

ORIGINAL PAPER

Ecology, Morphology, Phylogeny and Taxonomic Revision of Giant Radiolarians, *Orodaria* ord. nov. (Radiolaria; Rhizaria; SAR)



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The phylogenetic affiliation of Oroscoenidae, a family of Radiolaria possessing large siliceous skeletons, was thoroughly revised in this study. Plankton sampling was conducted over seven years in seven sea areas of the Northern Hemisphere. Molecular analyses on the obtained specimens revealed that living oroscoenids are phylogenetically different from hitherto-known radiolarian orders. The detailed examinations by Microfocus X-ray Computed Tomography and Scanning Electron Microscopy clarified that oroscoenids and the related family (Thalassothamnidae) have unique skeletal characteristics different from other radiolarian orders. Judging from their phylogenetic distinctiveness and the difference in the morphology and ecology, Oroscoenidae and Thalassothamnidae should be classified in a single order distinct from other radiolarian orders, and consequently, a new order, *Orodaria*, is established.

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Introduction

Oroscoenidae [Haeckel, 1887](#) is a family of Radiolaria, belonging to the phylum Retaria of the supergroup Rhizaria (SAR). Oroscoenids have large cells wholly covered with siliceous skeletons. Although biological studies on other large rhizarians have been vigorously conducted (e.g., [Ikenoue et al. 2019](#); [Biard and Ohman 2020](#)), there are only limited numbers of studies on oroscoenids as extant plankton (e.g., [Riecke 1910](#)). Yet, oroscoenids are often detected in Cenozoic strata ([Friend and Riedel 1967](#); [Kling 1971](#); [Norris et al. 2014](#); [Palmer 1988](#), [Resig et al. 1970](#)), and these radiolarians have a potential to be used as environmental indicators connecting the present and past.

The systematic study of oroscoenids involves (1) nomenclatural and (2) phylogenetic problems, which obstruct the taxonomic arrangement of this group. (1) The family Oroscoenidae is also known as Orosphaeridae [Haeckel, 1887](#), but the validity of this family name is questionable because the type genus *Orosphaera* [Haeckel, 1882](#) is a nomen dubium. (2) The phylogenetic affiliation of oroscoenids is also controversial. Oroscoenids (the family Oroscoenidae) were initially classified under Phaeodaria ([Chediya 1959](#); [Haeckel 1887](#)), but later studies assigned them to different radiolarian orders: Spumellaria (e.g., [Riedel and Sanfilippo 1977](#)), Collodaria (e.g., [Haecker 1908](#); [Hollande and Enjumeat 1953](#); [Petrushevskaya 1984](#)), or Entactinaria ([De Wever et al. 2001](#); [Kiessling 1999](#); [Kozur and Mostler 1982](#)). A recent study incorporating sequence analysis reported that the radiolarian phylogenetic tree contains a large clade composed of oroscoenids (monophyletic), Collodaria (monophyletic) and Nassellaria (paraphyletic), and suggested that oroscoenids could be classified as an independent order ([Nakamura et al. 2020](#)). This recent study, however, analyzed only two individuals, and further study with more data is indispensable to solve the controversy concerning the phylogeny of oroscoenids. [Nakamura et al. \(2020\)](#) also suggested that the rare radiolarian family Thalassothamnidae [Haecker, 1906](#), which is classified in the same order as Oroscoenidae ([De Wever et al. 2001](#)), is possibly related to Collodaria. Nevertheless, the position of Thalassothamnidae is also an unresolved issue in the radiolarian taxonomy.

In order to collect specimens of extant oroscoenids, plankton surveys targeting on large radiolarians were conducted over seven years in seven sea areas in the Northern Hemisphere. Fossil spec-

imens were also collected from the ocean sediment. The obtained specimens were examined by Microfocus X-ray Computed Tomography (MXCT) and Scanning Electron Microscopy (SEM) to understand their morphology in detail, and the data were compared with the information extracted from almost all documents concerning oroscoenids. The phylogenetic relationship of this group was thoroughly examined, and its taxonomic affiliation was rearranged in this study.

Results

Field Sampling and Molecular Analysis (Plankton Specimens)

The investigations were conducted in a large area in the North Pacific ([Fig. 1](#)), and 154 bulk plankton samples were collected ([Supplementary Material Table S1](#)). From these samples, only 13 individuals of Oroscoenidae and 1 individual of Thalassothamnidae were obtained ([Fig. 2](#), [Supplementary Material Fig. S1](#); [Tables S1–S2](#)). Most of these individuals were found from the mesopelagic (200–1,000 m) or bathypelagic (1,000–4,000 m) zones ([Supplementary Material Table S2](#)).

The specimens Or5, Or6 and Or7 were found from the inside of salps and gelatinous zooplankton ([Supplementary Material Fig. S2a–b, d, e–f](#); [Table S2](#)), and within Or6, there was a foraminiferan cell ([Supplementary Material Fig. S2b–c](#)). No symbiont-like algae were observed inside of any oroscoenid specimens by our morphological observations and dissection under the light microscope.

The SSU (18S) rRNA sequences were obtained from 9 specimens, and long sequences from the SSU to the LSU (28S) rRNA gene (i. e., SSU - ITS1 - 5.8S - ITS2 - LSU) were successfully read in 8 specimens ([Supplementary Material Table S2](#)). The oroscoenids formed an independent clade distinct from Nassellaria and Collodaria in the phylogenetic trees inferred from the concatenated alignment of SSU and LSU (D1 and D2 regions) ([Fig. 3](#), [Supplementary Material Fig. S3](#)) and from the alignment of SSU ([Supplementary Material Fig. S4](#)). Approximately 3,000 nucleotides were obtained in 8 oroscoenid specimens, but only 1 or 2 nucleotides were different from each other (the similarities among the 8 specimens were 99.93–99.97%) ([Supplementary Material Table S3](#)). These different nucleotides were not seen in variable regions, suggesting that they come from sequencing errors. Remarkable subclades

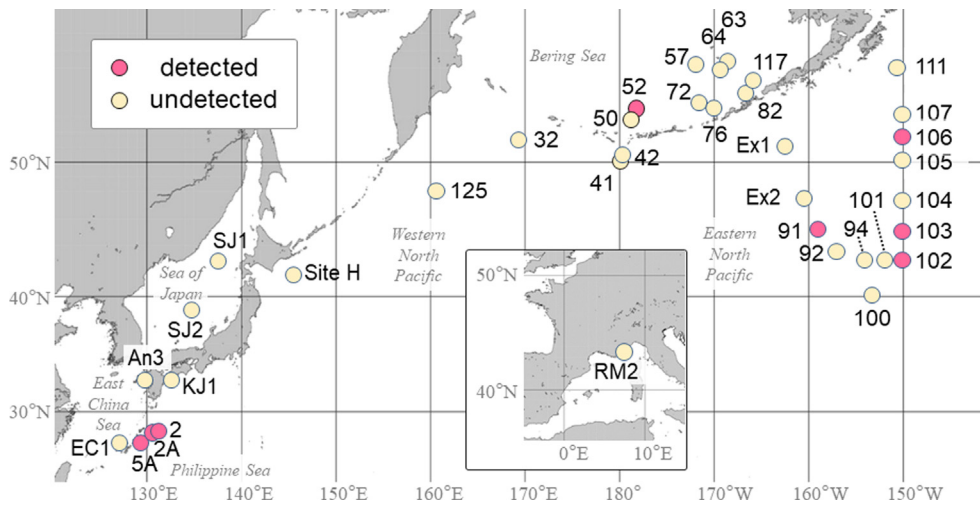


Figure 1. Plankton sampling stations of the investigations conducted in 2011–2017. The detailed information on each station is shown in [Supplementary Material Table S1](#).

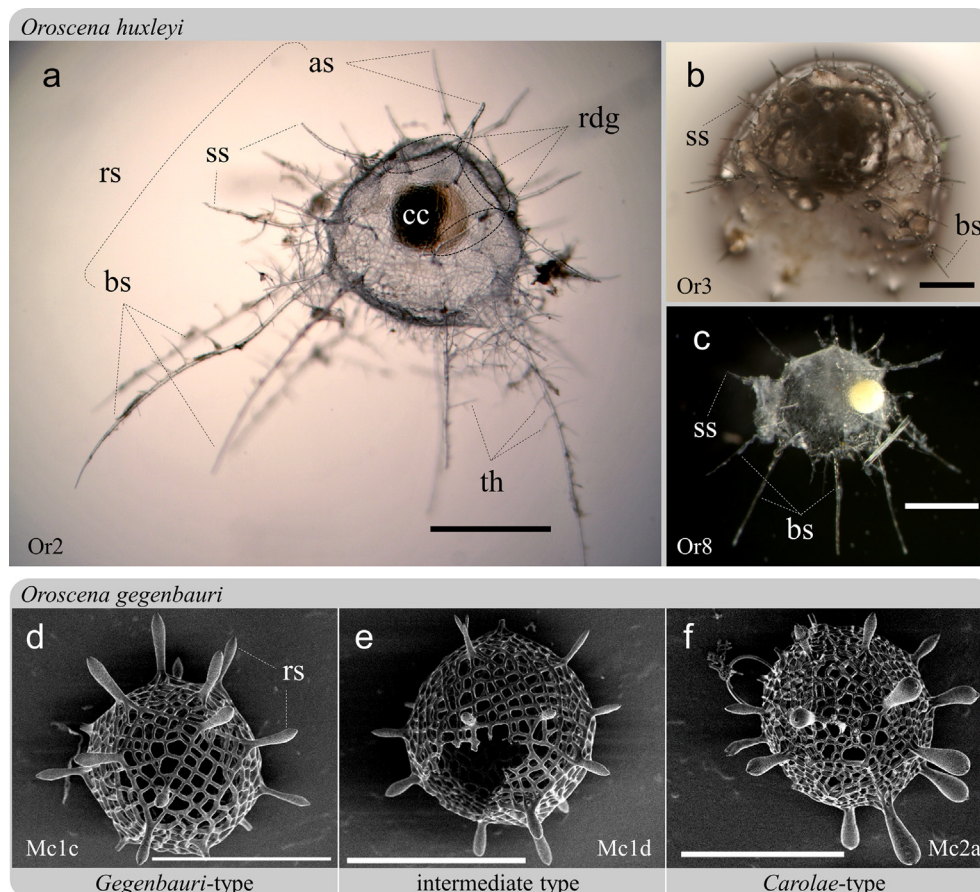


Figure 2. Images of *Orosцена huxleyi* and *Orosцена gegenbauri* (Orosценidae) analyzed in this study. rs: radial spine. as: apical spine. ss: side spine. bs: basal spine. rdg: ridge. th: thorn. cc: central capsule. The ridges with radial spines compose the primary spines, and the thorns compose the secondary spines. Three morphological categorizations are also shown for *Orosцена gegenbauri*. Note that the skeleton of Or1 is covered with the soft part (protoplasm and gelatinous substance) (b), whereas the soft part and some spines were removed by acid treatment in Or2 (a). All scale bars are 1 mm.

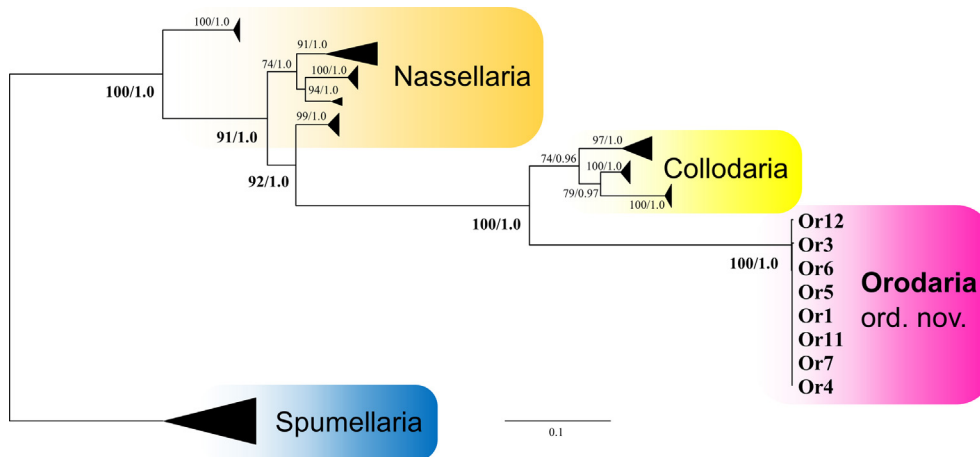


Figure 3. Maximum-likelihood (ML) phylogenetic trees of Orodaria ord. nov. and related radiolarian orders. The tree was derived from a concatenated alignment of SSU and LSU (D1, D2) rRNA. Numbers at nodes indicate ML bootstrap support values and Bayesian posterior probabilities (BPP). Only values higher than 70% (ML) and 0.7 (BPP) are shown. The uncollapsed tree is shown in [Supplementary Material Figure S3](#).

were not recognized within the large oroscenid clade both in the SSU+LSU and SSU trees (Fig. 3, [Supplementary Material Figs S3–S4](#)). We were unable to obtain the sequence data for Thalassothamnidae (*Cytocladus tricladius*, [Haecker, 1908](#): the specimen Coe7) ([Supplementary Material Fig. S1n](#)). The DNA had probably been decomposed before the sampling.

Morphological Examination (Plankton and Fossil Specimens)

The maximum diameter of Orosценidae was 6.54 mm (the specimen Ori1) (Fig. 4c–d, [Supple-](#)

[mentary Material Fig. S1m](#); Table S2), and that of Thalassothamnidae was 7.53 mm (the specimen Coe7) (Fig. 4e–f, [Supplementary Material Fig. S1n](#), Table S2).

The morphological examination with SEM and MXCT revealed the fine skeletal structure of Orosценidae. The general form of this group is apparently similar to that of the order Nassellaria, which possesses a conical shell completely covering the cell. However, the skeletal structure of orosценids is significantly different from the other radiolarian orders. The whole skeleton is generally composed of irregular polygonal structure, but there is a regu-

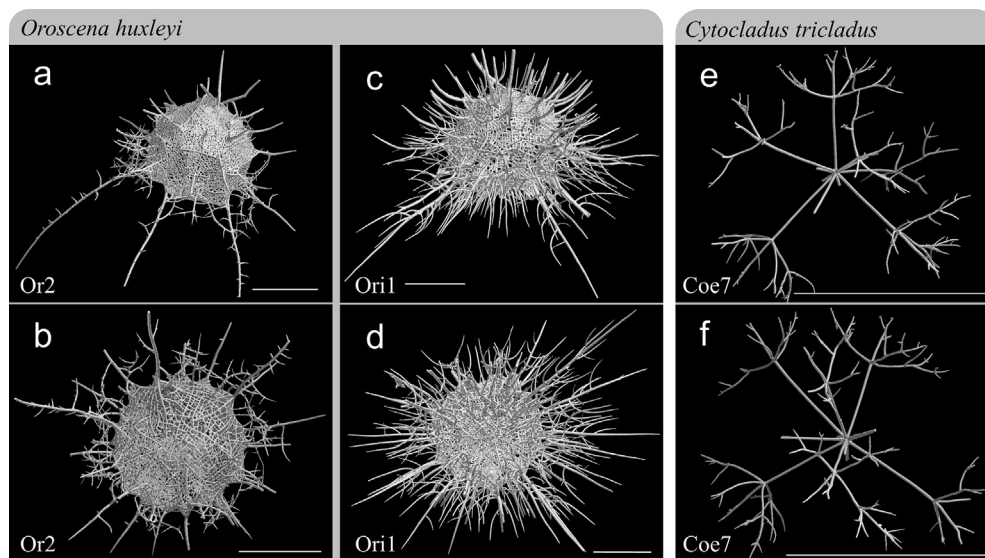


Figure 4. MXCT images of *Orosцена huxleyi* (Orosценidae) and *Cytocladus tricladius* (Thalassothamnidae). The side views (a, c, e) and ventral views (b, d, f) are shown. Scale bars are 1 mm (a–d) and 5 mm (e–f).

larity in the detailed structure: radial spines always extend from the ridges located in a regular meshwork structure (Figs 2, 4). A gelatinous substance (or possible cell membrane) covering whole skeleton was recognized in plankton specimens (Fig. 2b, Supplementary Material Fig. S1a, c, d, k). No mouth (i.e., large opening for feeding) was observed in any specimens (Fig. 4b, d).

Detailed morphological examination with SEM and MXCT revealed that all the oroscenids in the plankton specimens have the characteristics of *Orosцена huxleyi* Haeckel, 1887 (Supplementary Material Figs S1a–m, S5; Table S2). It is, nevertheless, noteworthy that the specimens Or1, Or2, Or3 and Or11 possess uncompleted external layers (Fig. 2a–b, 4b; Supplementary Material Fig. S1a, k), which are characteristics of another oroscenid species, *O. diplosphaera* Haeckel, 1887. The morphology of the specimen Ori1, which was analyzed with MXCT, generally corresponds to that of *O. huxleyi*. On the other hand, the ventral view of Ori1 (Fig. 4d) resembles the illustration of *O. arborescens* Haeckel, 1887, and their basal spines have a similar branching pattern.

Various types of spines and surface structures were observed in oroscenids from the deep-sea sediment. These oroscenids were identified as *O. gegenbauri* Haeckel, 1887 and *O. carolae* Friend & Riedel, 1967 on the basis of the general morphological characteristics (Fig. 2d–f, Supplementary Material Fig. S6; Table S4). The surface structures of the specimens Mc1b and Mc1c were, however, similar to those of *O. peruviana* Berry, 1929. Furthermore, the specimens Mc1d and Mc3a have characteristics that are intermediate between *O. gegenbauri* and *O. carolae* (Fig. 2e; Supplementary Material Fig. S6c, j).

The morphological examination also clarified that the skeleton of *C. tricladius* (Coe7) is composed of twelve spines, branching from the central junction (Fig. 4e–f). This skeletal structure has a resemblance to that of an extinct genus, *Orostaurus* Friend & Riedel, 1967. Before the soft part was removed by acid treatment, the whole skeleton of Coe7 was covered with a gelatinous substance (or possible cell membrane) (Supplementary Material Fig. S1n). The central capsule (including nuclei) of this species is not spherical but tubular, and it branches from the center along the radial spines (Supplementary Material Fig. S1n).

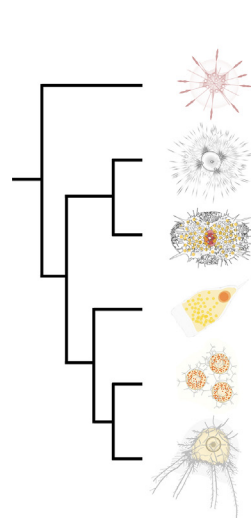
Discussion

Ecology

Despite our large effort in field sampling (Fig. 1; Supplementary Material Table S1), only 13 individuals of oroscenids were obtained (Supplementary Material Tables S1–S2), implying that their abundance is very low in the present ocean (simple occurrence probability is 0.05, since the number of total plankton samples is 154, and the number of samples in which oroscenids were detected is 8) (Supplementary Material Table S1). Fossils of oroscenids are commonly or abundantly found from the Oligocene to lower Middle Miocene in the northeastern Atlantic (Norris et al. 2014), but only a small number of survivors dwell in the deep sea of today's ocean.

Oroscenids are distributed in the mesopelagic and bathypelagic zones (Supplementary Material Table S1), and no algal symbionts have been detected so far. By contrast, collodarians are chiefly distributed in the epipelagic zone (Biard and Ohman 2020), and collodarians and nassellarians generally have symbiotic algae (Biard et al. 2015) (Fig. 5). There are, consequently, large differences between oroscenids and their well-known relatives from the ecological viewpoint.

Although the predator–prey relationships of radiolarians and phaeodarians are largely wrapped in mystery (Nakamura and Suzuki 2015a, b; Suzuki and Not 2015), a piece of information was clarified by this study. Oroscenids would be eaten by gelatinous zooplankton such as salps (Supplementary Material Fig. S2a–b, d, e–f; Table S2). Salps are generally filter-feeding plankton, and they were also reported to feed on phaeodarians (Gowing 1989; Raymont 1983). Nassellarians, a sister group of oroscenids, generally bear a shell-type skeletal structure with a mouth-like opening, inside of which the prey is digested (e.g., Matsuoka 2007). In contrast, oroscenids have no mouth-like opening (Fig. 4b, d), implying that they possibly digest the prey outside of the skeleton. The foraminiferan individual found from the inside of Or5 (Supplementary Material Fig. S2b–c) was presumably eaten by this oroscenid, but it is also possible that the foraminifer fed on the oroscenid from the inside or that they perhaps had a symbiotic relationship.



The figure shows a phylogenetic tree on the left with six main branches corresponding to the orders listed in the table. To the right of the tree are small illustrations of representative organisms from each order: Acantharia (a star-like organism with long spines), Taxopodia (a spherical organism with oar-like spines), Spumellaria (a spherical organism with a complex shell), Nassellaria (a spherical organism with a shell), Collodaria (a spherical organism with a shell or spicules), and Orodaria (a spherical organism with a shell and long spines).

order	body size* (μm)	skeleton type	main skeletal component	colony formation	symbiotic algae
Acantharia	50–1,000	shell**	SrSO_4	-	+
Taxopodia	200–800	oar-like spines	$\text{SiO}_2 \cdot n \text{H}_2\text{O}$	-	-
Spumellaria	50–2,000	shell**	$\text{SiO}_2 \cdot n \text{H}_2\text{O}$	-	+
Nassellaria	50–300	shell**	$\text{SiO}_2 \cdot n \text{H}_2\text{O}$	-	+
Collodaria	100–1.5 m*	dispersed shell/spicules or naked	$\text{SiO}_2 \cdot n \text{H}_2\text{O}$	+	+
Orodaria ordo. nov.	1,000–7,000	shell**	$\text{SiO}_2 \cdot n \text{H}_2\text{O}$	-	-

Figure 5. General characteristics of all extant radiolarian orders. The information was referred from Suzuki and Aita (2011), Suzuki and Not (2015) and Nakamura et al. (2019). The topology is based on Nakamura et al. (2020). * Length or diameter of a colony. ** Wholly-covered shell composed of numerous rods forming meshwork. +: reported in some species. -: not reported.

Morphology and Phylogeny

Rhizaria are known for gigantism: the members of xenophyophores (Foraminifera) are the largest unicellular benthos, while the largest unicellular zooplankton are Phaeodaria (e.g., Swanberg et al. 1986). Although *C. tricladius* (Coe7) examined in this study (Supplementary Material Table S2) is smaller than the largest Phaeodaria (ca. 8 cm), the present species has the largest body among Radiolaria.

The morphology of oroscenids is largely different from those of their well-known relatives, i.e., Collodaria and Nassellaria. Oroscenids have a shell-type skeleton wholly covering the cell, unlike collodarians which bear dispersed shell/spicules or possess no skeleton (Fig. 5). Most of nassellarians also have a shell-type skeleton, but their shell sizes are much smaller than those of oroscenids (Fig. 5). The colonial form, in which numerous individual cells are covered with gelatinous substance, is known in collodarians. Yet, the colonial form has never been reported for oroscenids.

Nakamura et al. (2020) suggested that oroscenids could be classified as an order different from Collodaria. The current study analyzed seven more individuals of Oroscoenidae (Supplementary Material Table S2), and it was confirmed that living oroscenids are monophyletic and sister to a robust Collodaria clade (Fig. 3; Supplementary Material Fig. S3–S4). Considering this reciprocal monophyly and the differences in the ecology and morphology, oroscenids are highly distinct from collodarians (and

from nassellarians), and Oroscoenidae should be taxonomically separated from the other two orders. One possibility is that the large clade composed of oroscoenids, collodarians and nassellarians is treated as a single highly diverse order, with oroscoenids distinguished from the others at a lower taxonomic level (e.g., family, superfamily or suborder). However, since Collodaria are currently accepted as an independent order (e.g., De Wever et al. 2001; Suzuki et al. (in press)), our view is that oroscoenids should also be classified as an independent order (Supplementary Material Table S4). The analogy between the family Thalassothamnidae (including *C. tricladius*) (Fig. 4e–f) and *Orostaurus* (Oroscoenidae) was suggested by Friend and Riedel (1967). The morphological similarity of these two group were confirmed by our morphological analyses, and therefore, we proposed that Thalassothamnidae is classified to the same order as Oroscoenidae.

Genus-level Classification

Some specimens of oroscoenids apparently have morphological characteristics of different species (Supplementary Material Table S2). Yet, the sequences of these oroscoenids were almost identical (Supplementary Material Table S3), and their phylogenetic differences could be relatively small. Judging from this point, all living oroscoenid specimens analyzed in this study would be classified into one single species, whose name should be *Oros-*

cena huxleyi [Haeckel, 1887](#) due to the Principle of Priority and the questionable existence of the name-bearing type of the genus *Orosphaera* (see also the taxonomic section below). The morphological diversity of analyzed specimens would be the intra-specific variation of *O. huxleyi*. This species may be the only extant species among oroscenids, since all the other members are reported from the sea sediment, and they have never been collected by our plankton sampling ([Supplementary Material Table S2](#)).

Morphological characteristics of *O. gegenbauri* and *O. carolae* were observed in oroscenids from the sediment. According to [Friend and Riedel \(1967\)](#), *O. gegenbauri* differs from *O. carolae* in the following three points: (1) lower spines tend to branch, (2) greater prominence of ribs and (3) more orderly arrangement of rectangular pores. These differences are, however, obscure, and specimens having intermediate characteristics of these two species were recognized in this study (Mc1d and Mc3a) ([Fig. 2e](#); [Supplementary Material Fig. S6c, j](#), [Table S2](#)). Considering the high morphological diversity in *O. huxleyi* mentioned above, the morphological variation in the fossil specimens would be an intra-specific differences, and *O. carolae* should be unified with *O. gegenbauri*. The observed morphological diversity of *O. gegenbauri* possibly comes from the difference in the ontogenetic stage, the living environment and the geologic age.

Taxonomy

Considering the results of this study, the order- and family-level classification of Oroscenidae and its related taxa is emended as follows (see also [Supplementary Material Table S4](#)):

Supergroup **Rhizaria** Cavalier-Smith, 2002, sensu emend. Cavalier-Smith, 2003

Phylum **Retaria** Cavalier-Smith, 1999, stat. Cavalier-Smith, 2002

Infraphylum **Radiolaria** Müller, 1859, sensu emend. Krabberød et al., 2017

Class **Polycystinea** Ehrenberg, 1839, stat. Levine et al., 1980

Order **Orodaria** Nakamura & Suzuki ord. nov.

Diagnosis: Skeleton composed of solid siliceous primary spines and secondary spines (thorns). Primary spines form spherical or subspherical shell and meshwork zone in some species. Gelatinous substance (or possible cell membrane) covers whole skeleton.

Etymology: “oro-” is from the type family of this order, Orosocenidae (“oro-“ comes from Greek *ορο*, hill or mountain) and “-daria” is from the suffix of “Collodaria”, the group phylogenetically related to this new order.

Type family: Orosocenidae [Haeckel, 1887](#)

Included families: Orosocenidae [Haeckel, 1887](#); Thalassothamnidae Haecker, 1906

Remarks: A total of 42 names at order level in Polycystinea have been proposed in previous papers (e.g., [Ehrenberg 1839, 1847, 1876](#); [Haeckel 1887](#); [Müller 1859](#)) but no appropriate taxon names have been proposed for grouping Orosocenidae and Thalassothamnidae (excluding Collodaria). A new order name is, therefore, proposed herein. Note that the following two taxa have similar composition: superfamily Orosphaericae [Campbell, 1954](#); suborder Orosphaeridea Van de Paverd, 1995.

Family **Orosocenidae** [Haeckel, 1887](#) sensu emend. Nakamura & Suzuki herein ([Figs 2, 4a–d](#), [Supplementary Material Fig. S1a–m](#), [S5a–i](#), [S6](#))

Synonyms: family Orosphaerida [Haeckel, 1887](#); family Orosphaeridae [Haecker, 1908](#)

Revised diagnosis: Spherical or subspherical solid skeleton composed of primary spines (ridges and radial spines) forming irregular polygonal structures with regular meshwork zones. Radial spines extend from the ridges located in meshwork zones. External layer develops in some species. Skeleton empty (no internal layer) containing central capsule. Gelatinous substance (or possible cell membrane) covers whole skeleton. No algal symbionts reported so far.

Geological range: Eocene to the present

Type genus: *Orosцена* [Haeckel, 1887](#) (type species: *Orosцена gegenbauri* [Haeckel, 1887](#), subsequently designated by [Campbell \(1954\)](#))

Included genera: *Orosцена* [Haeckel, 1887](#); *Orostaurus* [Friend & Riedel, 1967](#); *Oropelex* [Friend & Riedel, 1967](#)

Nomina dubia in this family: subfamily Oronida [Haeckel, 1887](#); *Orosphaera* [Haeckel, 1882](#); *Orodicytium* [Haeckel, 1887](#); *Orona* [Haeckel, 1887](#); *Oronium* [Haeckel, 1887](#)

Remarks: The family name, Orosphaeridae, is replaced with Orosocenidae due to the nomen dubium status for the genus name, *Orosphaera*, and the questionable existence of the name-bearing type of

this genus. *Orodapis* Friend & Riedel, 1967 was classified to the family Oroscoenidae (Orosphaeridae in the original text), but this genus has a complex internal spicular system, and no polygonal structures exist on the surface. Considering these points, *Orodapis* is excluded from the family Oroscoenidae. Note that the following three taxa have similar composition: subfamily Oroscoenida Haeckel, 1887; subfamily Orosphaerinae Campbell, 1954; subfamily Oroscoeninae Campbell, 1954.

Family **Thalassothamnidae** Haecker, 1906 sensu emend. Nakamura & Suzuki herein (Fig. 4e–f; Supplementary Material Fig. S1n, S5j)

Synonym: family Cytocladidae Schröder, 1908

Revised diagnosis: Radiolaria with single skeleton composed of branching primary spines (radial spines) extended from the central junction. Gelatinous substance (or possible cell membrane) covers whole skeleton.

Geological range: possibly Holocene to the present

Type genus: *Thalassothamnus* Haecker, 1906 (type species: *Thalassothamnus genista* Haecker, 1906, subsequently designated by Campbell (1954)).

Included genera: *Thalassothamnus* Haecker, 1906; *Cytocladus* Schröder, 1906

Nomen dubium in this family: *Conostylus* Popofsky, 1907

Remarks: The genus *Triassothamnus* Kozur and Mostler, 1981 was also included in Thalassothamnidae, and this genus was found only from Triassic strata (De Wever et al. 2001; Kozur and Mostler 1981). In contrast, *Thalassothamnus* is reported from the present. This stratigraphic gap is larger than the geologic range of Oroscoenidae, the sister group of Thalassothamnidae. The skeletal structures of *Thalassothamnus* and *Triassothamnus* are relatively simple, and it is difficult to discuss their phylogenetic relationship. Nevertheless, their large stratigraphic gap would indicate that the skeletal structure of *Triassothamnus* is analogous to that of *Thalassothamnus*, and therefore, the former should be excluded from Thalassothamnidae. This family has branching primary spines extended from the central junction, and this structure apparently resembles that of the family Astracanthidae Haecker, 1906 belonging to Phaeodaria. However, the spines of Thalassothamnidae are solid (there are no spaces inside) (Nakamura et al. 2020), unlike hollow (tubular) skeleton of Phaeodaria.

Methods

Field sampling and treatment: Plankton sampling was conducted in 2011–2017 at 37 stations located in 7 sea areas of the Northern Hemisphere (Fig. 1; Supplementary Material Table S1). Radiolarians were manually isolated from the samples under a stereomicroscope or inverted microscope (e.g., TMS, Nikon, Japan). The isolated individuals were photographed with a digital camera (e.g., Nikon 1 V3, Nikon, Japan) attached on the microscopes (Supplementary Material Fig. S1) and individually preserved in tubes filled with approximately 2.0 mL of 99.9% ethanol. The tubes containing the specimens were stored at 4 °C.

Deep-sea sediments were also observed under the light microscope in order to examine the morphology of fossil radiolarians. The sediment samples were obtained in 1978 at sta. CP1 (5,225 m in depth) located in the eastern North Pacific (Supplementary Material Table S1). The ages of the sediment samples were estimated as Lower Miocene (23.03–15.97 million years ago), but they possibly included sediments of the Eocene (56.0–33.9 million years ago). Skeletons of oroscoenids were picked up from the samples and put on stubs for the SEM observation.

Molecular analysis: The ethanol-preserved plankton specimens were dissected by a sterilized scalpel, and the “central capsule” (protoplasmic body including nuclei) of each specimen was put into 100 µL of guanidine-containing extraction buffer (GITC buffer, Decelle et al. 2012). The DNA extraction, amplification and sequencing were performed following the protocol shown in Nakamura et al. (2015). A total of 7 primers were used, including 4 oroscoenid-specific primers newly designed in this study (Table S5). Polymerase Chain Reactions (PCR) were performed with a thermal cycler (Veriti, Applied Biosystems, U.S.A.) using the following protocol: The initial denaturation at 95 °C for 10 min, 45 cycles at 95 °C for 20 sec, 52 °C for 15 sec (SSU) or 53 °C (LSU) for 15 sec, and 72 °C for 70 sec (SSU) or 72 °C for 80 sec (LSU), with a final extension at 72 °C for 2 min. The amplified PCR products were purified using AMPure XP Kit (Beckman Coulter, U.S.A.). The sequencing was conducted with ABI PRISM 3130xl Genetic Analyzer (ABI, U.S.A.).

The obtained sequences were assembled using ChromasPro (TechneLysium Pty Ltd, Australia), and the alignments were manually checked. The SSU and LSU (D1, D2) rRNA sequences of other radiolarians were also retrieved from the NCBI database (Supplementary Material Table S6). Some SSU sequences which were too short (less than 1,500 nucleotides) or low quality were removed from the alignments since they may decrease the accuracy of the analysis. The SSU and LSU rRNA sequences were individually aligned using MEGA 7 (Tamura et al. 2011), and then, the alignments were concatenated using SeaView v.4 (Gouy et al. 2010). Although the concatenated alignment consisted of 2,305 nucleotides and 74 sequences (Supplementary Material Table S6), ambiguously aligned positions were removed, and the final data set containing 2,129 nucleotides was used for constructing the phylogenetic trees. The GTR + G (general time reversible + gamma) model was selected using jModelTest (Tanabe 2011), and Maximum-likelihood (ML) analysis with 1,000 bootstrap replicates (Felsenstein 1985) was carried out using RAxML (Stamatakis 2014). The GTR + G + I were selected for Bayesian analysis, and the Bayesian posterior probabilities (BPP) were calculated with MrBayes v.3.2.2. (Ronquist and Huelsenbeck 2003). The Markov chains were run for 1.0×10^7 generations, and every 100 generations were sampled. The first 1.0×10^6 generations were discarded after checking by a program Tracer v.1.6 (Rambaut and Drummond 2009). The remaining data reaching the steady state were used for building the consensus tree. The obtained phylogenetic trees were visualized using FigTree v.1.4.4 (Rambaut 2009), and Spumellaria were used as outgroup since this order is monophyletic and closely related to the three orders discussed in this study

(i.e., Nassellaria, Collodaria and Orodaria) (Nakamura et al. 2020). Additional phylogenetic trees were also constructed based on the alignments of SSU rRNA. The GTR + G + I and GTR + G models were selected for ML and neighbor-joining (NJ) methods, respectively. All positions containing gaps and missing data were eliminated. The Bootstrap values were estimated based on 1,000 pseudo-replicates.

Morphological observation and documentary survey: After the morphological observation by an inverted microscope, all the fossil specimens and a part of the plankton specimens were carefully observed with a SEM (JSM-6390LV with LaB6 gun, JEOL, Japan) to examine their skeletal morphology in detail (Supplementary Material Figs S5–S6). The conditions and parameters were the same as those in Nakamura et al. (2016).

For further understanding the three-dimensional (3D) structure, three plankton specimens were examined by the MXCT, which is a non-destructive 3D imaging technique. The specimens were analyzed by ScanXmate-DF160TSS105 (Comscantech, Japan) with a high-resolution X-ray setting (X-ray focus spot diameter of 0.8 μ m, X-ray tube voltage of 80 kV, detector array size of 1024x1024 pixel, 1800 projections /360°, 4 average/projection. The spatial resolution was ranged from 5.00–8.96 μ m/voxel). By collecting X-ray transmission images, a series of two-dimensional (2D) cross-sectional images were generated. The 2D images of each specimen were then stacked using an image analysis software (MolcerPlus, White Rabbit, Japan), and a 3D image was constructed, whose interior structure is observable from whole angles. The 3D data of MXCT analysis obtained in this study were stored in a marine biological database, BISMaL (<https://www.godac.jamstec.go.jp/bismal/j/>).

Almost all the documents reporting on oroscoenid radiolarians were thoroughly checked, and the information about this radiolarian group was extracted to comprehend their morphological and ecological characteristics.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

CRedit authorship contribution statement

Yasuhide Nakamura: Conceptualization, Investigation, Resources, Formal analysis, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Akihiro Tuji:** Formal analysis, Supervision. **Katsunori Kimoto:** Formal analysis, Methodology, Data curation. **Atsushi Yamaguchi:** Investigation, Resources. **Rie S. Hori:** Investigation, Resources. **Noritoshi Suzuki:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary Material

Supplementary Material (Figs S1–S6 and Tables S1–S6) is available for this study. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.protis.2021.125808>.

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