***Watanabea borysthenica sp. nov.*, including '*Watanabea pyrenoidosa*' *nom. inval.* (*Trebouxiaceae*, Trebouxiophyceae)**

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Hanagata *et al.* (1998: 226, figs 5, 10) described the genus *Watanabea* and its type species *W. reniformis* N.Hanagata, I.Karube, M.Chihara & P.C.Silva based on their investigations of the strain SAG 211-9b (Culture Collection of Algae of the University of Göttingen). This strain was originally identified as *Chlorella saccharophila* Krüger; however, Darienko *et al.* (2010) showed that this strain is different from the authentic strain of *Chlorella saccharophila* (SAG 211-9a). *Chlorella saccharophila* Krüger was referred to *Chloroidium* by Darienko *et al.* (2010). The SSU and ITS rDNA sequences of strain SAG 211-9b (FM958480) confirmed that *Watanabea reniformis* represented a separate lineage within the *Watanabea* clade of the Trebouxiophyceae.

We isolated a new strain from the sandstone of the Trahtemyriv Landscape Park, near the river bank of the Dnipro (Kyiv region, Ukraine), and deposited it as SAG 2550. The SSU and ITS rDNA sequence of this strain (MH499911) showed several differences when compared to the sequence of SAG 211-9b (2 bp in SSU, 34 bp in ITS-1 and 9 bp in ITS-2). Temraleeva, Moskalenko & Portnaya (2018) studied a strain (ACSSI 014), isolated from grey forest soil collected near Moscow (Russia). The SSU (MG696555) and the ITS-2 (MG523285) sequences of this strain differed only in one base in SSU, and in two bases in ITS-2 to those of strain SAG 2550. They described the strain ACSSI 014 as a new species of *Watanabea* and proposed the binary designation, '*Watanabea pyrenoidosa'*. Unfortunately, the publication of this new species did not comply with Art. 39.2 ICN (Turland *et al.*, 2018), as an English or Latin diagnosis or description was not provided and a type was not designated, and it is thus invalid.

Comparing the morphological features of all three strains, the cells of the strains SAG 2550 (Fig. 2) and ACSSI 014 (Fig. 3) have a readily visible pyrenoid surrounded by several starch grains, but a pyrenoid could not be observed in the cells of strain SAG 211-9b (Fig. 1). Other morphological differences are summarised in Table 1. Considered with the genetic differences, we conclude that both strains SAG 2550 and ACSSI 014 represent a new species of *Watanabea* as follows to include '*Watanabea pyrenoidosa*':

Watanabea borysthenica Darienko & Pröschold, *sp. nov.* (Fig. 2)
Description: Young cells ellipsoidal, ovoid, sometimes irregular, 4.2 x 2.5 - 5.9 x 3.8 µm. Cell wall thin. Chloroplast parietal, saucer-shaped, not lobed. Mature vegetative cells ellipsoidal, broadly ellipsoidal, ovoid, 4.2 x 5.7 - 7.6 x 8.8 µm. Chloroplast parietal, saucer-shaped, sometimes slightly incised, containing a readily visible pyrenoid surrounded by many starch grains. Nucleus located in the middle of cell. Unilateral thickness of cell wall sometimes observed. Reproduction by 2-4-8 autospores, liberated by rupture of sporangial cell wall. Occasionally, unequal sized autospores observed.
Diagnosis: Differing from *Watanabea reniformis* by the presence of a pyrenoid surrounded by small starch grains and by differences in SSU and ITS rDNA sequences.
Holotype (designated here): The authentic strain SAG 2550 permanently cryopreserved at SAG in a metabolic inactive stage (see ICN Art. 40.3 Note 3).
Epitype (iconotype; designated here in support of the holotype): Fig. 2 in the present study.

Type locality: Epilithic on sandstone, Trahtemyriv Landscape Park, on the river bank of Dnipro, Kyiv region, Ukraine.
Etymology: The species name is derived from the Greek word *borysthenes*, the ancient Greek name for the river Dnipro (also known as the Dnieper).
Comment: Fučiková *et al.* (2014) found two strains (BCP-SEV1VF9 and BCP-SEV2VF2) named *Watanabea* sp., which were isolated from soil crusts collected in New Mexico (USA). The SSU rDNA sequences (KF693804 and KF693805) were identical to those of SAG 2550. Therefore, both strains probably belong to *Watanabea borysthenica*, but this requires confirmation by sequencing of the ITS.

Table 1. Comparison of the morphological features among the three *Watanabea* strains.

Feature	SAG 211-9b (Fig. 1)	SAG 2550 (Fig. 2)	ACSSI 014 (Fig. 3)
Cell shape	Young cells cylindrical to ellipsoidal, mature vegetative cells ellipsoidal, ovoid	Young cells ellipsoidal, ovoid, sometimes irregular, mature vegetative cells ellipsoidal, broadly ellipsoidal, ovoid	Young cells ellipsoidal, mature vegetative cells spherical
Cell size (µm)	3.5 x 5.0 - 7.0 x 12.0	4.2 x 5.7 - 7.6 x 8.8	3.5 x 5.0 - 7.0 x 10.0
Chloroplast shape	parietal, saucer-shaped, not lobed	parietal, saucer-shaped, not lobed	band-shaped, lobed
Pyrenoid	absent	present	present
Starch surrounding the pyrenoid?	n/a	present	present
Type of asexual reproduction	8-16 unequal-sized autospores	2-8 unequal-sized autospores	16-32 unequal-sized autospores

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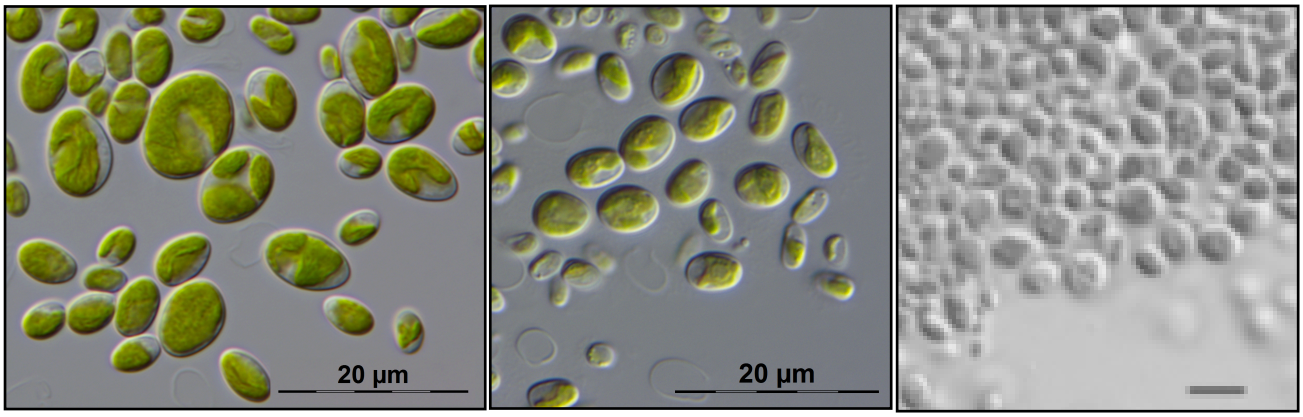

Figs 1-3: Morphology of *Watanabea* strains. **Fig. 1**. SAG 211-9b, *W. reniformis*, **Fig. 2.** SAG 2550, W. *borysthenica, sp. nov.,* (the present study). **Fig. 3**. *W. pyrenoidosa* after Temraleeva *et al.* (2018, fig. 1 h).