM-6390 with LaB6 gun, JEOL, Japan). They were mounted on specimen stubs under a stereomicroscope and were gently dried at 60°C in a dry heat sterilizer overnight, prior to coating with platinum.

To estimate the biomass of phaeodarians, the formalin-preserved samples were analyzed using an Optical Plankton Counter (OPC-1L, Focal Technology Corp., Canada). Almost all the zooplankton in these samples were first sorted out according to taxa, and subsamples of each taxon were prepared. The number of zooplankton in each subsample was then counted to estimate the abundance of each taxon. These subsamples were analyzed through the OPC, and the estimated spherical diameter (ESD) of each taxon was measured. From the mean ESD, the wet-mass biomass (mg WM m$^{-3}$) of each taxon was calculated on the supposition that the specific gravity of zooplankton is equal to that of water.

**Molecular analysis**

Two ethanol-preserved specimens of this phaeodarian species, one from Sta. SJ3 and another one from Sta. SJ4, were analyzed in this study. These specimens were observed under the inverted stereomicroscope to confirm that there was no contamination due to other organisms on or in the specimens, and images were recorded as evidence for the morphological identification. After being dissected with a sterilized scalpel, their central capsules (protoplasmic body with nucleus) were transferred into 50 $\mu$L of GITC buffer, preserved in $^{-80}$C overnight and then heated at 70°C for 20 min to break the wall of the central capsule. DNA was purified with a chemagic DNA Plant kit (Chemagen, Germany).

The PCR amplification for the small subunit (SSU) ribo-
somal DNA was carried out using a thermal cycler (Gene-Amp PCR system 9700, ABI, U.S.A.). The reaction volume was 25 µL, and the solution consisted of 1.0 µL template DNA, 0.35 µM of each primer, 12.5 µL Ampdirect Plus (Shimazu, Japan), 9.625 µL H₂O and 0.125 µL KAPA 3G Plant DNA Polymerase (Nippon Genetics Co. Ltd, Japan). Two primer sets were used (Table 1). PhaeoF and PhaeoR were phaeodarian-specific primers and designed for this study. PCR reactions were performed using the following protocol: initial denaturation at 95°C for 5 min, 40 cycles at 95°C for 30 sec, 50°C for 30 sec and 72°C for 60 sec with a final extension at 72°C for 7 min. The amplified PCR products were purified with ExoSAP-IT (USB Corporation, U.S.A.). Sequencing reactions were conducted using an ABI PRISM 3130xl Genetic Analyzer (ABI, U.S.A.).

The obtained sequences were assembled using ChromasPro (Technelysium Pty Ltd, Australia), and the alignments were checked manually. The sequences were registered in the DNA Data Bank of Japan (S3J: AB820365, SJ4: AB820366). In order to examine the phylogenetic relationship between the present species and other phaeodarians, the sequences of nine other species were obtained from the Basic Local Alignment Search Tool (BLAST) at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analyses of the obtained sequences were conducted using the computer program MEGA 5 (Tamura et al. 2011). The General Time Reversible (GTR) model plus Gamma with the shape parameter for among-site rate variation (G) was selected based on the lowest BIC (Bayesian Information Criterion) score, and the substitution nucleotide matrix parameters were calculated. A maximum-likelihood (ML) tree was constructed with the selected model by the program. Bootstrap values (Felsenstein 1985) for neighbor-joining (NJ) and ML methods were estimated, and the two calculations were performed for 1000 replicates and 100 replicates, respectively.

Results

Systematic descriptions

*Aulographis japonica* Nakamura, Tuji et Suzuki sp. nov.

*Examined specimens:* 60 individuals from Sta. SJ1 and 60 individuals from Sta. SJ2.

Dimensions: Malacoma is 1.11–1.59 mm in diameter ($\bar{x}$=1.37, n=50). Central capsule is 0.22–0.27 mm in diameter ($\bar{x}$=0.24, n=100). Terminal branch is 18.1–25.0 µm ($\bar{x}$=21.7 µm, n=33) long.

_Type material:_ All type material from sample OS11019 in Plankton Laboratory, Hokkaido University.

_Holotype:_ A mounted stub MPC-03709 (Fig. 4A–F).

_Paratypes:_ A mounted slide MPC-03710 (Fig. 2A–C). An ethanol-fixed specimen MPC-03711 (Fig. 2D–F).

The specimens with the acronym MPC are deposited in the Micropaleontology Collection, Department of Geology and Paleontology, National Museum of Nature and Science, Japan. Other paratypes and extracted DNA are kept at the Center for Molecular Biodiversity Research, National Museum of Nature and Science, Japan.

_Type locality:_ Sta. SJ1 (41°59′N, 138°21′E) in the Sea of Japan.

_Description:_ Malacoma spherical, large, consisting of two or rarely three central capsules (Figs. 2A, 2D, 3A), phaeodium, and spherical calymma. Central capsule globular or sometimes distorted, and deep brown in color. Phaeodium consisting of aggregated small brown granules, concentrated in center of malacoma, and generally bigger than central capsule. Scleroma composed of spherical veil and several hundred radial spines. Spherical veil comprised of very thin, tube-like, and cylindrical shaped tangential needles forming a hollow sphere. Radial spines long, cylindrical and distally tapered; 1/8 to 1/12 exposed out from spherical veil. Distal end of radial spine forming distal crown, having three or four terminal branches each with four to six peripheral teeth and sometimes one apical tooth on distal end. Peripheral tooth conical, directed obliquely outward and surrounding end of spathilla (Figs. 2C, 2F, 3C, 4B–F).

_Systematic remarks:_ This new species is characterized by the radial spines possessing several obliquely outward-directed peripheral teeth on the spathilla of the terminal branch, and by the absence of a dentate terminal branch. Peripheral teeth never bend back like a fishhook. Among the 32 previously described *Aulographis* species, similar morphological characters to the new species are recognized in the following six species: *A. flammabunda* Haeckel, 1887, *A. hexancistra* Haeckel, 1887, *A. mohri* Tibbs, 1976, *A. pentastyla* Haecker, 1908, *A. tetranicistra* Haeckel, 1887 and *A. triama* Haeckel, 1887. *Aulographis japonica* sp. nov. is easily distinguished from *A. flamma-
bunda by having pronounced branched distal crowns and from *A. triama* which has irregularly-ramified spathilla. This new species differs from *A. hexancistra* and *A. mohri* which have a dome-shaped tip on the spathilla, and from both *A. pentastyla* and *A. tetrancistra*, which have spiny spathilla but no conical teeth. The morphological characters distinguishing it from the remaining three species are listed in Table 2 and Fig. 5.

**Etymology:** Derived from the distribution area of this new species.

**Molecular phylogenetic analysis**

The phylogenetic tree constructed with the two *Aulographis japonica* sp. nov. specimens and nine phaeodarian species registered in the NCBI database (Fig. 6) showed that the species analyzed were dividable into two groups, i.e. the species (*Coelodendrum ramosissimum* and *Conchellium capsula*) belonging to the order Phaeoconchia and the other eight species of the other orders. The latter group was subdividable into four clades, one of which solely contained *A. japonica*.

Fig. 2. Paratype specimens (MPC-03710: A–C, MPC-03711: D–F) of *Aulographis japonica* sp. nov. A, D: Whole cell. B, E: Radial spines with terminal branches. C, F: Terminal branches with peripheral teeth directed outward and upward.
Vertical distribution of biomass

The sea surface temperature at Sta. SJ1 was 13.4°C. The temperature decreased with depth and became almost constant ca. 0.5°C below 400 m depth (Fig. 7A). The water temperature at Sta. SJ2 was 20.2°C at the sea surface and then decreased. The temperature below 400 m depth was nearly constant, ca. 0.5°C. The salinity at Sta. SJ1 was 33.8 at the sea surface and increased to ca. 34.0 with limited fluctuation down to 250 m depth. The salinity became almost constant, ca. 34.0, below 250 m depth. The salinity at Sta. SJ2 greatly varied from 33.9–34.5 above 250 m, while below 250 m it was almost stable at ca. 34.0. Considering the temperature of ca. 0.5°C and salinity of ca. 34.0, the water masses below 400 m at the two stations are classified as typical Japan Sea Proper Water (JSPW) (Sudo 1986).

Autographis japonica sp. nov. was found throughout the water column below 250 m depth at Sta. SJ1 and SJ2, whereas it was never found above 250 m depth. Zooplankton collected with the VMPS below 250 m at both stations consisted mainly of copepods (e.g. Neocalanus spp.), Autographis japonica, euphausiids, ostracods and amphipods. The biomass of A. japonica below 250 m was higher in shallower layers than in deeper layers at both stations (Fig. 7B, E). At Sta. SJ1, the biomass reached the maxi-
mum of ca. 40.0 mg WM m\(^{-3}\) in the 500–750 m layer and became nearly constant (2.0–4.0 mg WM m\(^{-3}\)) below 750 m (Fig. 7B). At Sta. SJ2, the maximum biomass of the species was 10.7 mg WM m\(^{-3}\), in the 500–750 m layer, and became lower ranging 0.7–3.1 mg WM m\(^{-3}\) in the deeper layers (Fig. 7E). The proportion of \textit{A. japonica} with respect to the total zooplankton biomass below 250 m ranged from 7.8–24.5% at Sta. SJ1 (Fig. 7C). In contrast, the relative proportion comprised by this species showed a different pattern at Sta. SJ2, where it was relatively low in the layers between 250 m and 2,000 m water depth, with a peak of 11.3% in 500–750 m layer. However, the relative propor-
A new phaeodarian species from the Sea of Japan

The present result of the molecular phylogenetic analysis is very mysterious because the relationships in the tree differ greatly from the morphology-based, current systematics of the Phaeodaria. For example, \textit{Protocystis xiphodon} and \textit{P. tridens} of the order Phaeogromia were closer to \textit{Aulosphaera trigonopa} of the order Phaeosphaeria than to \textit{Challengeron diodon} and \textit{Medusetta arcifera}, which belong to the same order as \textit{Protocystis} spp. In addition, \textit{Aulacantha scolymantha} of the same family Aulacanthidae as the present species was closer to \textit{Aulosphaera trigonopa} and \textit{Protocystis} spp., which belong to different orders, than to the present species. Since such a discrepancy between the present results and current systematics is very unlikely, the mysterious result is possibly attributable to some unknown problems with the genetic data used here.

Species belonging to the genus \textit{Aulographis} are distributed worldwide in the deep sea, below 200 m (Reshetnyak 1966). Reshetnyak (1966) examined the phaeodarian fauna in the Sea of Okhotsk, the Bering Sea and the Kuril–Kamchatka region, but she did not record any species referable to \textit{A. japonica}. We carefully examined plankton samples collected from 0–3,000 m depth in the western North Pacific in 2007 and stocked in Hokkaido University, but never found \textit{A. japonica} in any of these samples (our unpublished data). These facts suggest that the distribution of this species is restricted to the Sea of Japan. In addition, the present result, that \textit{A. japonica} was never collected from the layers above 250 m depth but was abundant in the 250–500 m and deeper sampling layers, suggests the species is endemic to JSPW.

Meyer (1933) examined the vertical distribution of phaeodarians in the Atlantic and classified them according to the water temperature at the sampling depths. He noted that the distribution of phaeodarians is presumably dependent on the water temperature because the species found in deep waters at low latitudes were also collected from shallower waters in high-latitudes in the North Atlantic. Considering that \textit{Aulographis} species were distributed in cold waters in the Atlantic (Meyer 1933), the distribution of

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**Fig. 5.** Schematic illustrations of terminal branches of different species belonging to the genus \textit{Aulographis}.

**Fig. 6.** Phylogenetic tree of \textit{Aulographis japonica} sp. nov. and other phaeodarian species based on SSU 18S rDNA alignments and the Maximum-likelihood (ML) method. The two specimens of \textit{A. japonica} sp. nov. are shown with the sampling stations and indicated in bold letters. The specimens from Sta. SJ3 and SJ4 were registered in DNA Data Bank of Japan (SJ3: AB820365, SJ4: AB820366). Species retrieved from NCBI shown with their accession numbers. Numbers at nodes indicate NJ (Neighbor Joining)/ML bootstrap support values (only values higher than 50 are shown).
*A. japonica*, which was collected only from a cold water mass, i.e. JSPW, may also depend on water temperature. *Aulographis japonica* showed a remarkable maximum biomass in the mesopelagic zones at the two stations. This tendency was also observed for the families Aulosphaeridae, Sagosphaeridae, Aulacanthidae and Coelodendridae in the subarctic North Pacific (Steinberg et al. 2008). Phaeodarians are presumed to feed upon detrital particles sinking from the epipelagic zone (Gowing 1986, 1989). It is possibly better for them to inhabit zones as shallow as possible in the JSPW to efficiently obtain the organic matter sinking from the upper layers, and this is probably one reason for the highest abundance having occurred in the uppermost layer of the JSPW. Phaeodarians are hypothesized to be generalist feeders (Gowing 1986, 1989). Probably because of this generalist type of feeding, the present species was able to maintain a certain level of biomass despite the increasing depth. Considering its high relative biomass, especially in deep waters, this species undoubtedly plays an important role in matter cycles in this area.

Despite its large contribution to total biomass, to our knowledge this is the first record of a phaeodarian species in the Sea of Japan. Basic information such as the distribution or the ecology of phaeodarians still remains unknown. The lack of taxonomic information on phaeodarians in the Sea of Japan is probably one reason the abundant species *A. japonica* was unknown until this study. Future studies should focus on ecological aspects such as the life cycle and regional distribution of this quantitatively important species to better understand plankton ecology in the Sea of Japan.

**Fig. 7.** Vertical distribution of temperature and salinity (A, D), biomass of *Aulographis japonica* (B, E) and proportion of this species compared to the total zooplankton biomass sampled (C, F) at Sta. SJ1 and at Sta. SJ2 in the Sea of Japan during June 2011. T: temperature; S: salinity.

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