Filamentous eukaryotic algae with a possible cladophoralean affinity from the Middle Ordovician Winneshiek Lagerstätte in Iowa, USA

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Abstract

Previous studies on the Darriwilian (Middle Ordovician) Konservat-Lagerstätte of the Winneshiek Shale in Iowa (USA) have reported various animal and trace fossils. A search for "small carbonaceous fossils" (SCFs) in palynological samples from the Winneshiek Shale has now led to the discovery of several different kinds of organic-walled microfossils. Here we report on a particular group of filamentous microfossils that occur abundantly throughout the exposed and subsurface successions of the Winneshiek Shale. The fossils are characterised by large, elongated cells (220-600 µm in length, 60-240 µm in diameter) with thin and delicate walls and occasional branching. The cells often contain dark internal bodies, most likely condensed protoplasmic remains. Together, these features identify the fossils as eukaryotic rather than cyanobacterial in origin. In particular, the cell size, cross-walls and branching pattern are shared with particular forms of benthic ulvophycean green algae, a group with a long but sporadic fossil record that is otherwise restricted to Proterozoic Lagerstätten. The new specimens therefore expand the known diversity of local primary producers in the palaeoenvironment of the Winneshiek Shale, and suggest that the apparent dearth of delicate filamentous green algae in the Phanerozoic record may be, in part, an artefact of low preservation potential combined with destructive processing techniques.

Keywords: Winneshiek Lagerstätte, Middle Ordovician, Algae, Ulvophyceae, Small Carbonaceous Fossils

1. Introduction

The Darriwilian (Whiterockian, Middle Ordovician) Winneshiek Shale near the city of Decorah in Winneshiek County, northeastern Iowa (USA) has gained attention for its exceptionally preserved fossils (Liu et al., 2006). Previous reports from this Konservat-Lagerstätte (*sensu* Seilacher, 1970) have documented the presence of arthropods, linguloid brachiopods, mollusks, conodonts, possible jawless fish, and bromalites and other trace fossils (Liu et al., 2006, 2007, 2009, Briggs et al., 2015; Lamsdell et al., 2015a, 2015b; Liu et al., 2017). The lack of many elements of typical Ordovician open marine faunas, along with sedimentological indications, have led to an interpretation of the Winneshiek Shale as representing a restricted, possibly brackish, shallow marginal-marine environment (Liu et al., 2006, 2013; Witzke et al., 2011). During the Middle Ordovician, this area was located near the equator, on the extensively flooded shelf of the palaeocontinent Laurentia (Liu et al., 2009, their fig. 4). Previous palynological investigations of the Winneshiek Shale using standard processing techniques have yielded a diversity of acritarchs and coenobial green algae, representing planktonic primary productivity (P. Zippi, 2011; unpublished report). Here we report on giant-celled, filamentous, eukaryotic algae from the Winneshiek Lagerstätte. These were isolated using a low-manipulation hydrofluoric acid technique designed for extracting SCFs ("small carbonaceous fossils"; Butterfield and Harvey, 2012), which are organic micro- and mesofossils that tend to be more fragile and larger than conventional palynomorphs (such as acritarchs or land-plant derived spores and pollen grains). The fossil record of comparable algae is rather limited, and essentially restricted to Proterozoic Lagerstätten (Butterfield et al., 1988, 1994; Butterfield, 2004; Podkovyrov, 2009). In stark contrast, similar modern-day algae are extremely abundant and wide-spread in both marine and fresh water environments. They are important as primary producers at the base of the trophic web and fulfill various other ecological roles. The new Winneshiek specimens expand the known record of delicate filamentous green algae and demonstrate the utility of gentle processing techniques for gaining a more complete picture of ancient ecosystems.

2. Geological setting

The Winneshiek Shale is a greenish brown or medium to dark grey, slightly sandy, well laminated shale unit (Liu et al., 2006; Wolter et al., 2011). It is restricted to a roughly circular area with a diameter of ~5.6 km around the city of Decorah and mostly in the subsurface (Fig. 1; Lamsdell et al., 2015a). Multiple geological features indicate that the small circular basin was formed by a meteorite impact (Liu et al., 2009; McKay et al., 2011), and this has been confirmed by the results of geophysical surveys conducted by the U.S. Geological Survey (Koontz and McKay, 2013). The only known exposure of the Winneshiek Shale is in a small section which is mostly covered by the Upper Iowa River (Liu et al., 2006). This section was excavated by geologists of the Iowa Geological Survey during the summer of 2010 by temporarily damming the river and using earth-moving equipment to dig into the riverbed, exposing a total thickness of about 4 m of the upper Winneshiek Shale. This 4 m section was sampled in detail. From two drill cores and more than 20 other wells with rock chips, the total thickness of the shale unit has been determined as 17-27 m (Wolter et al., 2011). The complete Ordovician sedimentary succession in the Decorah region was obtained from local geological data and from investigations of wells, some of which penetrated the Cambrian. The Winneshiek Shale is disconformably overlain by the St. Peter Sandstone, which is widely distributed in the Upper Mississippi Valley of USA, and overlies a local un-named unit of conglomerate, sand and shale, including thick and massive impact breccia (Liu et al., 2009; McKay et al., 2011).

3. Material and methods

The material used for the present study includes nineteen samples (prefixed WS) from the excavated 4 m section of the upper Winneshiek Shale and nine samples (prefixed H2) from a drill core near the outcrop. The specimens described and illustrated herein are reposited in the University of Iowa Paleontology Repository, Department of Earth and Environmental Sciences (labelled SUI). The rock samples (a few tens of grams each) were treated with hydrofluoric acid in a technique similar to the one employed and described by Butterfield & Harvey (2012, supplementary information), but for safety reasons, we used two rounds of diluting and decanting, before filtration with a mesh size of 51 μm. Fossils were hand-picked with a pipette from the residue in water and placed on glass cover slips for permanent palynological slides or on stubs for SEM analysis. Photographs were made with a ZEISS AxioCam MRc mounted on an Axioplan2 microscope or a ZEISS AxioCam Erc5s mounted on an Axio Imager A2. Twenty-three well-defined specimens were examined.

4. Results

Parts of giant-celled algae (Fig. 2,3) were found in most Winneshiek Shale samples from the excavated section and from the borehole H2 (Fig. 4). The cells occur isolated or connected in uniserial filaments without an envelope, sometimes with preserved branching. Chains of two distinct cells (Fig. 3(1,3,4)) and filaments with an indistinct number of cells (Fig. 3(2,7,8)) were observed. In samples where they are present, a few well-defined specimens typically co-occur with abundant, smaller fragments. The cell walls are smooth and unsculptured, very thin and flexible. They are often wrinkled, and fold easily during manipulation under water. The walls appear transparent and colourless or very light brown, indicating a low degree of thermal maturation, a finding consistent with unpublished Rock-Eval analysis of the shale and with the pale colouration of co-occurring (thin-walled) organic-walled algal microfossils and acritarchs (P. Zippi, 2011; unpublished report). They are only faintly visible in bright field microscopy. The largest fragment measures a total length of 1.25 mm (Fig. 3(7)). Individual cells have an observed length of 220-640 μm, with rectangular to near-sphaerical outlines. Cells with convex margins are more frequently observed as isolated cells (Fig. 2). Chained cells can have convex (Fig. 3(1)) or straight (Fig. 3(4)) margins, or both (Fig. 3(3)). The maximum width of the flattened cells is in the range of 90-380 μm. To attain the original diameter, we can apply a correcting factor of 2/π, assuming a perfect flattening of a cylindrical form. This gives values for original cell diameters of about 60-240 μm. The contacts between cells are often constricted relative to the middle of the cells, indicating that these parts potentially retained the original diameter during sedimentary compaction due to constraining cross walls. Cross walls are not always visible. Visible cross walls, constrictions, or contact areas in isolated cells occur in 29 specimens (e.g., Fig. 2(1-2,4,6-7),3(1,3-5)), and show a width range of 70-240 μm, consistent with the diameters calculated from maximum cell widths. This indicates an original cylindrical shape for most of the known specimens. Occasionally, individual cells may show a difference between the maximum width and the width of cell-cell contact areas larger than expected from compaction of simple cylinders, pointing to barrel-shaped cells in life (Fig. 2(7)). Two comparatively small and well-rounded specimens of isolated cells show no or at most one contact area (Fig. 2(3,5)). If this is not a preservational effect, these cells would have had a spherical or ovoid shape in life and possibly represent a single-celled stage in the life cycle (zygotes or zoospores).

Most of the specimens preserve internal bodies within the cells. These inclusions appear grainy in texture and are light to dark brown in colour, sometimes nearly opaque. Under the SEM, they are seen to be much thicker than the surrounding material and marked by a considerable, granular relief, whereas the rest of the cell appears very smooth and flat. They can have oval/ovoid, rectangular, band-like or similar shapes, often somewhat reflecting the appearance of the surrounding cell (Fig. 2,3(1-2, 8)). Their size in relation to the cell varies between specimens. The identity of similar cell inclusions preserved in organic-walled microfossils (including chert-hosted fossils) has been much discussed (see Pang et al., 2013 for a recent review). Proposed origins of the internal bodies include taphonomically condensed cytoplasmic remains, protoplasm (the entire cell content, including cytoplasm, nucleus and plasma membrane) that was biologically condensed in preparation for encystment, nuclei, mitochondria, chloroplasts, or pyrenoids, which are proteinaceous structures in chloroplasts that are involved in CO_2 fixation and often covered by starch (Oehler, 1977; Niklas & Brown, 1981; Dejax et al., 2001; Pang et al., 2013). Pang et al. (2013) argued that nuclei do not have a better preservation potential than cytoplasm, and therefore would not be expected to preserve as discrete features. In the Winneshiek specimens, an interpretation of internal bodies as cytoplasm or protoplasm is supported by the way in which their form seems to mirror to some extent the outer shape of the cell; more well-rounded cells contain an ovoid body, longer cells contain ribbon-like bodies (compare Fig. 2(1-5,7) and (6)). In contrast, pyrenoids tend to be globular and would be expected to preserve as approximately circular bodies that may occasionally co-occur with remnants of other cell contents such as cytoplasm. Since the variable forms of the internal bodies is also inconsistent with encystment, they are interpreted as post-mortem condensed cell contents. It should be noted that similar structures frequently occur in other algal microfossils and acritarchs in the Winneshiek Shale, which indicates that their preservation is not dependent on a taxon-specific biological process, but due to the taphonomy of this Lagerstätte.

The branching of the cells appears to be subapical, judging from few, incomplete specimens with branching (Fig. 3(7, 8)). In these cases, cross-walls are not conspicuous. Consequently, we cannot determine the branching position on the stem cells.

At least one specimen bears an outgrowth (now superimposed on the main filament) in the form of a tube with and opening at the distal end (Fig. 3(4,6)). The same specimen and one other also carry more rounded bulging structures that may either be external or prominent internal bodies (Fig. 3(4,5)). The tubular outgrowth appears brownish, internally granular, but outwardly smooth, and less transparent than the main cells. It could be interpreted as an early stage of branching or as an epiphyte, and also resembles antheridia (male reproductive organs) of, e.g., recent vaucheriacean algae.

5. Discussion

In general, filamentous microfossils may be produced by green, red, and brown algae, cyanobacteria, and fungi. However, cyanobacteria have considerably smaller cells than the fossils described herein. Fungal hyphae also tend to be much narrower than the Winneshiek fossils (by an order of magnitude), and we do not observe any fungal synapomorphies in our material (cf. Smith 2016; Bengtson et al. 2017). Instead, the closest comparisons are with various kinds of filamentous eukaryotic algae.

Large-celled filamentous fossils from the Svanbergfjellet Formation (Neoproterozoic, Spitsbergen) have been interpreted as the Gongrosira-phase of a vaucheriacean alga (Xanthophyceae, Ochrophyta) (Butterfield, 2004). Vaucheriacean cells occur in an envelope, as loose chains with strongly constricted connections or detached as distinct individuals. By contrast, our specimens never have an envelope, and chained cells are well connected. In addition, cross-walls occur in *Vaucheria* only in connection with sexual reproduction, whereas they are common in our material, and almost never in association with anything that could be a sexual reproductive organ – the exception being an outgrowth observed in just one of the Winneshiek specimens, which is reminiscent of the antheridia of *Vaucheria* (Fig. 3(4,6)).

The Winneshiek fossils are more closely comparable to particular groups of chlorophyte green algae. In particular, the large size and elongate shape of the Winneshiek cells, their uniserial arrangement, the lack of evidence for a mucilaginous sheath, the regular presence of cross-walls, and the occurrence of branching, are shared with certain members of the the Ulvophyceae (Chlorophyta), specifically in the orders Cladophorales (compare e.g. *Cladophora vandenhoekii* Norris and Olsen 1991) and Ulotrichales (e.g., *Acrosiphonia*). *Acrosiphonia* is common in the intertidal zone in cold to temperate regions, where its filaments form mats or tufts held together by rhizoids or hook-like branches. The Winneshiek algae lack rhizoids or hooks, but these are also not shared by all filamentous Ulotrichales. A compelling comparison is with the genus *Cladophora*, which is known as mostly benthic forms on hard substrates, and sometimes also as floating masses from modern-day freshwater, brackish and marine environments in high to low latitudes (Zulkifly et al., 2013). The Cladophorales have a limited fossil record, but have been reported from the Mesoproterozoic Lakhanda Formation of Siberia (Podkovyrov, 2009) and the Neoproterozoic Svanbergfjellet Formation on Spitsbergen (Butterfield et al., 1988, 1994). We note, however, that the extant genus *Cladophora*, as well as *Proterocladus* from the Neoproterozoic (Butterfield et al., 1994), are marked by thick cross-walls, while those in specimens from the Winneshiek Lagerstätte are very thin. *Cladophora* also has a particular branching pattern with budding at the apical end of axial cells – a feature which cannot be confirmed in our current material.

On balance, the regular presence in the Winneshiek fossils of cross-walls with minimal constriction and the lack of a sheath or of typical vaucheriacean life cycle stages, most strongly support an ulvophycean green algal affinity (Cladophorales or Ulotrichales). Furthermore, the branching pattern and absence of structures to connect filaments points to the Cladophorales rather than the Ulotrichales. Given the sparseness of comparable forms in the fossil record and the paucity of available morphological characters, any systematic assignment must remain tentative. Of course, similar sets of features may have existed in early representatives of various algal groups that are hitherto entirely undocumented. Nevertheless, the occurrence of similar fossils in Konservat-Lagerstätten from the Proterozoic, which occur in organic-rich shales deposited in shallow or marginal marine environments that are broadly comparable to the depositional environment of the Winneshiek Shale (Butterfield et al., 1988, 1994; Butterfield, 2004; Podkovyrov, 2009), is consistent with a continuous but sporadically sampled history of comparable filamentous marine benthic green algae from the Mesoproterozoic to the present day.

In analogy to modern-day species of *Cladophora*, which act as ecosystem engineers, the Winneshiek algae could have had various important effects on their ecosystem, e.g., as a food source, by increasing sedimentation rate as bafflers, as a habitat or refuge for smaller organisms, and by binding nutrients (Zulkifly et al., 2013). Interactions between the algae and the animal groups presently known from the Winneshiek Shale are currently not evident, but it can be assumed that these and similar algae were more widespread in the marginal seas during the Middle Ordovician, and an integral part of the trophic web at the time, even though they have not been observed before.

The delicate nature of such fossils seems to require rare conditions similar to the mentioned Lagerstätten, and their preservation would further be limited by the destructive effects of diagenesis and any subsequent deformation. However, as demonstrated by the wide distribution of Cambrian SCFs, similarly small organic remains are likely to be more commonly preserved than equivalent macrofossils, and need not be limited to known Konservat-Lagerstätten (Butterfield and Harvey, 2012). Since they are not usually conspicuous on the bedding plane and are easily destroyed by standard palynological maceration techniques, the lack of observations so far is undoubtedly in part an artefact of preservation, sampling, and preparation technique. Non-calcifying algae are particularly under-represented in the current fossil record in comparison to their expected original abundance. The systematic application of more gentle processing techniques therefore has the potential to fill an important gap in our knowledge of ancient ecosystems.

6. Conclusions

Besides arthropod cuticles, acritarchs and a diverse assemblage of various microscopic algal groups, the Winneshiek Lagerstätte contains abundant but cryptic remains of a distinct type of giant-celled, filamentous eukaryotic alga. The fossils most likely represent ulvophyceaen green algae and perhaps Cladophorales or Ulotrichales, which currently have a poor or non-existent fossil record, respectively. New material preserving additional features is required to elucidate the details of both morphology and affinity. The preservation of relatively large, connected fragments despite their fragility suggests the possibility that even complete thalli might be preserved in the Winneshiek Shale, and the careful examination of bedding planes, as well as acid-extracted material, may prove fruitful. More generally, we advocate the use of a gentle SCF-type processing procedure alongside traditional palynological methods to fill gaps in the fossil record of Phanerozoic ecosystems and in particular of non-calcifying green algae.

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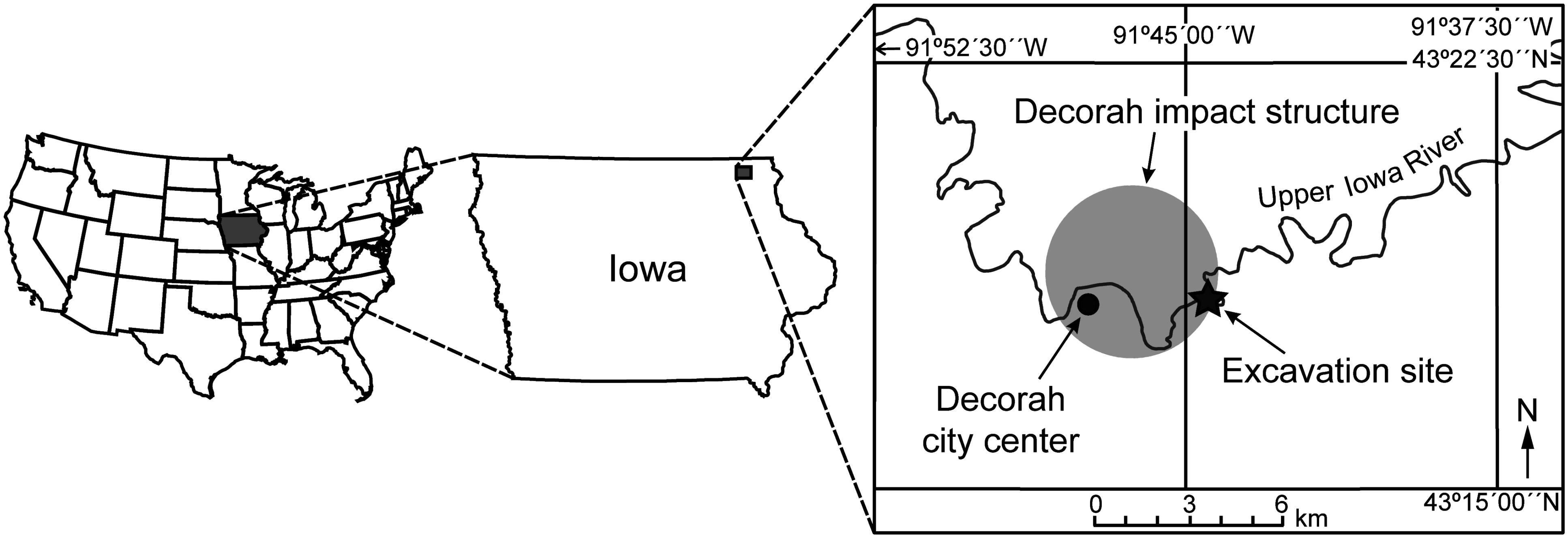
Figure captions

Fig. 1. Location map. Shows the position of the excavated outcrop of the Winneshiek Shale in the bed of the Upper Iowa river and the extent of the Decorah impact crater.

Fig. 2. Isolated giant cells of filamentous algae from the Winneshiek Shale. Scale bars = 200 μm. **1.** SUI 143683-1 (Sample H2 10.5'). **2.** Cell with rimmed contact area (lower end). SUI 143651-1 (Sample WS-6). **3.** Oval cell without evident contact area. SUI 143621-2 (Sample WS-12). **4.** Cell with darkened contact area (lower end) and bottleneck-like opposing end. SUI 143621-4 (Sample WS-12). **5.** Well-rounded cell with one possible contact area (lower end) . SUI 143591-1 (Sample WS-18). **6.** Longest observed single cell. Note straight outline and bandlike intracellular body. SUI 143621-3 (Sample WS-12). **7.** Well-rounded cell with obliquely flattened contact area, apparently preserving remnants of the wall of the neighboring cell (upper right). SUI 143651-2 (Sample WS-6).

Fig. 3. Filamentous fragments of giant-celled algae from the Winneshiek Shale. Scale bars = 200 μm. **1.** SUI 143651-3 (Sample WS-6). **2.** Ribbon-like fragment with elongate, paired internal bodies. SUI 143646-1 (Sample WS-7). **3.** Two connected cells showing both straight (lower cell) and convex (upper cell) outlines. SUI 143621-1 (Sample WS-12). **4-6.** At least two connected cells with outgrowths. SUI 143616-1 (Sample WS-13). **5.** Close-up of a ovoid outgrowth or internal body. **6.** Close-up of a tubular outgrowth bearing an opening. **7.** Largest fragment, bearing two(?) branchings. SUI 143688-2 (Sample H2 15'). **8.** Large fragment with branching. Note elongate intracellular body in the branch. SUI 143688-1 (Sample H2 15').

Fig. 4. Samples and stratigraphic distribution of giant-celled filamentous algae in the Winneshiek Shale. **A.** excavated outcrop in the Upper Iowa River. **B.** H2 drill core.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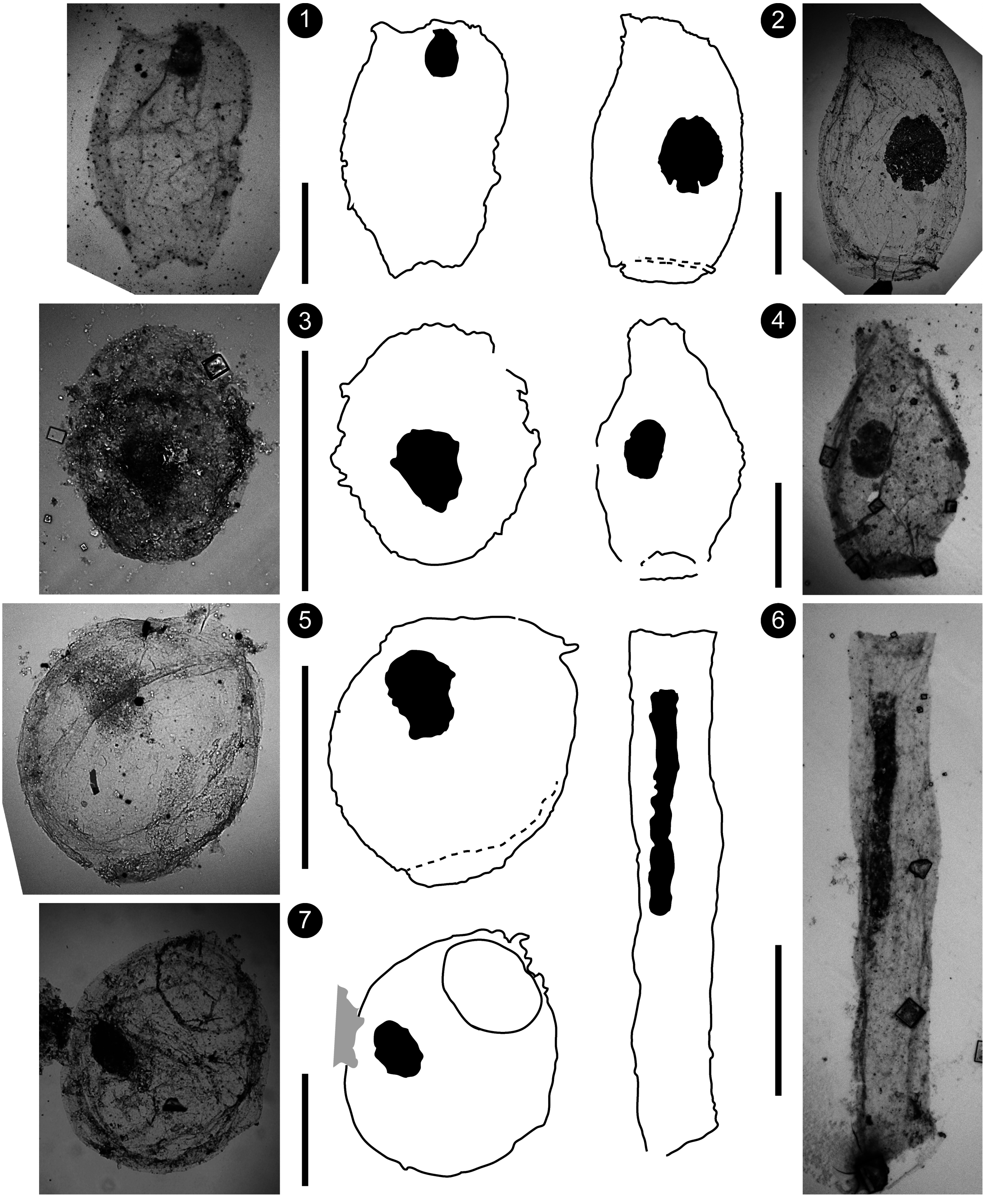

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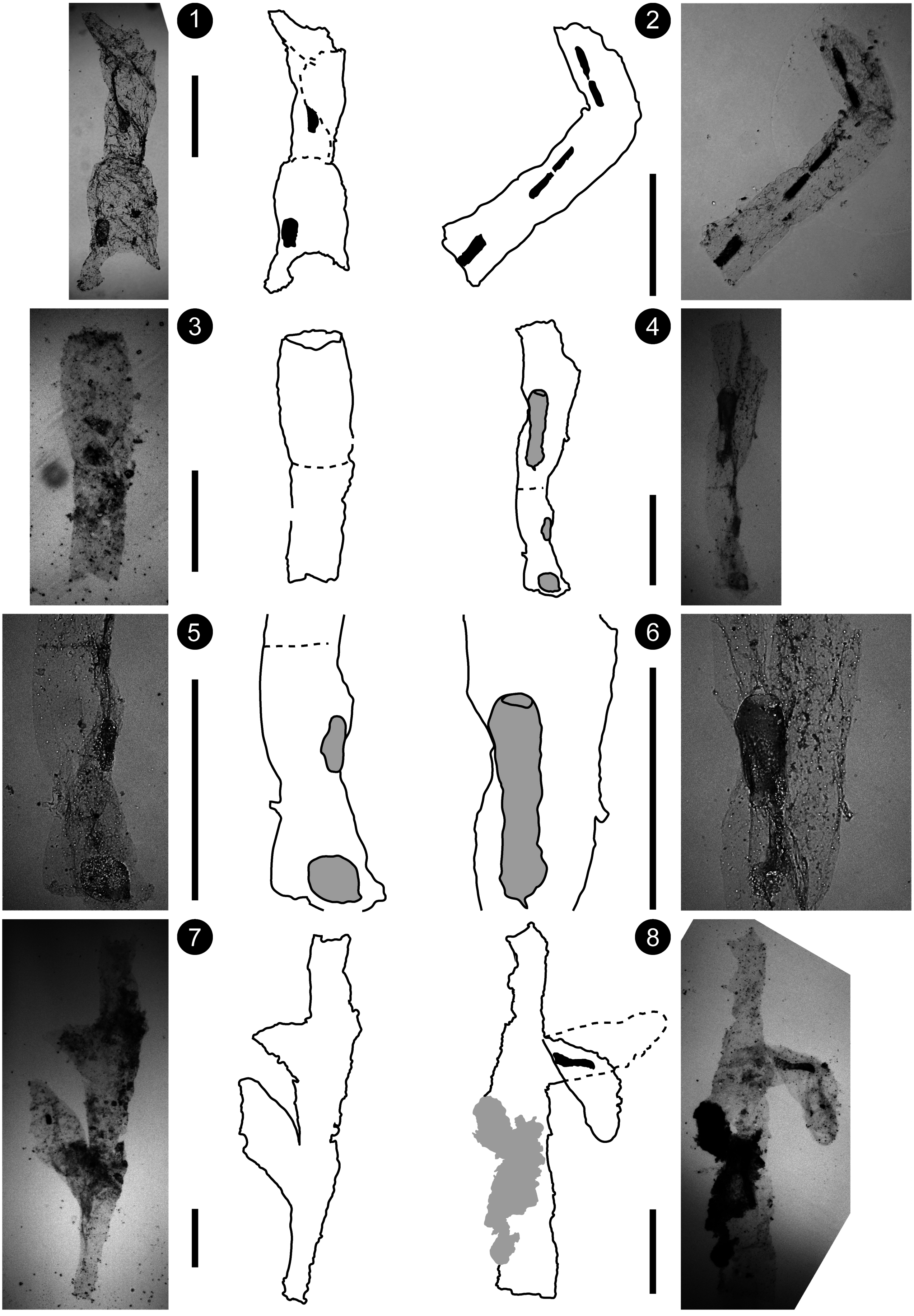
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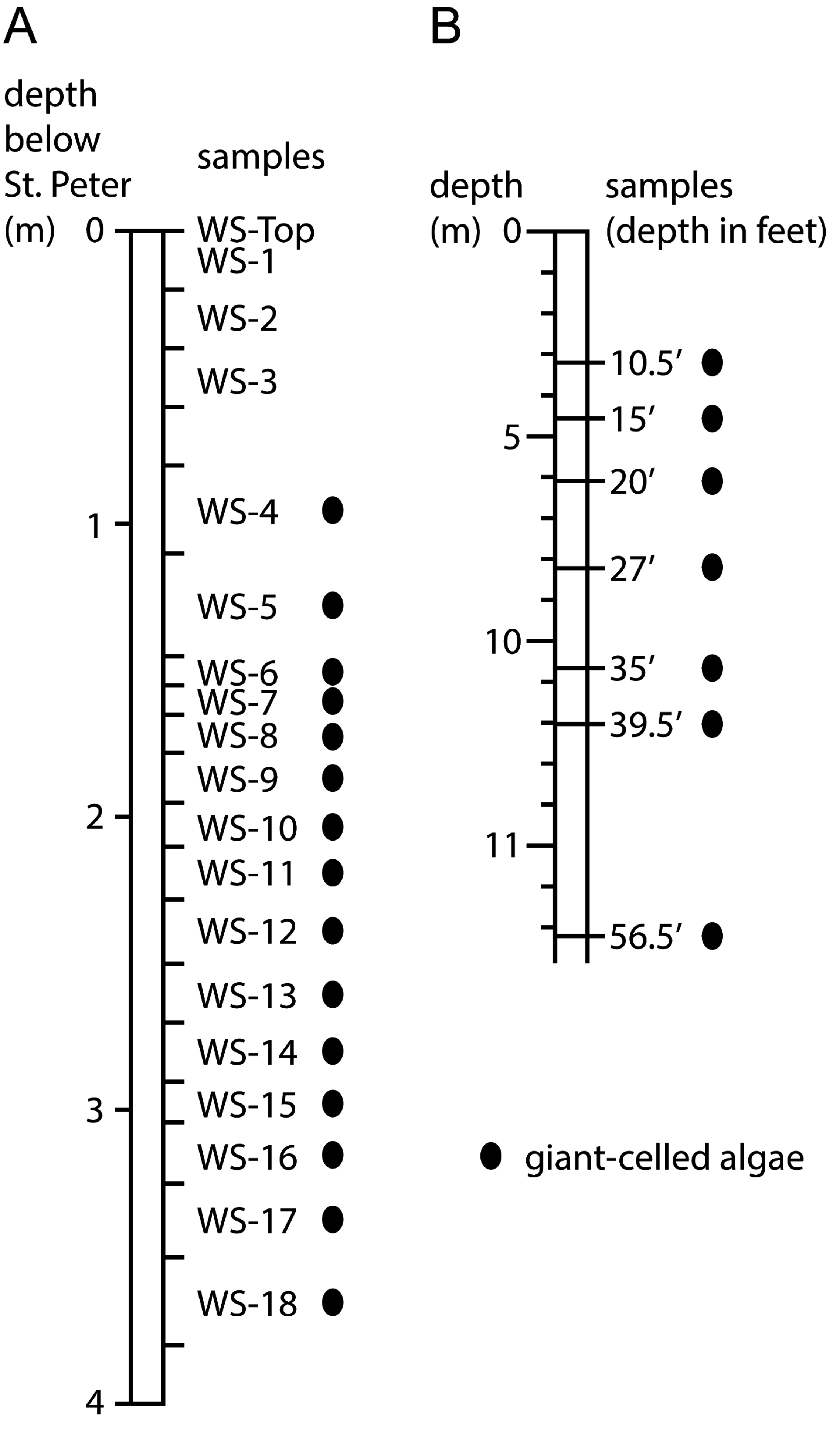
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