
The establishment of *Diplosphaera epiphytica* sp. nov. and its distinction from *Chlorella sphaerica* Tschermak-Woess

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Karbovska & Kostikov (2012: 41, fig. 1 [a-h]) proposed the new combination *Diplosphaera sphaerica* (Tschermak-Woess) Karbovska & Kostikov for *Chlorella sphaerica* Tschermak-Woess. This species was described by Tschermak-Woess (1988: 136, figs 1-3, 5) as a photobiont of the lichen *Pseudocyphellaria carpoloma* (Delise) Vainio, growing as an epiphyte on the palm tree *Rhopalostylis sapida* Wendl & Drude (*Arecaceae*) from Waweira, North Island of New Zealand. An authentic strain material of *Chlorella sphaerica* Tschermak-Woess was independently deposited by E. Tschermak-Woess on 15 December 1986 in the Culture Collection of Algae of the University of Göttingen (SAG) under the number SAG 11.88, and on 2 January 1987 in the Culture Collection of Algae of the University of Texas (UTEX) under the number UTEX 2485. The strain SAG 11.88 was later rendered axenic, whereas the strain UTEX 2485 was always subcultured without any further treatment. Friedl & O’Kelly (2002) found that SAG 11.88 belonged to the *Prasiola*-clade of the Trebouxiophyceae, seemingly closely related to *Stichococcus bacillaris* Nägeli and *Prasiola crispa* (Lighthoot) Kützing based on phylogenetic analyses of the SSU rDNA sequences. Our BLAST N search in GenBank of sequences (SSU: AJ416105 and ITS-2: KX094829), however, clearly showed that SAG 11.88 is closely related to SAG culture 49.86 *Diplosphaera* sp. (*Prasiolaceae*) with an identity of 99%. Hodač *et al.* (2016) also demonstrated, based on these sequences, that SAG 11.88 belonged to *Diplosphaera*. Later, this strain was included in the Culture Collection of Algae at the Kyiv State University (ACKU) as ACKU 533-06. Karbovska & Kostikov (2012) compared ACKU 533-06 with two strains of *Diplosphaera*, which were originated from SAG collection (ACKU 880-09 = SAG 49.86 and ACKU 879-09 = SAG 48.86) and concluded that all these strains were closely related; they then proposed the transfer of *Chlorella sphaerica* to *Diplosphaera*. They also found some morphological features, which differ to those of the original description by Tschermak-Woess (1988). However, the UTEX strain was never investigated and verified. We have compared both strains SAG 11.88 and UTEX 2485 by light microscopy (see Figs 1 and 2), and they clearly represent two separate organisms. Whilst SAG 11.88 (Fig. 2) clearly belongs to the genus *Diplosphaera*, UTEX 2485 (Fig. 1) corresponds to the original description and holotype of *Chlorella sphaerica* (Fig. 3). The morphological differences are summarized in Table 1.

Our morphological investigation of SAG 11.88 confirm the findings of Karbovska & Kostikov (2012). We did not, however, observe a pyrenoid surrounded by starch grains. By contrast, UTEX 2485 showed clear differences to SAG 11.88 but did show great similarities to the original description of *Chlorella sphaerica*. Considering all these results, the combination of *Diplosphaera sphaerica* by Karbovska & Kostikov (2012) was an incorrect taxonomic assignment because it is based on observations of a culture that did not correspond with the type material of *Chlorella sphaerica*. Accordingly, we propose to describe strain SAG 11.88 as a new species:

Feature	UTEX 2485 (Fig. 1)	SAG 11.88 (Fig. 2)	Original description (Fig. 3)
Cell shape	spherical, sometimes irregular	young cells ellipsoid, old cells spherical	spherical, sometimes ellipsoid
Cell size	3.5 - 7.5 - (9.5) μm	2.8 - 7.9 μm in diameter, 3.1 - 7.9 μm in length x 2.2 - 6.5 μm in width	3 - 9.5 μm
Chloroplast shape	parietal to band-shaped, often removed from the cell wall	cup-to band-shaped, not removed from the cell wall	parietal to band-shaped, often removed from the cell wall
Pyrenoid	present	absent	present
Starch surrounding the pyrenoid	several	absent	two to several
Satellite pyrenoid	present	absent	present
Mucilage	absent	present	absent
Type of asexual reproduction	4-8 unequal sized autospores	vegetative cell division	4-16 unequal sized autospores
Cell packages	absent	present	absent

Table 1. Comparison of cultures of *Chlorella sphaerica* Tschermak-Woess with the original description.

Diplosphaera epiphytica Darienko & Pröschold, *sp. nov.*

Description: Young cells ellipsoid, 3 - 7.9 μm in length x 2.2 - 6.5 μm in width, mature cells spherical, 2.8 - 7.9 μm in diameter, gathered into cell packages of 2-4-8 cells surrounded by mucilage. Cell packages disintegrating in old cultures. Chloroplast cup- to band-shaped without a pyrenoid. Reproduction by means of vegetative cell division.

Holotype (designated here): Fig. 2 of the present paper (ICN Art. 40.5 Melbourne Code, McNeill *et al.* 2011).

Type locality: unknown, from culture; probably from “Waweira [Hill] Scenic Reserve, New Zealand”, 17 March 1984, leg. J.K. Bartlett

Etymology: The epithet *epiphytica* indicates that *Diplosphaera* usually occurs as epiphytes on bark of trees or on lichens.

Comment: strain SAG 11.88 has not been successfully cryopreserved at SAG. This strain was probably a second alga in the original culture of *Chlorella sphaerica*, which Tschermak-Woess sent to the culture collections, but this remains uncertain.

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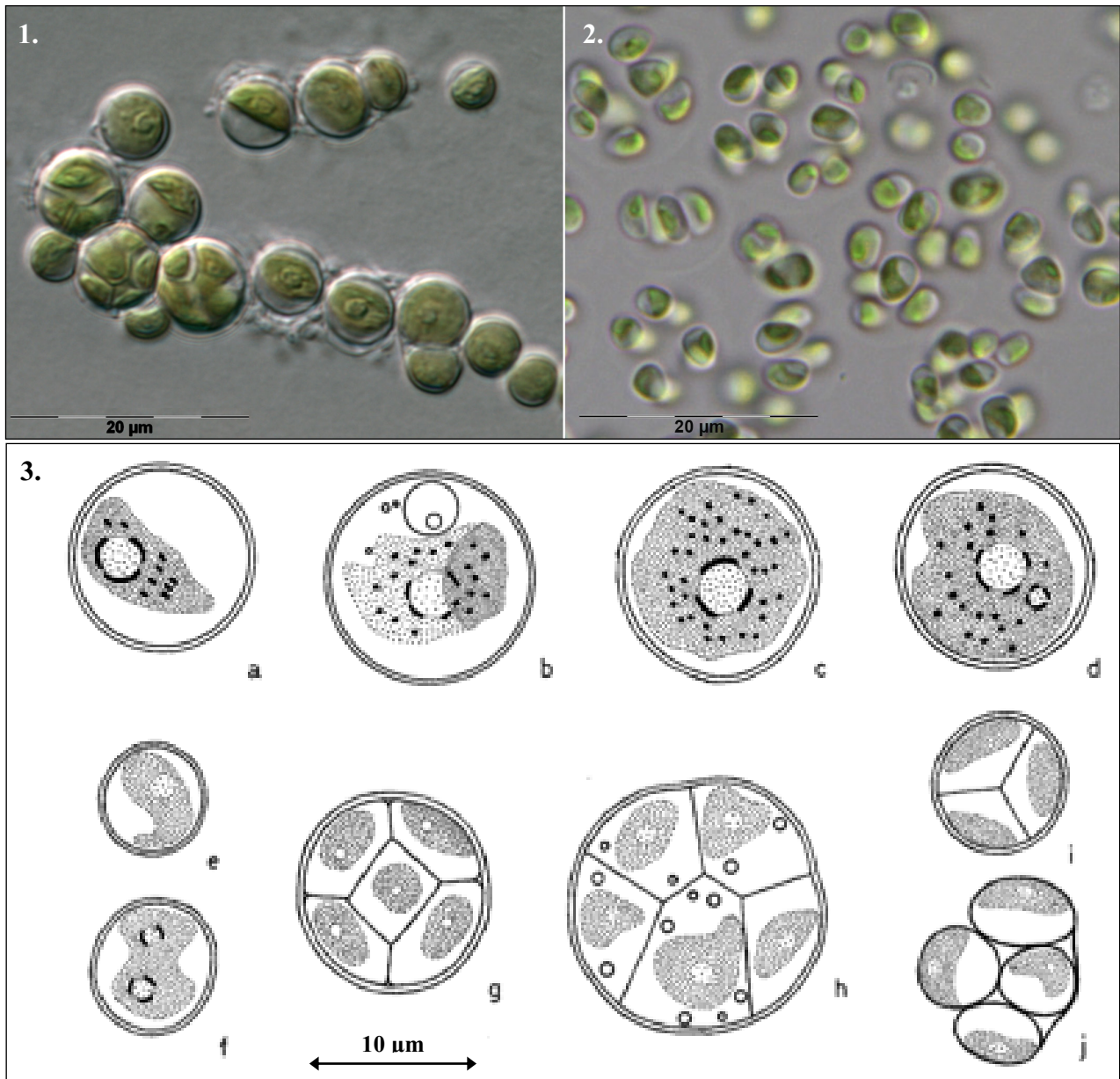
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Figures: Morphology of the strains UTEX 2485 (1.) and SAG 11.88 (2.) compared with the holotype (3.) of *Chlorella sphaerica* after Tschermak-Woess (1988).