

SHORT COMMUNICATION

First record of the freshwater green alga *Scotinosphaera austriaca* from the Baltic Sea

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Article info

Received: 12 Aug. 2018
Revision received: 3 Oct. 2018
Accepted: 5 Oct. 2018
Published: 14 Dec. 2018

Corresponding Editor

Judita Koreiviene

Abstract. The freshwater green microalga *Scotinosphaera austriaca* has been recorded from the brackish waters of the Gulf of Gdańsk (Baltic Sea). Morphological characters and genetic data were analyzed to confirm the taxonomic affiliation. This species bears features unusual in representatives of *Ulvophyceae* present in the Baltic Sea.

Key words: *Scotinosphaera*, *Ulvophyceae*, Baltic Sea

Introduction

A typically freshwater taxon belonging to *Scotinosphaera* (*Chlorophyta*) was recorded in the brackish waters of the Gulf of Gdańsk (Baltic Sea). The complicated cell cycle of *Scotinosphaera austriaca*, characterized by significant morphological variation, can hinder its identification and may account for the rarity of its observations. An unusual feature of the species is the size of the spherical mature vegetative cell, reaching up to 200 μm in diameter. Mature cells can be seen with the naked eye, which is quite rare for green microalgae, and these settle very quickly to the bottom. This report provides morphological and molecular identification of *Scotinosphaera austriaca*, representing the first record in the Baltic Sea.

Material and methods

Green spheres (Fig. 1A) were observed in coastal surface water (salinity 6.7 PSU) samples after their exposure for a few months in a shaded place at ca. 17°C. The cells were sonicated, washed twice with distilled water and transferred onto f/2 nutrient-enriched agar plates for further purification. Newly isolated cells were transferred to sterilized liquid f/2 medium prepared from Baltic seawater of the same salinity. The resulting cultures are currently maintained under constant light and temperature conditions (50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 17°C) in the Culture Collection of Baltic Algae (CCBA, BA-178).

The growth of the species was also checked in a series of salinities (3.4, 6.7, 17.2 PSU).

Morphological observations were performed using a Nikon ECLIPSE 80i light microscope with Nomarski interference contrast, additionally equipped with a 200W Lumen200 fluorescence illuminator as a source of light and combined with G-2A green excitation filter block for epifluorescence microscopy. The successive phases in the cell cycle were observed for 5 consecutive days in a few selected cells kept on a wet mouth microscope slide sealed with wax. The location and shape of chloroplasts was determined using epi-fluorescence microscopy. Lugol's solution was used to stain deposits of starch on the outskirts of the pyrenoids. For lipid staining, Sudan III dye in 70% ethanol solution was used after pre-soaking cells in the same solvent to improve the penetration of the dye within the cell (O'Brien & McCully 1981).

The cells of the studied species were centrifuged, frozen and thawed several times and mechanically disrupted by vortexing together with glass beads in a plastic reaction tube (Eppendorf 1.5 ml). DNA was extracted using a Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland) following the manufacturer's recommendations. The V4 18S rDNA fragment was amplified using primers and following the procedure described by Zimmermann et al. (2011). The polymerase chain reaction (PCR) was performed using 2xMix PCR PLUS reaction mixture (A&A Biotechnology, Gdynia, Poland). Subsequently, Sanger sequencing was conducted by Macrogen® (Macrogen Europe, Amsterdam, the Netherlands). The obtained sequence was edited in BioEdit (Hall 1999).

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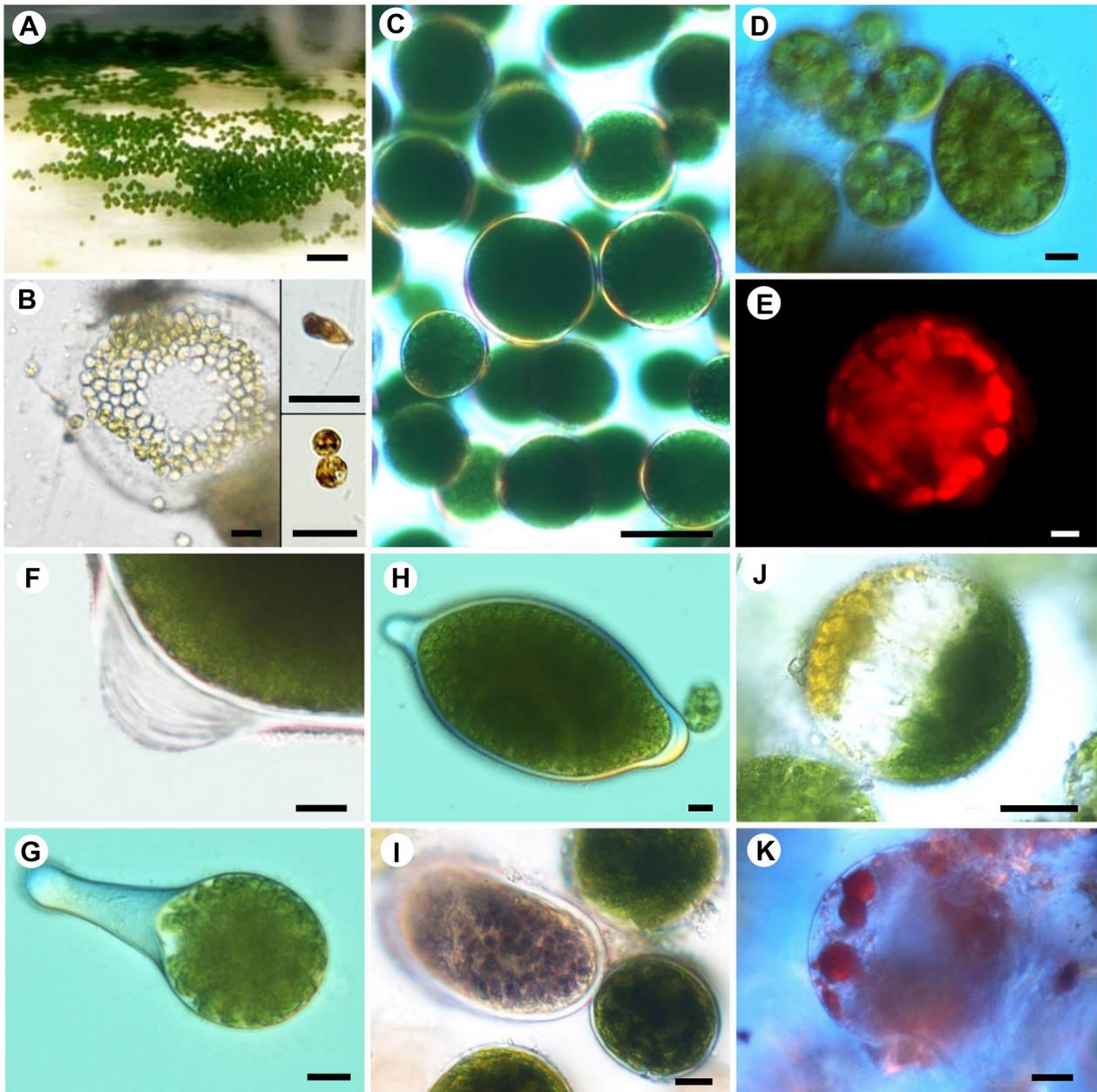


Figure 1. *Scotinosphaera austriaca*. A – adult vegetative cells; B – auto-sporangium releasing daughter cells, zoospores (top right), autospores (bottom right); C, D – globular and ovoid shaped cells; E – reticulate chloroplasts radiating to the cell peripheries; F – apically localized, lamellated thickenings of the cell wall; G, H – cell wall protuberances; I – iodine stained pyrenoids; J – oil droplets grouped on one side of the cell. K – Sudan III stained oil droplets. Scales: A = 1 mm; C, J = 100 μ m; B, D–I, K = 10 μ m.

Results and discussion

After reaching the target size (ca 200 μ m), the cell (auto-sporangium/zoosporangium) opens and releases daughter cells (autospores/zoospores) around the empty cell wall (Fig. 1B), beginning the new cell cycle (Wujek & Thompson 2005). The cell wall, built of cellulose, is fairly distinct around the cells, which mostly are globular (Fig. 1C) but may also be ellipsoidal or ovoid (Fig. 1D), ranging from 12 to 213 μ m in diameter or in length. The characters observed for *Scotinosphaera* included reticulate chloroplasts radiating to the cell peripheries (Fig. 1E) and apically localized, lamellated thickenings of the cell wall (Fig. 1F). A mature cell may possess one (Fig. 1G) or two (Fig. 1H) cell wall protuberances. Pyrenoids are regularly scattered around the cell (Fig. 1I, iodine

staining); that character is diagnostic for *S. austriaca* (Punčochářová 1992).

In addition to morphological features, the V4 subregion of the 18S rDNA sequence was used to verify the species. By running the obtained sequence (382 bp; GenBank accession number MK005260) against the GenBank database it was found to be identical to the HE860253 sequence assigned to *S. austriaca*, strain CAUP H 5304, confirming the morphological identification.

The genus *Scotinosphaera* forms a distinct and highly divergent clade within *Ulvophyceae*, warranting the establishment of a new order, *Scotinosphaerales* (Škaloud et al. 2013). Ulvophyceae green algae are best known for their macroscopic representatives, found mainly in marine coastal environments; some of them have adapted to freshwater habitats. Cocquyt et al. (2010) suggested that

the ancestral ulvophyte could be unicellular and that the multicellular macroscopic growth forms were achieved independently in various lineages. Skaloud et al. (2013) demonstrated the close relationship of *Scotinosphaerales* with other early-diverging ulvophycean orders, and proposed that non-motile unicellular freshwater organisms played an important role in the early diversification of *Ulvophyceae*. However, the fact that the *Scotinosphaera* have also been found in marine waters may potentially shed some light on the course of these processes.

Some interesting features of this species can be used in aquaculture. Green algae are strong candidates for energy-rich biomass production, as lipids can constitute up to 40% of their dry biomass. Figure 1J shows mature *S. austriaca* cells after centrifugation. The centrifugal force affected the protoplast, revealing large amounts of orange oil droplets grouped on one side of the cell. Sudan III dye was used to confirm the presence of lipids (Fig. 1K). The cell size and strong cell wall, which most often are obstacles to green algae harvesting and extraction, may in this case be quite beneficial. While small cells (up to 10 μm) are difficult to disrupt physically, large ones are very amenable to crushing and releasing their contents.

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