

SHORT COMMUNICATION

***Gazelletta kashiwaensis* sp. nov. (Medusettidae, Phaeodaria, Cercozoa), Its Morphology, Phylogeny, Distribution, and Feeding Behavior**Yasuhide Nakamura^a , Rei Somiya^b, Masaru Kanda^c, Atsushi Yamaguchi^{d,e}, Akihiro Tuji^a & Rie S. Hori^f^a Department of Botany, National Museum of Nature and Science, Tsukuba 305-0005, Japan^b Faculty of Environmental & Symbiotic Sciences, Prefectural University of Kumamoto, Kumamoto, 862-8502, Japan^c Kuroshio Zikkan Center, Otsuki Town 788-0343, Japan^d Graduate School of Fisheries Sciences, Hokkaido University, Hakodate 041-8611, Japan^e Arctic Research Center, Hokkaido University, Sapporo 001-0021, Japan^f Graduate School of Science and Engineering, Ehime University, Matsuyama 790-8577, Japan**Keywords**

Kuroshio; Rhizaria; SAR; Thecofilosea; unicellular zooplankton.

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ABSTRACT

A phaeodarian morphotype, characterized by the feet surrounded with forked pedal spines with anchor-like structures, was collected in the subtropical North Pacific. Considering the morphological and phylogenetic uniqueness, this morphotype is described as *Gazelletta kashiwaensis* sp. nov. The distribution of this new species is possibly affected by the Kuroshio Current. The feeding behavior of living phaeodarians was first filmed: the present new species floated in the water column stretching “protoplasmic webs” and collected diatoms by repeating the expansion and retraction of “pseudopodium-like tentacles”.

SHELL-BEARING unicellular zooplankton support the microbial loop and have a great influence in the ocean material cycles. Phaeodarians, possessing siliceous skeleton, are a group of such unicellular zooplankton. This group is distributed in the world oceans from the surface to deep waters (Kling and Boltovskoy 1999; Takahashi 1991; Takahashi and Anderson 2000), and they are thought to play valuable roles in the marine ecosystem and silica cycle because of its high biomass (Lampitt et al. 2009; Nakamura et al. 2013; Riemann 1989; Steinberg et al. 2008). Phaeodarians are supposed to be one of the most important contributors to the biomass of Rhizaria, which occupy a high proportion of the total plankton abundance worldwide (Biard et al. 2016; de Vargas et al. 2015).

Phaeodarians had been considered to be closely related to Radiolaria (Haeckel 1887); however, it was confirmed that Phaeodaria are a monophyletic group within Thecofilosea of the phylum Cercozoa (Nakamura et al. 2015; Nikolaev et al. 2004; Polet et al. 2004; Sierra et al. 2013; Yuasa

et al. 2005). Thecofilosea were for a long time represented by only very few sequences, but their high genetic and morphological diversities are being revealed (Dumack et al. 2017a,b). Thecofiloseans have recently been clarified to be major algae grazers showing their significance in soil systems (Seppey et al. 2017), so that phaeodarians may play a similar role in marine ecosystems by grazing algae.

There is only fragmentary information on the feeding ecology of phaeodarians, despite their ecological importance. Organic particles such as fecal pellets and detritus were found inside of their central capsules (Gowing 1989; Nöthig and Gowing 1991), and phaeodarians were thought to be heterotrophic omnivores (González 1992; Gowing 1986, 1989; Gowing and Bentham 1994; Swanberg et al. 1986). Although the feeding ecology of radiolarians, formerly believed as sister group of phaeodarians due to the similar morphology, was partially clarified (Matsuoka 2006, 2007; Sugiyama et al. 2008), there is only one document on the feeding behavior of phaeodarians: some individuals floated in the water column by maintaining the

neutral buoyancy and collected living plankton, such as copepods and small salps, with their pseudopodia or skeletal projections (Swanberg et al. 1986). Large part of the distribution and species diversity is also wrapped in mystery, and the knowledge of species-level distribution has not been accumulated in the Pacific region, partly because the previous studies were mainly conducted in the Atlantic region by European researchers (Nakamura and Suzuki 2015a).

There is a paucity of information especially in the subtropical North Pacific (Nakamura and Suzuki 2015b), although this sea area is known for high species diversity affecting the local ecosystem and fisheries. The investigations targeted on phaeodarians were, therefore, conducted in the area, and some fragile phaeodarians were collected by our modified sampling method (described in Materials and Methods). The morphology of these specimens was different from that of the hitherto-known species. We also succeeded to observe the feeding behavior of this morphotype, and this is the first record of the feeding process of phaeodarians (a part of the data were presented in a conference [Hori 2015]). The information on feeding behavior and the discovery of an undescribed species in the subtropical North Pacific would be worth reporting in order to further clarify the ecology and diversity of marine protists. Consequently, this morphotype is described as a new species, and its distribution and feeding behavior are briefly discussed in this study.

MATERIALS AND METHODS

Sampling and treatment

Investigations were conducted from 2008 to 2017 at 14 stations located in the subtropical area of the western North Pacific, affected by the Kuroshio Current (Fig. S1; Table S1). Samples were obtained in different seasons at Sta. KJ1 in the Philippine Sea. Plankton were collected mainly by a plankton net with bottle-type cod end to protect samples. The plankton net was towed at relatively low speed (< 1 m/s) in order to collect undamaged phaeodarians. Immediately after each sampling, phaeodarian individuals were manually isolated under a stereomicroscope and an inverted microscope. The isolated individuals were incubated at ca. 27 °C for several hours in wells of cell culture plates, and their behavior was recorded by a video camera (Everio, JVC Kenwood, Japan) attached to the inverted microscope. The morphological characters of the specimens were also photographed with a digital camera (Nikon 1 V3, Nikon, Japan). After the observation, the specimens were individually preserved in tubes filled with ca. 1.5 ml of 99.5% ethanol and stored at -20 °C. For detailed examination of their morphology, the specimens were also observed and photographed with a Scanning Electron Microscope (SEM, JSM-6390LV with LaB6 gun, JEOL, Japan) according to the method in Nakamura et al. (2016). The 18S rDNA of four specimens (Ty03, Gaz1,

Gaz5, and Gaz6) was analyzed to confirm the phylogenetic uniqueness of this morphotype. The single-cell PCR was conducted following the protocol written in Nakamura et al. (2015), and phylogenetic trees were constructed with other cercozoan sequences obtained from NCBI database (Table S2).

RESULTS AND DISCUSSION

Gazelletta kashiwaensis sp. nov. Nakamura et Hori

urn:lsid:zoobank.org:act:0551DB74-F276-4BCA-B78B-32BA10ACE39B

Diagnosis. Dome hat-shaped and thorny, with 5–8 (often 6–7) straight feet extended from the peristome. Two proximal spines located at the basal part of each foot (Fig. S2a–c, S3b). Feet surrounded by irregularly arranged pedal spines (Fig. 1a–b, S3b). Pedal spines forked, and their distal part bear two double-layered spathillae. Each spathilla possesses four teeth, forming anchor-like structure (Fig. 1b–c, S3b). The transverse section of the distal part of foot hexagonal, with small thorns on the surface (Fig. S2e). Central capsule globular and brown to orange in color, suspended in the extracapsular zone. Phaeodium composed of small brown granules, and concentrated near the central capsule (Fig. S2f; Movie S1).

Dimensions. The whole skeleton is 910–1,372 µm in diameter (\bar{x} = 1,171, n = 6). The diameter of the dome is 82–124 µm (\bar{x} = 98, n = 7), and the length of the foot is 343–584 µm (\bar{x} = 479, n = 12).

Remarks. *Gazelletta kashiwaensis* sp. nov. is characterized by the presence of the forked pedal spine with anchor-like structure at the distal part. This genus currently contains 12 species with discriminable illustrations (Table S3) (*Gazelletta atlantica* Borgert 1901 and *G. fragilis* Borgert 1902 were later re-classified into the genera *Planktonetta* and *Nationaletta*, respectively. *Gazelletta furcata*, *G. penicillata*, *G. pectinate*, and *G. pinnata* were all described by Haeckel (1887) without illustrations, and the former two species have never been reported since the first description. These species are judged as *nomina dubia* here and excluded from Table S3). The skeleton of *G. kashiwaensis* sp. nov. is composed of an irregular honeycomb structure with numerous “chambers” (Fig. S2f, S3), which is also seen in the other members of the genus *Gazelletta*, but this new species can easily be distinguished from the other species by the form of the feet. The present new species is different from *G. hexanema*, *G. macronema*, *G. orthonema*, *G. cyrtanema*, and *G. bifurca*, because these five species have feet without pedal spines. The rest of the *Gazelletta* species possess pedal spines, but *G. studeri*, *G. schleinitzii*, *G. melusina*, and *G. pentapodium* bear pedal spines without spathilla. The spathilla of *G. trispathilla* bears six teeth, while that of *G. robusta* is a bunch of short spines. *Gazelletta dendronema* has scattered and arborescent pedal spines. The domes of these three species are much bigger (more than double in diameter)

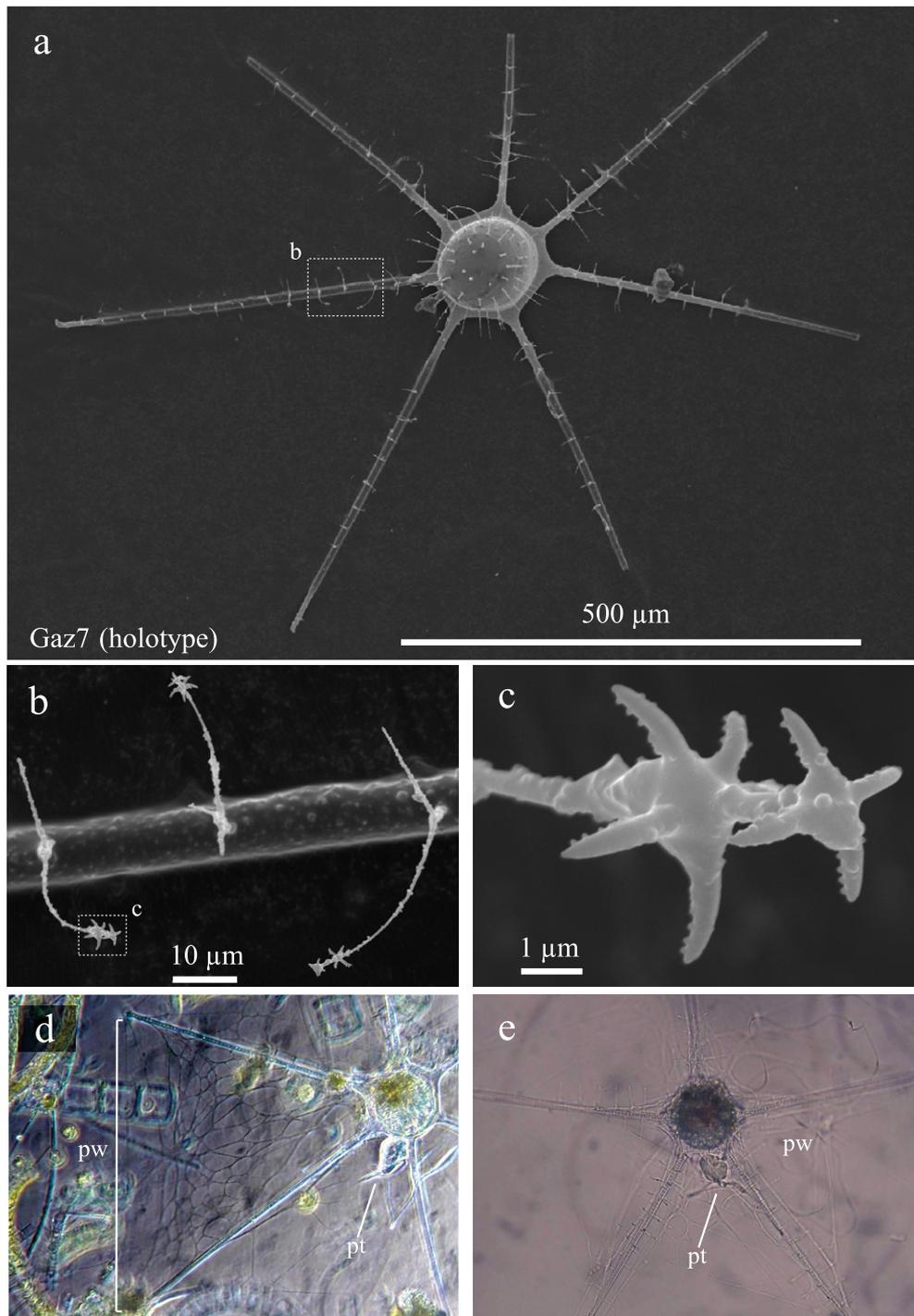


Figure 1 SEM and LM images of *Gazelletta kashiwaensis* sp. nov. (a)–(c) specimen Gaz7, holotype, MPC-33143. (a) whole cell. (b) a part of a foot. (c) anchor-like spathillae. (d)–(e) specimens Gaz13 and Gaz9, showing the protoplasmic webs (pw) and pseudopodium-like tentacles (pt).

than that of the new species, and they are distinguishable from the viewpoint of cell size. The species of the genus *Gazelletta* generally possess 6 feet, and only the present new species and *G. pentapodium* have variable number of feet (Table S3).

All the sequences of *G. kashiwaensis* sp. nov. (Ty03, Gaz1, Gaz5, and Gaz6) formed a single clade different

from the other phaeodarian species in the phylogenetic tree (Fig. S4). The 18S rDNA sequences of this new species are registered on NCBI database (Table S4).

Etymology. The specific epithet is derived from *Kashiwa-jima*, the island near which the holotype was collected.

Examined specimens. Twenty-eight individuals including 10 SEM specimens.

Type series. Holotype (MPC-33143, Gaz7): a SEM specimen (Fig. 1a–c). Paratype1 (MPC-33144, Tk2): a SEM specimen (Fig. S2a–c). Paratype2 (MPC-33145, Ta4): a SEM specimen (Fig. S2d). The type series are deposited in the Micropaleontology Collection, Department of Geology and Paleontology, the National Museum of Nature and Science, Japan. The specimen information is shown in Table S4.

Type locality. Sta. KJ1 (32°46′09″N, 132°36′00″E) located in the subtropical area of the western North Pacific (Off *Kashiwa-jima* island in Kochi prefecture, Japan).

Distribution. All specimens of *G. kashiwaensis* sp. nov. have so far been collected from two stations (Stas. KJ1 and TM2) in the subtropical area of the western North Pacific (Fig. S1; Table S1). Some large plankton distributed near these two stations are thought to be flowed by the Kuroshio Current (e.g. jellyfish; Toshino 2017), and consequently the distribution of the present new species is presumably also affected by this current. This new species was collected only during winter (November–March) at the Sta. KJ1, and this seasonality is possibly caused by the seasonal fluctuations of environmental factors in the Kuroshio Current.

Feeding behavior. During the incubation shortly after the sampling, individuals of *G. kashiwaensis* sp. nov. floated in the water column and stretched “protoplasmic webs” between the feet (Fig. S3a; Movie S1–S2). These webs would be used to change the float direction and depth, and to collect their prey. They sometime extended “pseudopodium-like tentacles” protruding from the “oral aperture” (Fig. S3a). The proximal part of the tentacles was generally swollen, and from this part, one or several long tentacles extended. The tentacles became coiled up during the extension process. After the expansion of 45–60 s, the tentacles retracted to the original position. The expansion and retraction were repeated, and some centric diatoms were occasionally drawn into the oral aperture during the retraction process. Judging from the behavior observed in this study and the information on the feeding ecology (González 1992; Swanberg et al. 1986), the phaeodarians of the genus *Gazelletta* float in the water column presumably adjusting the direction and depth by means of the protoplasmic webs. They actively catch their prey by the pseudopodium-like tentacles, and this feeding strategy with amorphous tentacles is different from those of radiolarians, which catch prey by the axial projection and terminal cone (order Nassellaria) or by contraction–extension movement of numerous axopodia (order Spumellaria) (Matsuoka 2006, 2007; Sugiyama et al. 2008). The feeding behavior of phaeodarians observed in this study could be valuable knowledge to clarify the ecological diversity of rhizarians.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Stations of plankton sampling between 2008 and 2017 in the subtropical area of the western North Pacific.

Figure S2. SEM and LM images of *Gazelletta kashiwaensis* sp. nov.

Figure S3. Schematic illustrations of *Gazelletta kashiwaensis* sp. nov.

Figure S4. Phylogenetic tree of phaeodarians based on 18S ribosomal DNA alignments and the Neighbor Joining (NJ) method.

Table S1. Detailed information on the stations, where plankton were collected in this study.

Table S2. List of the cercozoan sequences used for the phylogenetic analysis in this study.

Table S3. Comparison of morphological characteristics between the species belonging to the genus *Gazelletta*.

Table S4. Specimens of *Gazelletta kashiwaensis* sp. nov. shown in this study.

Movie S1. *Gazelletta kashiwaensis* sp. nov. (specimen Gaz13) stretching “protoplasmic webs” between the feet and extending “pseudopodium-like tentacles”. The video is four times faster than real time.

Movie S2. *Gazelletta kashiwaensis* sp. nov. (specimen Gaz9) stretching “protoplasmic webs” between the feet and extending “pseudopodium-like tentacles”. The video is four times faster than real time.