The distinctions between *Chlamydomonas incerta* Pascher and *Chlamydomonas globosa* J.W. Snow and their taxonomic consequences

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Two strains of the Culture Collection of Algae of the University of Göttingen (SAG), SAG 7.73 and SAG 81.72, were originally identified as *Chlamydomonas incerta* Pascher and *C. globosa* J.W**.** Snow, respectively. Kroes (1971) isolated the strain SAG 81.72 from a bog pool in the nature preserve Hartertse Venen (Netherlands) in 1967 and named it *Chlamydomonas globosa* without describing any morphological features. The strain SAG 7.73 was isolated by Hindák from Cuba (basin in Jardin Zoológico, Habana) in 1965. The morphology of this strain was investigated by Ettl (1965: 276, fig. 1; 1983: 228, fig. 194) in detail and identified as *Chlamydomonas incerta*. The ITS rDNA sequences of both strains were identical (see Pröschold *et al.* 2005). Nakada *et al.* (2010: 75; fig. 3) studied an isolate from Japan and compared this strain (NIES 2462) with SAG 7.73. They concluded that both strains belong to the same species based on identical morphology and sequences (SSU, ITS and rbcL), and determined these strains as *Chlamydomonas globosa* J.W**.** Snow.

Our morphological investigations of the three *Chlamydomonas* strains found in some discrepancies in the identification as *C. globosa*, and these are summarised in Table 1 and demonstrated in Figs 1-3. Considering the original descriptions (Snow 1903, Pascher 1927), both species have similar cell morphology (spherical cell shape, cup-shaped chloroplast, cell wall without papilla), but differ in cell size, flagella length, shape of pyrenoid and eyespot (cf. Table 1). *Chlamydomonas globosa* as described by Snow (1903: 375, pl. I, fig. III: 1-5; see Fig. 4) has a cell size between 5-7.8 µm, whereas *C. incerta* (Pascher 1927: 192-193, fig. 131; see Fig. 5) is larger (12-22 µm). Nakada *et al.* (2010) reported only a cell size of 5-12 µm among the strains SAG 7.73 and NIES 2462 and named both *C. globosa*. By contrast, we found that only zoospores and young vegetative cells of all three strains have this cell size; the vegetative cells mostly have a diameter of 8-15 µm, sporangia up to 16.5 µm in diameter. These discrepancies are likely to be the result of different cultivation methods and observation dates. Nakada *et al.* (2010) observed the strains three days after inoculation. We examined the morphology of the strains in two-week-old cultures. The small cell sizes could only be observed in young cultures where the cells formed many daughter cells per cell. This could be the reason that the cell sizes differed in the two studies. Ettl (1965, 1979) investigated the strain of *C. incerta* (SAG 7.73) and found the same morphological features and similar cell size that we discovered here (see Fig. 6). In addition, the other differing features (flagella length, shape of pyrenoid and eyespot; see Table 1 and Figs. 1-3) correspond with the original description of *C. incerta*. Ettl (1976) described the differences between *C. incerta* and *C. globosa* and concluded that both are separate species. He never proposed that both species were representative of a single species. As a consequence of our study, we propose the re-establishment of *C. incerta* for those strains as follows:

Chlamydomonas incerta Pascher, Die Süßwasserflora Deutschlands, Österreichs und der Schweiz, **4**: 193-194, fig. 131, 1927.

Syntype localities: Haffkrug near Neustadt (Schleswig-Holstein, Germany); Ischl (Upper Austria, Austria)

Lectotype (designated here): Fig. 131 in Pascher (1927). (ICN Art. 9.2 Melbourne Code, McNeill *et al*. 2011).

Comment: It is presumed that for the strains SAG 81.72 and SAG 7.73, one of the strains was replaced by the other (Harris *et al.*, 1991, Coleman & Mai, 1997, Nakada *et al.*, 2010). However, the AFLP patterns of the two strains are different (Darienko & Pröschold; unpublished data). Ettl (1965: 276-279, fig. 1) described *Chlamydomonas incerta* var. *macropyrenoidosa* from small pond in Glaselsdorf near Zittau (Germany), which differs in a larger pyrenoid and smaller eyespot. However, the morphology of this variety falls into the phenotypic plasticity of the three investigated strains.

The taxonomic status of *C. globosa* remains unresolved. Snow (1903) found *C. globosa* among cell clusters of *Pleurococcus regularis* Atari (currently referred to *Coelastrum microporum* Nägeli). This species normally does not produce mucilage, but the figures of this species (Snow 1903: pl. II: fig. IX:1-5) showed cells surrounded by mucilage. Therefore, it is possible that *C. globosa* are zoospores of a tetrasporalean alga, but further study is required.

We thank Michael Guiry for revising and editing of the manuscript.

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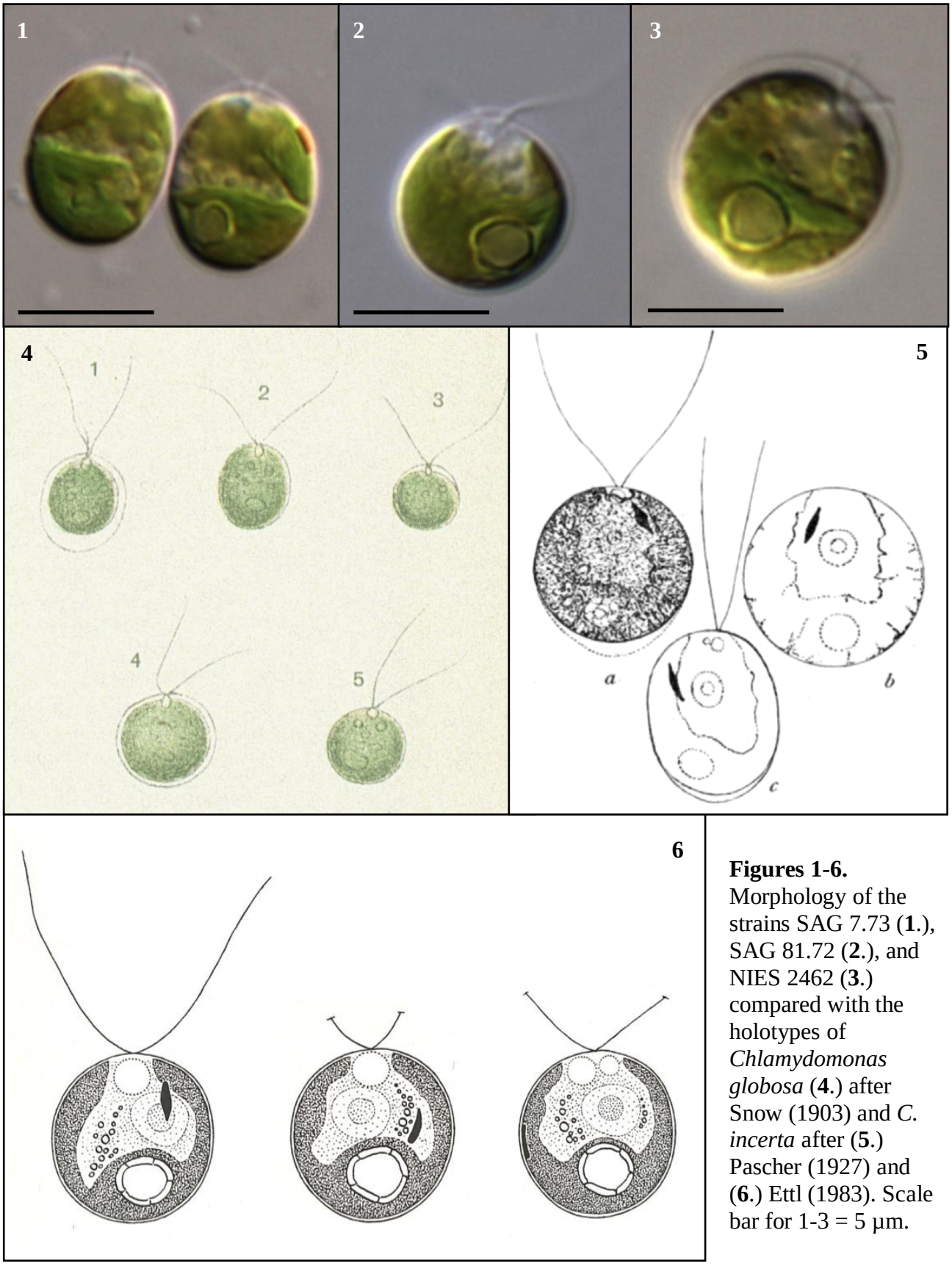
Feature	**SAG 7.73** **SAG 81.72** **NIES 2462** (Figs 1-3)	Original description of *C. globosa* (Fig. 4)	Original description of *C. incerta* (Fig. 5)
Cell shape	spherical - broadly ellipsoid	spherical	spherical - broadly ellipsoid
Cell size	8.0-9.5 x 9.7-12.4 µm (11.9-15.1 µm)	5-7.8 µm	12-22 µm
Cell wall papilla	no	no	no
Contractile vacuoles	2 apical	1 apical *	2 apical
Flagella length	slightly longer as the cell	1 1/2 times longer as the cell	slightly longer as the cell
Position of the nucleus	above the middle of the cell	in the middle of the cell	above the middle of the cell
Chloroplast shape	cup-shaped	cup-shaped	cup-shaped
Pyrenoid	medium, round-slightly ellipsoid, in basal position	small, round-slightly ellipsoid, in basal position	medium, round-slightly ellipsoid, in basal position
Eyespot	elliptic in anterior position	dot-like in anterior position	elliptic in anterior position
Cell division	longitudinal without 90° rotation of the protoplast	longitudinal without 90° rotation of the protoplast	longitudinal without 90° rotation of the protoplast
Number of zoospores	2-4	4	2-4
Size of zoospores	6.6-7.4 x 10.0-10.6 µm	n.d.	n.d.
Size of sporangia	15.6-16.5 µm	n.d.	n.d.

Table 1. Morphological comparison of cultures of *Chlamydomonas globosa* J.W. Snow and *C. incerta* Pascher with the original descriptions. * Pascher (1927) and Ettl (1976) observed that Snow probably did not observe the second contractile vacuole.



Figures 1-6. Morphology of the strains SAG 7.73 (**1**.), SAG 81.72 (**2**.), and NIES 2462 (**3**.) compared with the holotypes of *Chlamydomonas globosa* (**4**.) after Snow (1903) and *C. incerta* after (**5**.) Pascher (1927) and (**6**.) Ettl (1983). Scale bar for 1-3 = 5 µm.