

## ORIGINAL ARTICLE

# Morphology, Systematics and Life Cycle of *Ozanimia fimbriatus* (Haptista: Centroplasthelida), With Notes on Evolution of Organic Skeleton in Centrohelids

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## ABSTRACT

The majority of centrohelids bear coverings that consist either of siliceous scales or organic spicules. Strains carrying organic spicules appear in all major clades of scale-bearing centrohelids. Sometimes they represent alternative life cycle stages of scale-bearing species, whereas in other cases such strains do not alternate their morphology. Studying this “siliceous-organic” polymorphism is instrumental to understanding centrohelid diversity and evolution. The genus *Chlamydatester* has unique organic coverings that are formed with a mucous sheath and seemingly lack skeletal elements. Two centrohelid strains matching the description of *Chlamydatester fimbriatus* were isolated in clonal cultures and studied with the use of light and electron microscopy. The mucous sheath was shown to be covered with short, crisscrossed spicules. One of the strains formed cysts with siliceous spine scales, whereas the other did not encyst. SSU rDNA-based molecular phylogenetics placed the strains into the scale-bearing genus *Ozanimia*, distant from the type species of *Chlamydatester*. The new combination *Ozanimia fimbriatus* (Penard 1904) Drachko was established. The implications of *Chlamydatester* non-monophyly and the role of the “siliceous-organic” polymorphism in encystment were discussed.

## 1 | Introduction

Centrohelids (Centroplasthelida Febvre-Chevalier and Febvre 1984) are a monophyletic group of amoeboid axopodial protists closely related to haptophytes (Burki et al. 2016). Centrohelid cells usually bear an external skeleton consisting of separate elements that can be either organic or siliceous. Siliceous elements, traditionally referred to as “scales” (Dürschmidt and Patterson 1987a), often have a complex shape and ultrastructure, whereas organic elements, referred to as “spicules”, have a uniform spindle-shaped appearance. Most centrohelids carry

scales and thus are usually described and identified using scale morphology (Zlatogursky and Klimov 2016).

Initially, centrohelids with organic coverings were considered to represent the ancestral character state (Mikrjukov 2002). However, molecular phylogenetic studies have revealed that at least some of these species occupy positions inside families and genera of centrohelids that usually bear scales (Cavalier-Smith and von der Heyden 2007; Zlatogursky 2016). This was explained as a result of regressive evolution from complex skeletal elements to simple organic ones that apparently occurs

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independently in different centrohelid taxa (Zlatogursky 2016). Soon after, it was demonstrated that some centrohelids are capable of switching between organic and siliceous coverings within a clonal culture (Zlatogursky et al. 2018). Yet another interpretation suggested that centrohelids may display organic spicules in only one stage within the life-cycle, and every centrohelid with organic spicules is actually able to transform into a scale-bearing stage (Drachko et al. 2020; Gerasimova et al. 2023). Contrary to this, observations of long-term cultures of several spicule-bearing *Acanthocystis* strains (Zlatogursky 2016) as well as a spicule-bearing strain of *Raphidocystis contractilis* (Wan et al. 2023) did not reveal any morphological polymorphism. This contradiction remains unresolved. Meanwhile, the accumulation of phylogenetic data shows that spicule-bearing centrohelids are incredibly widespread, appearing within the majority of families and family-level clades of the centrohelid phylogenetic tree (Shishkin-Skarð et al. 2022; Zagumyonnyi et al. 2021, 2022). Switching between different types of siliceous coverings has also been documented. Although only one species of centrohelids, *Raphidiophrys heterophryioidea*, has been shown to vary between different types of siliceous coverings in trophic cells (Drachko et al. 2020), the formation of diverse cyst coverings is more widespread. Some centrohelids, such as *Raphidiophrys elongata* (Drachko et al. 2021) and *Raineriophrys erinaceoides* (Zlatogursky 2013) employ modified plate-scales that form thick and robust cyst walls. Some produce complex structures, such as the monolythic cyst of *Khitsovia mutabilis* that has an emergence pore (Zagumyonnyi et al. 2022) or the mosaic cyst wall of *Raphidiophrys heterophryioidea* (Drachko et al. 2020). Overall, the biodiversity of centrohelids is complex and entangled; in order to correctly describe and explain it, prolonged observation of centrohelids in cultures is crucial.

The genus *Chlamyaster* Rainer 1968 has unique coverings that do not consist of siliceous scales and organic spicules like those in other centrohelids. Instead, the *Chlamyaster* cells are surrounded by a thick mucous sheath. Three species of *Chlamyaster* were described: the type species *C. sterni* (Penard 1904), Rainer 1968 with a smooth sheath; *C. laciniatus* (Penard 1904), Rainer 1968 with a fimbriated sheath and numerous axopodia; and *C. fimbriatus* Dürschmidt et Patterson, 1987, which has a fimbriated sheath similar to *C. laciniatus* but is smaller and has significantly fewer axopodia. Like those in the spicule-bearing centrohelids, *Chlamyaster* was initially interpreted as an ancestral lineage of centrohelids (Mikrjukov 2002), but a subsequent molecular phylogeny (Cavalier-Smith and von der Heyden 2007) placed *C. sterni* deep within Pterocystidae A, a major scale-bearing clade, which suggests that the peculiar mucous sheath was a secondary acquisition. Yet little else is known about the three species. The morphology of their coverings was studied exclusively by means of light microscopy, that is, there are no SEM studies, and in a TEM study of *C. fimbriatus* (Dürschmidt and Patterson 1987b) the coverings are not shown. The phylogenetic position of *C. laciniatus* and *C. fimbriatus* is unknown and data on any kind of morphological polymorphism or encystment are missing for these species. However, the unique morphology of *Chlamyaster* invites re-examination of this long-established genus in the broader context of centrohelid diversity and evolution.

Here, we isolate from clonal cultures two strains (FA21.2 and SR21.3) matching the description of *Chlamyaster fimbriatus*,

study their morphology and culture dynamics, as well as uncover their phylogenetic placement.

## 2 | Materials and Methods

### 2.1 | Cultures and Samples

Two strains of *Ozanimia fimbriatus* were studied from freshwater samples. The sample containing strain FA21.2 was collected from the upper layer of sediments of Serdobolsky pond, St. Petersburg, Russia (N 59.995100, E 30.332200) on August 28, 2021. The sample containing strain SR21.3 was collected from the upper layer of sediments of the channel near the lake Sestroretsky Razliv, Sestroretsk, Russia (N 60.088716, E 29.955532) on November 6, 2021. Sample inoculation and establishment of clonal cultures were performed as described by Drachko et al. (2021) for *Raphidiophrys elongata*. The culture of *Neobodo* sp. was used as prey. The cultures were studied in 24-well plates containing 1 mL of culture medium per well. The number of cells per 1 mL of medium was counted manually under 100× magnification. The cultures of FA21.2 and SR21.3 were maintained from November 2021 to May 2024.

### 2.2 | Microscopy

Light and scanning electron microscopy (SEM) images were obtained as described by Drachko et al. (2021) for active cells of *Raphidiophrys elongata*.

### 2.3 | Molecular Phylogeny

SSU rDNA of individual cells was amplified with single-cell PCR as described by Zlatogursky and Klimov (2016). For strain SR21.3, two overlapping fragments of the SSU rDNA were amplified separately: for the 5' fragment, eukaryote-specific primers (forward Thx25F 5'-CATA TGCTTGCTCAAAGATTAAGCCA-3' and reverse S14R 5'-AAGTTTCAGCCTTGCGACCA-3') were used, for the 3' fragment eukaryote-specific primers (forward S12.2 5'-GATCAGATACCGTCGTAGTC-3' and reverse Helio1979R 5'-CACACTTACWAGGAYTTCCTCGTSA-3') were used (Cavalier-Smith and von der Heyden 2007; Pawlowski 2000). For strain FA21.2, only the 3' fragment was amplified with the use of S12.2 and the Helio1979R primers. PCR and purification were performed as described by Drachko et al. (2021). For SR21.3, both fragments were cloned in *Escherichia coli* JM107 strain, using the pTZ57R/T vector and the InsTAclone PCR cloning Kit (Thermo Scientific), whereas the product for FA21.2 was not cloned. Sequencing was performed on an ABI Prism 3500 xl sequencer (Applied Biosystems), using the M13F (50-GTAAAACGACGGCCAGTG-30) and M13R (50-CAGGAAACAGCTATGAC-30) primers for the 3' fragment of SR21.3, the Thx25F and S14R primers for the 5' fragment of SR21.3, S12.2 and the Helio1979R primers for the 3' fragment of FA21.3.

A multiple alignment was performed using the L-INS-I algorithm implemented in MAFFT v.7.526 (Kato and Standley 2013) for 169 centrohelid taxa and 57 outgroup taxa (Zlatogursky 2016).

Then the sequences were trimmed using -gt 0.3 -st 0.001 parameters in TrimAl version 1.4 (Capella-Gutiérrez et al. 2009). Maximum likelihood phylogenetic reconstruction was performed using RAxML v. 8.2.12 (Stamatakis 2014) with 1854 unambiguously aligned positions and the GTR substitution model. We used 25 among-site rate categories and assessed clade stability with 1000 rapid bootstrap replicates.

### 3 | Results

#### 3.1 | Culture Dynamics and Encystment

Within 2 weeks after isolation, clonal cultures of *Ozanimia fimbriatus* (both FA21.2 and SR21.3 strains) reached the maximum abundance of about 70–100 cells per 1 mL of culture medium; later, the number of cells ceased to increase. After 1.5–2 months, the abundance of prey and subsequently of trophic heliozoan cells started to decrease. At this point, the cultures of the FA21.2 strain began to produce globular cysts surrounded by a thick, robust wall. After 3 months, only cysts were found in the FA21.2 strain plate wells. When transferred to fresh culture medium, the cysts of strain FA21.2 produced trophic cells within 2 days. When left in the old culture, the cysts survived for up to 3 months. The cultures of the SR21.3 strain showed different dynamics after the 2-month mark: even though the cultures were starving, no cysts were observed. The number of trophic cells steadily decreased until only several cells remained in a culture well. These cells were 2–3 times smaller than usual (around 5 μm) and did not feed since almost no prey remained. After 5 months since establishing a clonal culture, all SR21.3 cells died, and no cysts were produced.

To compare the strains' reaction to unfavorable conditions in a controlled environment, 10 cells each from the cultures of FA21.2 and SR21.3 in the growth phase were isolated in individual wells of a 96-well plate. The wells were filled with Prescott-James medium (Prescott and James 1955) that did not contain any nutrients or prey. All FA21.2 cells encysted within 3–6 days. SR21.3 cells did not encyst; instead, they steadily decreased in size in the course of 2–3 weeks and ultimately died. Up to the very end, they preserved the general morphology of a trophic cell.

#### 3.2 | Morphology of Trophic Cells

The trophic cells of both *O. fimbriatus* strains displayed a similar morphology under the light microscope (Figure 1A,D). The cell diameter was 11–19 μm (average 14 μm;  $N=19$ ) in FA21.2 and 10–16 μm (av. 12 μm;  $N=48$ ) in SR21.3. The cell was enclosed in a mucous sheath about 2.5 μm thick. On the outside, the sheath bore one layer of thin, short attachments that crisscrossed at a sharp angle with each other. At lower magnifications, it made the cell coverings appear fimbriated, as if bearing a row of conical protrusions. The cells were often flattened against the bottom of the plate well and firmly adhered to the substratum, so that their extraction required scraping them off with a glass capillary. During 2.5 years of observations, we never observed trophic cells with siliceous or otherwise distinct coverings.

In dried cell preparations studied with the SEM (Figure 1B,C,E), the mucous sheath retained the general shape of the cell. The

sheath was covered with short spicules 2–5 μm (av. 3.5 μm;  $N=48$ ) in FA21.2 and 3–4.5 μm (av. 3.5 μm;  $N=48$ ) in SR21.3. The spicules were attached to the amorphous surface of the sheath in bundles consisting of 4–8 spicules diverging from one point, which explains their appearance as an ordered layer of crisscrossed attachments in a live cell.

#### 3.3 | Morphology of FA21.2 Cysts

Cysts were similar in size to the trophic cells, namely 10–16 μm (av. 13.3 μm;  $N=14$ ). The cyst was covered with a robust multi-layer cyst wall, which was highly refractive when viewed under the light microscope (Figure 2A). The cyst wall was covered with irregularly arranged spine-scales of varying length. Such scales were never observed in trophic cells. Unlike trophic cells, the cysts were never flattened and were not attached to the substratum.

When dried, the cyst wall retained its globular shape (Figure 2B). The cyst coverings comprised two types of siliceous scales typical for *Ozanimia* (Figure 2B–F): plate-scales and spine-scales. In addition to these siliceous scales, some cysts (Figure 2B,D) carried a small number of organic spicules remaining from a trophic cell. The plate-scales were thin, circular (diameter 0.7–3 μm; av. 1.8 μm;  $N=48$ ), lacked a distinct texture and marginal rim, and possessed a circular thickening in the center of the scale (Figure 2B,F). The edges of the scale did not roll inwards with the formation of a hollow marginal rim. The spine-scales varied in length significantly (2.1–8.7 μm, av. 5.8 μm;  $N=48$ ). The spine-scale consisted of a smooth straight shaft of uniform width (0.1–0.3 (av. 0.2) μm;  $N=45$ ) that ended in 2–4 sharp teeth. The shaft was eccentrically situated on a basal plate with a diameter of (0.1–0.3 μm; av. 0.2 μm;  $N=45$ ) (Figure 2B–E). The basal plate formed an indentation at the attachment point of the shaft and lacked a marginal rim (Figure 2D,G). In artificially broken cysts, a cross-section of their walls was visible (Figure 2C). The cyst wall consisted of 3–5 layers of plate-scales and was 0.2–0.4 μm (av. 0.25 μm;  $N=11$ ) thick.

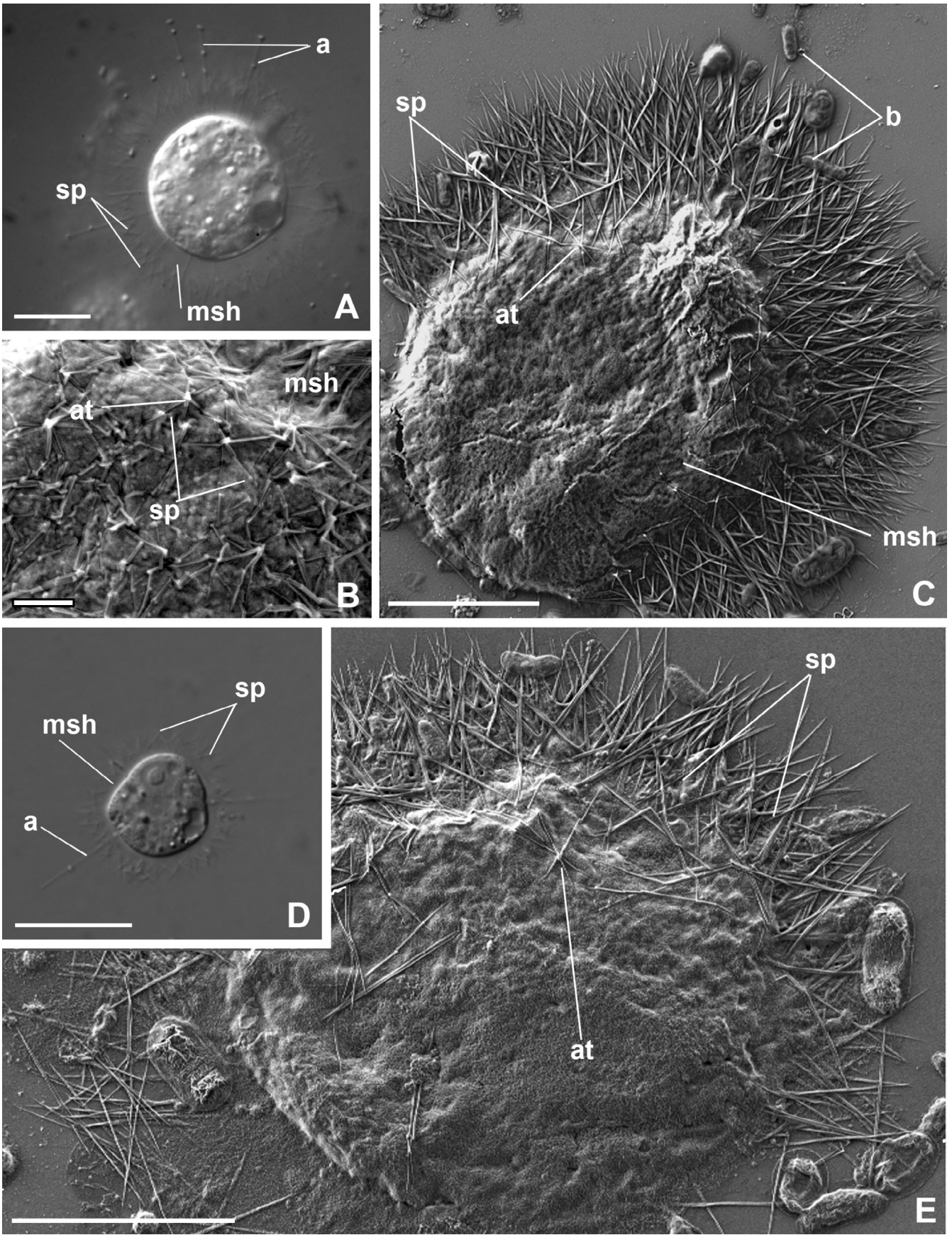
#### 3.4 | Molecular Phylogeny

For SR21.3, the full 1596-bp sequence of the SSU rDNA was obtained, and for FA21.2, a 624-bp 3' fragment of the SSU rDNA was obtained. This 624-bp fragment showed 100% nucleotide similarity between both strains. Molecular phylogenetic analysis robustly placed both strains within the genus *Ozanimia* (Figure 3).

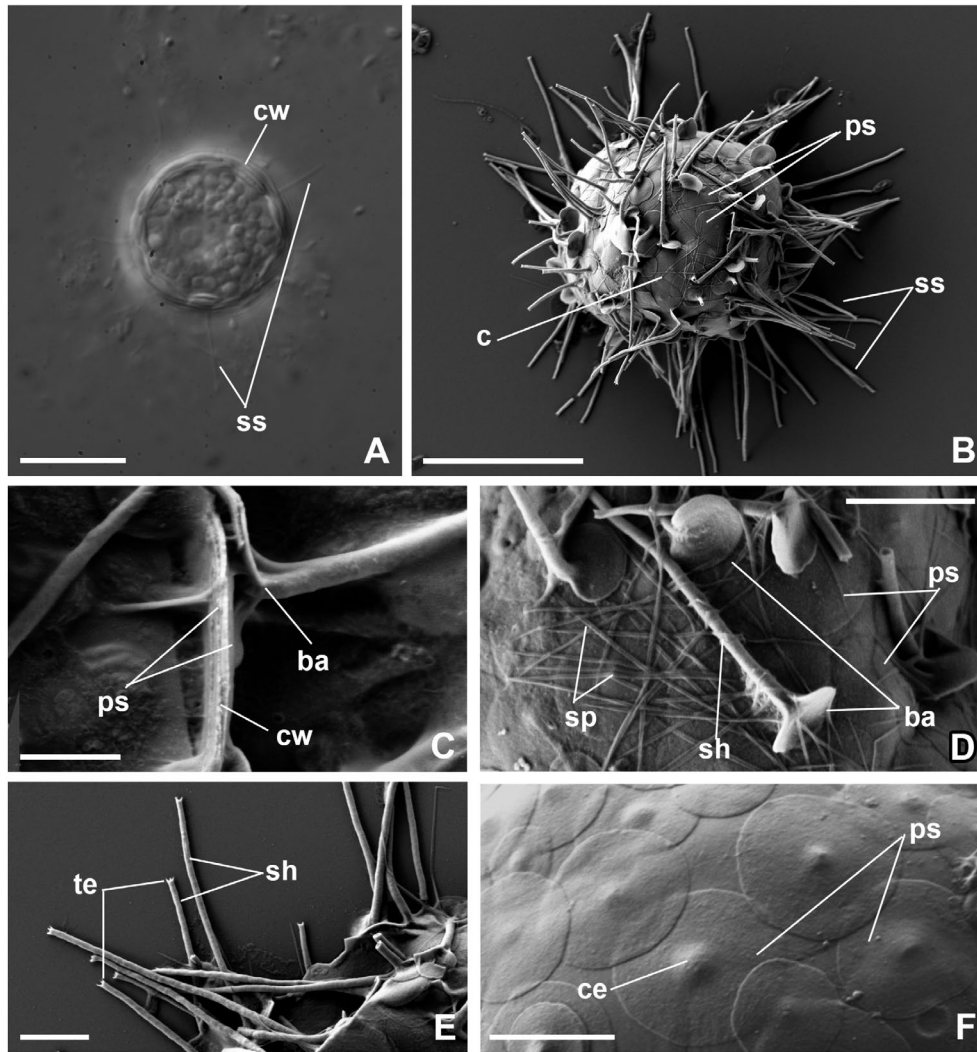
### 4 | Discussion

#### 4.1 | Taxonomy and Identification

Here, we assign both strains studied to the same species. Most centrohelid species are distinguished by the structure of the external skeleton of their trophic cells. These morphological species are then verified by comparison of molecular data; particularly, the 100% sequence similarity of 3' fragment of SSU rDNA we see in the case of FA21.2 and SR21.3 is considered sufficient evidence for the strains belonging to the same species



**FIGURE 1** | Trophic cells of *Ozanamia fimbriatus* comb. nov. Strain FA21.2 (A–C), strain SR21.3 (D, E). DIC (A, D), SEM (B, C, E). Scale bars: (A) 10  $\mu$ m, (B) 1  $\mu$ m, (C, E) 5  $\mu$ m, (D) 20  $\mu$ m. a, axopodia; at, attachment point of the spicules; b, bacteria; msh, mucous sheath; sp., spicule.



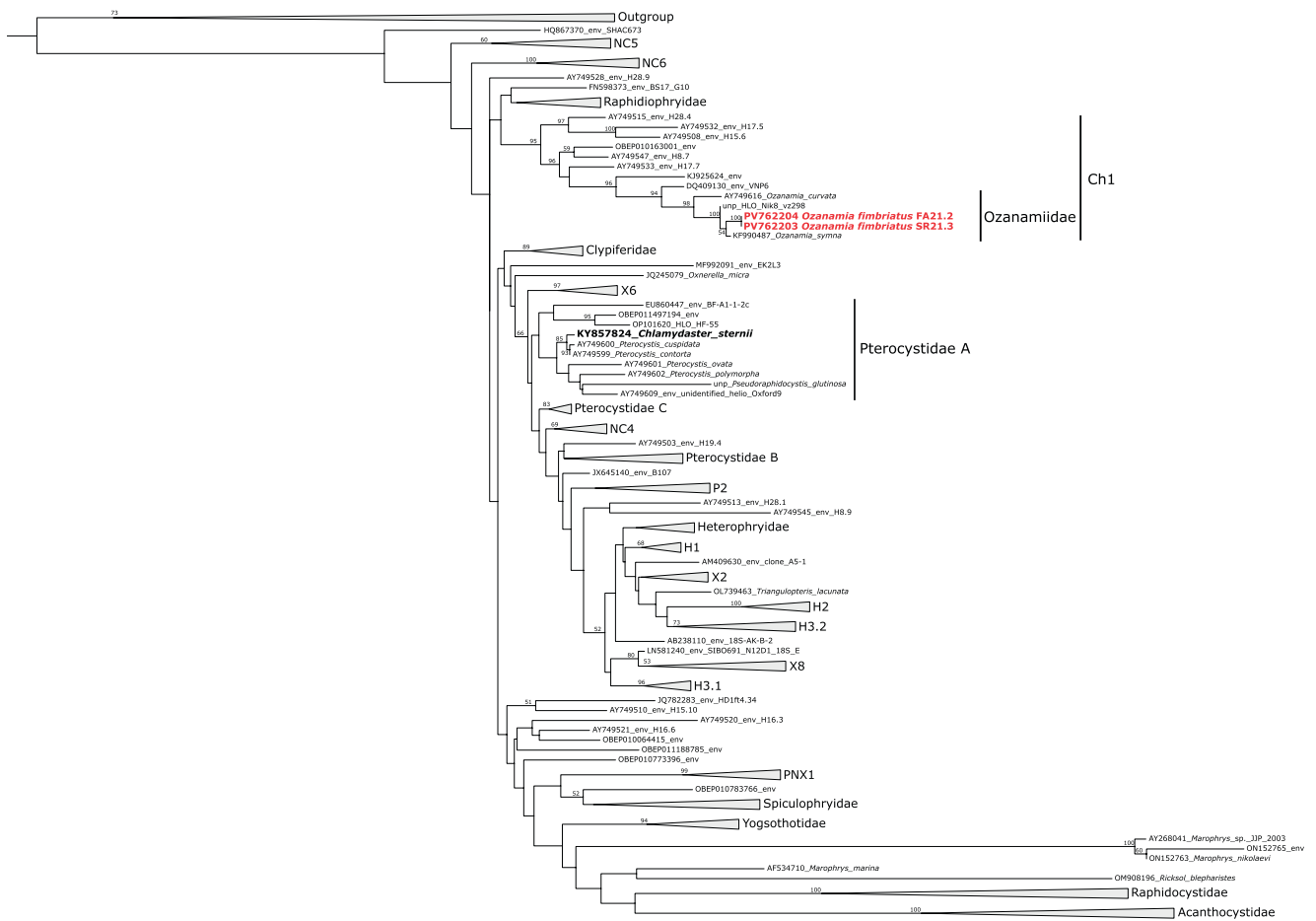
**FIGURE 2** | Cysts of *Ozanamia fimbriatus* comb. nov., strain FA21.2. DIC (A), SEM (B–F). Scale bars: (A, B) 10  $\mu\text{m}$ , (C) 1  $\mu\text{m}$ , (D–F) 2  $\mu\text{m}$ . ba, base of spine-scale; c, cyst; ce, central thickening of plate-scale; cw, cyst wall; ps, plate-scales; sh, shaft of spine-scale; sp., spicules; ss, spine-scales; te, teeth.

(Zlatogursky and Klimov 2016). Although the spicule-bearing trophic cells of FA21.2 and SR21.3 are morphologically similar, the biggest distinction between them is that one strain, unlike the other, forms cysts with siliceous coverings. However, it was demonstrated that centrohelids are able to cease forming siliceous skeletal elements within a morphological species as in the case of *Raphidocystis glabra* (Zlatogursky et al. 2018) or even within a clonal culture, as in *Raphidocystis contractilis* (Wan et al. 2023). Thus, we argue that such variations of the life cycle are not a viable criterion for discerning species of centrohelids. The morphological similarity of trophic cells and the molecular data we obtained should be enough to consider strains FA21.2 and SR21.3 the same species.

Although phylogenetic analysis firmly placed this species within the genus *Ozanamia*, its identification and naming proved difficult, since different stages of its life cycle possess characteristics of different centrohelid taxa. The morphology of the trophic cell is in accordance with the diagnosis of *Chlamyaster fimbriatus* Dürschmidt et Patterson 1987. The most distinctive feature of this diagnosis is the fimbriated, cone-covered mucous sheath

surrounding the cell. The coverings of *C. fimbriatus* have never been studied with SEM; however, our observations of a fimbriated sheath under the light microscope match perfectly to the available drawings and microphotographs (Dürschmidt and Patterson 1987b). The fact that the sheath's fringe consists of crisscrossed spicules and not some kind of conical outgrowths is difficult to assess even at 1000 $\times$  magnification, and the authors of older works might have been unable to observe its true structure. The coverings of *C. fimbriatus* and *C. laciniatus* are similar; however, these species differ in cell size [diameter ca. 30  $\mu\text{m}$  in *C. laciniatus* (Penard 1904) vs. 11–28  $\mu\text{m}$  in *C. fimbriatus* (Dürschmidt and Patterson 1987b)] and the number of axopodia unusually numerous axopodia, namely around 80, are depicted in an illustration by Penard vs. 5–15 axopodia are visible at a time when observing *C. fimbriatus* at 1000 $\times$  magnification (Dürschmidt and Patterson 1987b).

The structure of the cyst coverings in the FA21.2 strain corresponds to the general skeletal morphology of the genus *Choanocystis* Penard 1904, which is characterized by simple plate-scales and spine-scales that consist of a heart-shaped base



**FIGURE 3** | Maximum likelihood tree for SSU rDNA of 169 centrohelid sequences and 57 outgroup species (1854 sites, GTRCAT; 1000 bootstrap iterations, 25 rate classes). The strains of *Ozanamia fimbriatus* are marked in bold red. *Chlamydsterni* is marked in bold.

and eccentrically situated hollow shaft. Currently, *Choanocystis*-like centrohelids were proved to be an assemblage of at least four distant lineages: Ozanamiidae (Shishkin-Skarð et al. 2022), Meringosphaeridae (Zlatogursky et al. 2021), *Cernunnos* (Gerasimova et al. 2023) and Marophryidae (Fukuda 2014). Thus, “*Choanocystis*” is one of the most ubiquitous centrohelid morphotypes. Out of all described *Choanocystis*-like species, the spine-scales of FA21.2 cysts resemble most those of *Choanocystis mylnikovi* Gerasimova 2022 and *Choanocystis perpusilla* Petersen et Hansen, 1960 (phylogenetic position is unknown for both of these species). However, the scales of *Ch. perpusilla* are much shorter (2–4.5 µm) (Mikrjukov 2002), and the spine-scales of *Ch. mylnikovi*, whereas having a similar length range (2.0–12.9 µm), possess invariably two teeth (Gerasimova 2022), whereas in FA21.2 the number of teeth varies. The circular plate-scales of FA21.2 deviate from the ovate plate-scales of the remaining *Choanocystis*-like centrohelids.

In the strain FA21.2, only the cysts have siliceous coverings, and it is known that in many centrohelids, siliceous cyst-scales often differ from those of a trophic cell. Cyst structure has never been studied with scanning electron microscopy in *Choanocystis*-like centrohelids (and, specifically, *Ozanamia*). Thus, it is impossible to use the cyst scales as distinguishing feature.

It is a common practice to identify and describe centrohelids based on their scale-bearing form, if they have any, as

spicule-bearing cells are usually morphologically very similar (Drachko et al., 2022). However, as was stated above, we cannot reliably compare scale-bearing cysts of FA21.2 to known *Choanocystis*-like species, and the other strains of this species, such as SR21.3, may not be able to form cysts at all. Moreover, the organic coverings of the trophic cell have a unique, distinct, and complex structure. Due to these factors, we are inclined to retain the *fimbriatus* epithet, thus establishing a combination *Ozanamia fimbriatus*.

## 4.2 | Phylogenetic Placement of *O. fimbriatus* and the Ubiquity of *Chlamydsterni*

Phylogenetic analysis places the FA21.2 and SR21.3 strains within *Ozanamia*, the pterocystid *Choanocystis*-like genus situated in the Ch1 clade, which itself is sister to the family Raphidiophryidae (Figure 3). Currently, *Ozanamia* includes two more species, *O. curvata* (Cavalier-Smith and von der Heyden 2007) Shishkin-Skarð 2022 and *O. symna* (Zlatogursky 2014) Shishkin-Skarð et Zlatogursky 2022, both characterized by typical *Choanocystis*-like siliceous scales. *Ozanamia curvata* is sister to a clade formed by *O. symna* and *O. fimbriatus*.

The type species of *Chlamydsterni* is *C. sterna* (Penard 1904) Rainer, 1968, which is characterized by a smooth, non-fimbriated mucous sheath. *Chlamydsterni sterna* is placed in the

clade Pterocystidae A (Figure 3), which is phylogenetically quite distant from the Ozanamiidae [in the ranked taxonomy of centrohelids (Shishkin et al. 2018) these groups are attributed to different orders]. The clade Pterocystidae A also includes several scale-bearing *Pterocystis* species.

It appears that *Chlamydaster* is not a monophyletic, scale- and spicule-less group of centrohelids, as was presumed earlier (Mikrjukov 1996, 2002; Rainer 1968; Zlatogursky 2016). The characteristic mucous sheath of *O. fimbriatus* is intimately integrated with organic spicules, and this species is capable of producing siliceous scales in its life cycle. Given the close relationship of *C. sterni* and several *Pterocystis* species, some type of morphological polymorphism seems possible for members of this species, too. Overall, whereas *Chlamydaster*-type coverings are rare among centrohelids, they seem to have emerged at least two times independently in the evolution of this group, probably as a modification of a more traditional, spicule-based external skeleton.

All three species initially attributed to *Chlamydaster* are characterized by an unusually strong adhesion to the substratum, presumably facilitated by the mucous sheath (Dürschmidt and Patterson 1987b; Mikrjukov 2002; Rainer 1968). We noticed that *O. fimbriatus* cells are not only firmly adhered, but also flattened against the substratum, significantly deviating from the typical globular centrohelid cell shape. This matches the observations by Penard (1904) concerning *C. sterni* and *C. laciniatus*. We assume that some kind of ecological drive for increased adhesion [e.g., existing on macrophytes like the stalked centrohelid *Raphidocystis arborescens* (Mikrjukov 2002)] could have caused the convergent development of a mucous sheath in these species.

### 4.3 | Encystment in *O. fimbriatus* and the Question of “Permanently Organic” Centrohelids

Strain FA21.2 is the first member of the Ozanamiidae and the sixth centrohelid overall known to switch between fully siliceous and fully organic cell coverings in the course of its life cycle. However, this specific type of dichotomy between a spicule-bearing trophic cell and a scale-bearing cyst has not yet been observed in centrohelids. Moreover, encystment has never been reported from other spicule-bearing centrohelids even when observed in long-term cultures [three different strains of *Acanthocystis* (Zlatogursky 2016), a strain of *Raphidocystis contractilis* (Wan et al. 2023), *Marophrys nikolaevi* (Gerasimova et al. 2022)].

These observations indicate a close connection between the processes of encystment and silicification of skeletal elements in FA21.2 and probably in centrohelids in general. Cysts are crucial for survival and dispersal of heliozoa, and centrohelids tend to fortify their cyst wall with additional layers of siliceous scales (Drachko et al. 2021; Zagumyonny et al. 2022). The triggers inducing the switch between the formation of organic spicules and siliceous scales in trophic cells are unknown. It might represent an adaptation to fluctuating silica levels (Drachko et al. 2020) or to different phases of population growth (Drachko, unpublished data), however, encystment is the most expected and straightforward application of silicification process. Hence, one might

speculate that strains that, such as SR21.3, fail to encyst even under the conditions triggering encystment in a closely related strain, have truly lost the ability to silicify their skeleton.

With the accumulation of data on centrohelids, it becomes apparent that there is no simple way to explain the coexistence of strains with a “siliceous-organic” polymorphism and strains that form exclusively an organic skeleton. The two phenomena seem to be widespread and entangled; the transitions between the two often happen in rather closely related taxa, occasionally even within a single species (Wan et al. 2023). Although some authors presume that all spicule-bearing strains might have a cryptic scale-bearing stage (Gerasimova et al. 2023), we infer from the two *Ozanamia fimbriatus* strains described in this article the existence of “permanently organic” centrohelids.

## 4.4 | Taxonomic summary

**Haptista** Cavalier-Smith 2003.

**Centroplasthelida** Febvre-Chevalier et Febvre 1984.

**Pterocystida** Cavalier-Smith et von der Heyden 2007.

**Raphidista** Shishkin et Zlatogursky 2018.

**Ozanamiidae** Shishkin-Skarð 2022.

**Ozanamia** Shishkin-Skarð 2022.

***Ozanamia fimbriatus*** (Dürschmidt et Patterson 1987) Drachko comb. nov. Basionym: ***Chlamydaster fimbriatus*** Dürschmidt et Patterson 1987.

**Type SSU rDNA sequence:** GenBank accession number PV762203.

**Zoobank LSID:** urn:lsid:zoobank.org:pub:B54DD8E7-A9D9-48CF-A89A-A7CECB19EE10.

## 4.5 | Improved Diagnosis

Trophic cells are 10–19 µm in diameter. Cell body is surrounded by a thick mucous sheath carrying one layer of organic spicules. The spicules intersect at an acute angle, creating the appearance of conical outgrowths. Spicules are fusiform, 2–5 µm in length. Some strains produce cysts. Cysts are 10–16 µm in diameter, with siliceous coverings consisting of plate-scales and spine-scales. Cyst wall consists of 3–5 layers of plate-scales. Plate-scales are circular, about 2 µm in diameter, with central thickening and no hollow marginal rim. Spine scales are 2–8 µm in length, consisting of a basal plate and eccentrically situated shaft. Shaft is straight, smooth, ending in 2–4 teeth.

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### Data Availability Statement

The data that support the findings of this study are openly available in GenBank at <https://www.ncbi.nlm.nih.gov/nuccore/2993358730>.

### References

- Burki, F., M. Kaplan, D. V. Tikhonenkov, et al. 2016. “Untangling the Early Diversification of Eukaryotes: A Phylogenomic Study of the Evolutionary Origins of Centrohelida, Haptophyta and Cryptista.” *Proceedings of the Royal Society B* 283: 20152802. <https://doi.org/10.1098/rspb.2015.2802>.
- Capella-Gutiérrez, S., J. M. Silla-Martínez, and T. Gabaldón. 2009. “trimAl: A Tool for Automated Alignment Trimming in Large-Scale Phylogenetic Analyses.” *Bioinformatics* 25: 1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>.
- Cavalier-Smith, T., and S. von der Heyden. 2007. “Molecular Phylogeny, Scale Evolution and Taxonomy of Centrohelid Heliozoa.” *Molecular Phylogenetics and Evolution* 44: 1186–1203. <https://doi.org/10.1016/j.ympev.2007.04.019>.
- Drachko, D., V. Mikhailovskii, Y. Shishkin, and V. V. Zlatogursky. 2021. “Phylogenetic Position and Morphology of *Raphidiophrys elongata* sp. nov. Haptista: Centroplasthelida. With Notes on Cyst Wall Structure and Evolution.” *European Journal of Protistology* 81, no. 1: 125836. <https://doi.org/10.1016/j.ejop.2021.125836>.
- Drachko, D., Y. Shishkin, and V. V. Zlatogursky. 2020. “Phenotypic Masquerade: Polymorphism in the Life Cycle of the Centrohelid Heliozoan *Raphidiophrys heterophryioidea* (Haptista: Centroplasthelida).” *European Journal of Protistology* 73: 125686. <https://doi.org/10.1016/j.ejop.2020.125686>.
- Dürschmidt, M., and D. J. Patterson. 1987a. “On the Organization of the Heliozoa *Raphidiophrys ambigua* Penard and *R. pallida* Schulze.” *Annales Des Sciences Naturelles, Zoologie et Biologie Animale* 8: 135–155.
- Dürschmidt, M., and D. J. Patterson. 1987b. “A Light and Electron Microscopic Study of a New Species of Centroheliozoon, *Chlamydaster fimbriatus*.” *Tissue and Cell* 19: 365–376. <https://doi.org/10.1016/0040-816687.90032-2>.
- Fukuda, A. 2014. “Taxonomic Study of the Novel Heliozoa SRT127 Strain.” *Tsukuba Journal of Biology* 13: 13.
- Gerasimova, E. A. 2022. “Two New Brackish-Water Species of Centrohelid Heliozoans (Haptista: Centroplasthelida), *Choanocystis mylnikovi* sp. n. and *C. punctata* sp. n., From Russia.” *Protistology* 16: 10–20. <https://doi.org/10.21685/1680-0826-2022-16-1-2>.
- Gerasimova, E. A., Y. V. Mindolina, D. V. Tikhonenkov, et al. 2023. “Unexpected Ubiquity of Heart-Shaped Scale Morphotype in Centroplasthelida (Haptista): Ancestral Trait or Multiple Acquisitions?” *Journal of Eukaryotic Microbiology* 70, no. 6: e12992. <https://doi.org/10.1111/jeu.12992>.
- Gerasimova, E. A., L. V. Radaykina, D. G. Zagumyonni, D. V. Tikhonenkov, D. Drachko, and V. V. Zlatogursky. 2022. “Morphology and Spicules Elemental Composition of *Marophrys nikolaevi* Spec. Nov. (Haptista: Centroplasthelida).” *European Journal of Protistology* 84: 125888. <https://doi.org/10.1016/j.ejop.2022.125888>.
- Katoh, K., and D. M. Standley. 2013. “MAFFT Multiple Sequence Align Ment Software Version 7: Improvements in Performance and Us Ability.” *Molecular Biology and Evolution* 30: 772–780. <https://doi.org/10.1093/molbev/mst010>.
- Mikrjukov, K. A. 1996. “Revision of Genera and Species Composition of Lower Centroheliozoa. I. Family Heterophryidae Poche.” *Archiv für Protistenkunde* 147, no. 1: 107–113. <https://doi.org/10.1016/S0003-936596.80010-8>.
- Mikrjukov, K. A. 2002. *Centrohelid Heliozoans (Centroheliozoa)*, 1–132. KMK Scientific Presstime.
- Pawlowski, J. 2000. “Introduction to the Molecular Systematics of Foraminifera.” *Micropaleontology* 46: 1–12.
- Penard, E. 1904. *Les Héliozoaires d'eau Douce*, 341. H. Kündig. <https://doi.org/10.5962/bhl.title.1407>.
- Prescott, D. M., and T. W. James. 1955. “Culturing of *Amoeba proteus* on *Tetrahymena*.” *Experimental Cell Research* 8: 256–258. <https://doi.org/10.1016/0014-482755.90067-7>.
- Rainer, H. 1968. “Urtiere, Protozoa, Wurzelfüßler, Rhizopoda, Sonnentierchen, Heliozoa. Systematik und Taxonomie, Biologie, Verbreitung und Ökologie der Arten der Erde.” In *Die Tierwelt Deutschlands und der Angrenzenden Meeressteile nach ihren Merkmalen und Nach Ihrer Lebensweise. Teil 56*, edited by F. Dahl, M. Dahl, and F. Peus, 176. Gustav Fischer Verlag.
- Shishkin, Y., D. Drachko, V. I. Klimov, and V. V. Zlatogursky. 2018. “*Yogsothoth knorrus* gen. n., sp. n. and *Y. carteri* sp. n. (Yogsothothidae fam. n., Haptista, Centroplasthelida), with Notes on Evolution and Systematics of Centrohelids.” *Protist* 169: 682–696. <https://doi.org/10.1016/j.protis.2018.06.003>.
- Shishkin-Skarð, Y., D. Drachko, and V. V. Zlatogursky. 2022. “Shedding Light on the Origin of Acanthocystidae: *Ricksol blepharistes* gen. n., sp. n. (Ricksolidae Fam. n., Panacanthocystida, Centroplasthelida), with Notes on the Evolution of the Genera *Acanthocystis*, *Ozaniamia* gen. n. (Ozaniamiidae Fam. n.), and “*Heterophrys*-Like Organisms”.” *Organisms Diversity & Evolution* 23: 263–274. <https://doi.org/10.1007/s13127-022-00595-3>.
- Stamatakis, A. 2014. “RAxML Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies.” *Bioinformatics* 30: 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>.
- Wan, Y., M. Arikawa, A. Chihara, and T. Suzaki. 2023. “Siliceous Scales in the Centrohelid Heliozoan *Raphidocystis contractilis* Facilitate Settlement to the Substratum.” *European Journal of Protistology* 88: 125971. <https://doi.org/10.1016/j.ejop.2023.125971>.
- Zagumyonni, D. G., L. V. Radaykina, P. J. Keeling, and D. V. Tikhonenkov. 2022. “Centrohelid Heliozoans of Ukraine With a Description of a New Genus and Species (Haptista: Centroplasthelida).” *European Journal of Protistology* 86: 125916. <https://doi.org/10.1016/j.ejop.2022.125916>.
- Zagumyonni, D. G., L. V. Radaykina, and D. V. Tikhonenkov. 2021. “*Triangulopteris lacunata* gen. et sp. nov. (Centroplasthelida), a New Centrohelid Heliozoan From Soil.” *Diversity* 13, no. 12: 658. <https://doi.org/10.3390/d13120658>.
- Zlatogursky, V. V. 2013. “Puzzle-Like Cyst Wall in Centrohelid Heliozoans *Raphidiophrys heterophryioidea* and *Raineriophrys erinaeoides*.” *Acta Protozoologica* 52, no. 4: 229–236. <https://doi.org/10.4467/16890027AP.13.020.1117>.
- Zlatogursky, V. V. 2016. “There and Back Again: Parallel Evolution of Cell Coverings in Centrohelid Heliozoans.” *Protist* 167: 51–66. <https://doi.org/10.1016/j.protis.2015.12.002>.
- Zlatogursky, V. V., D. Drachko, V. I. Klimov, and Y. Shishkin. 2018. “On the Phylogenetic Position of the Genus *Raphidocystis* (Haptista: Centroplasthelida) With Notes on the Dimorphism in Centrohelid Life

Cycle.” *European Journal of Protistology* 64: 82–90. <https://doi.org/10.1016/j.ejop.2018.03.006>.

Zlatogursky, V. V., and V. I. Klimov. 2016. “Barcoding Heliozoa: Perspectives of 18S rDNA for Distinguishing Between *Acanthocystis* Species.” *Protist* 167, no. 6: 555–567. <https://doi.org/10.1016/j.protis.2016.09.004>.

Zlatogursky, V. V., Y. Shishkin, D. Drachko, and F. Burki. 2021. “The Long-Time Orphan Protist *Meringosphaera mediterranea* Lohmann, 1902 [1903] Is a Centrohelid Heliozoan.” *European Journal of Protistology* 68: e12860. <https://doi.org/10.1111/jeu.12860>.