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Source: Willdenowia, 28(1/2) : 239-247

Published By: Botanic Garden and Botanical Museum Berlin (BGBM)

URL: <https://doi.org/10.3372/wi.28.2820>

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WOLF-HENNING KUSBER

## **A study on *Phacus smulkowskianus* (Euglenophyceae) – a rarely reported taxon found in waters of the Botanic Garden Berlin-Dahlem**

### **Abstract**

Kusber, W.-H.: A study on *Phacus smulkowskianus* (Euglenophyceae) – a rarely reported taxon found in waters of the Botanic Garden Berlin-Dahlem. – Willdenowia 28: 239-247. 1998. – ISSN 0511-9618.

The morphological variation and ecology of a rarely reported but widely distributed flagellate, previously referred to as *Phacus similis* and *Euglena smulkowskiana*, was studied in detail during one year in a pond of the Botanic Garden Berlin-Dahlem. Its taxonomy and nomenclature are discussed and the new combination *Phacus smulkowskianus* is made.

### **Introduction**

In 1989 a small twisted euglenoid flagellate was found in the pond 'Teich Lichterfelde' in the Botanic Garden Berlin-Dahlem. This flagellate is not included in the keys of current monographs of *Euglenophyceae* (e.g., Huber-Pestalozzi 1955, Starmach 1983). It was identified as a *Phacus* species. To improve our knowledge of the taxonomy and ecology of this flagellate, its occurrence and morphological variability in Berlin-Dahlem was documented in a one-year study in 1996/97.

### **Material and methods**

The specimens were identified by light microscopy (LM) and scanning electron microscopy (SEM; critical point drying). Samples have been deposited at B.

Water temperature, pH, and conductivity were measured in situ with probes of WTW (Wissenschaftlich-Technische Werkstätten, Weilheim). Quantitative samples were collected with a Ruttner sampler near the shore at least fortnightly and preserved with Lugol's solution. Counts were carried out following Utermöhl's method (Utermöhl 1958, Rott 1981). Whole chambers (volumes between 12.5 ml and 52 ml) were counted to allow for detection of low abundances. Immediately after collecting, subsamples with living material were analysed.

### **Results**

#### **Morphology**

The studied *Phacus* species (Fig. 1) has a rigid pellicle. Apart from its helicoidal torsion, the cell shows bilateral symmetry. Cell size ranges are given in Tab. 1 and Fig. 2 (item 7). One

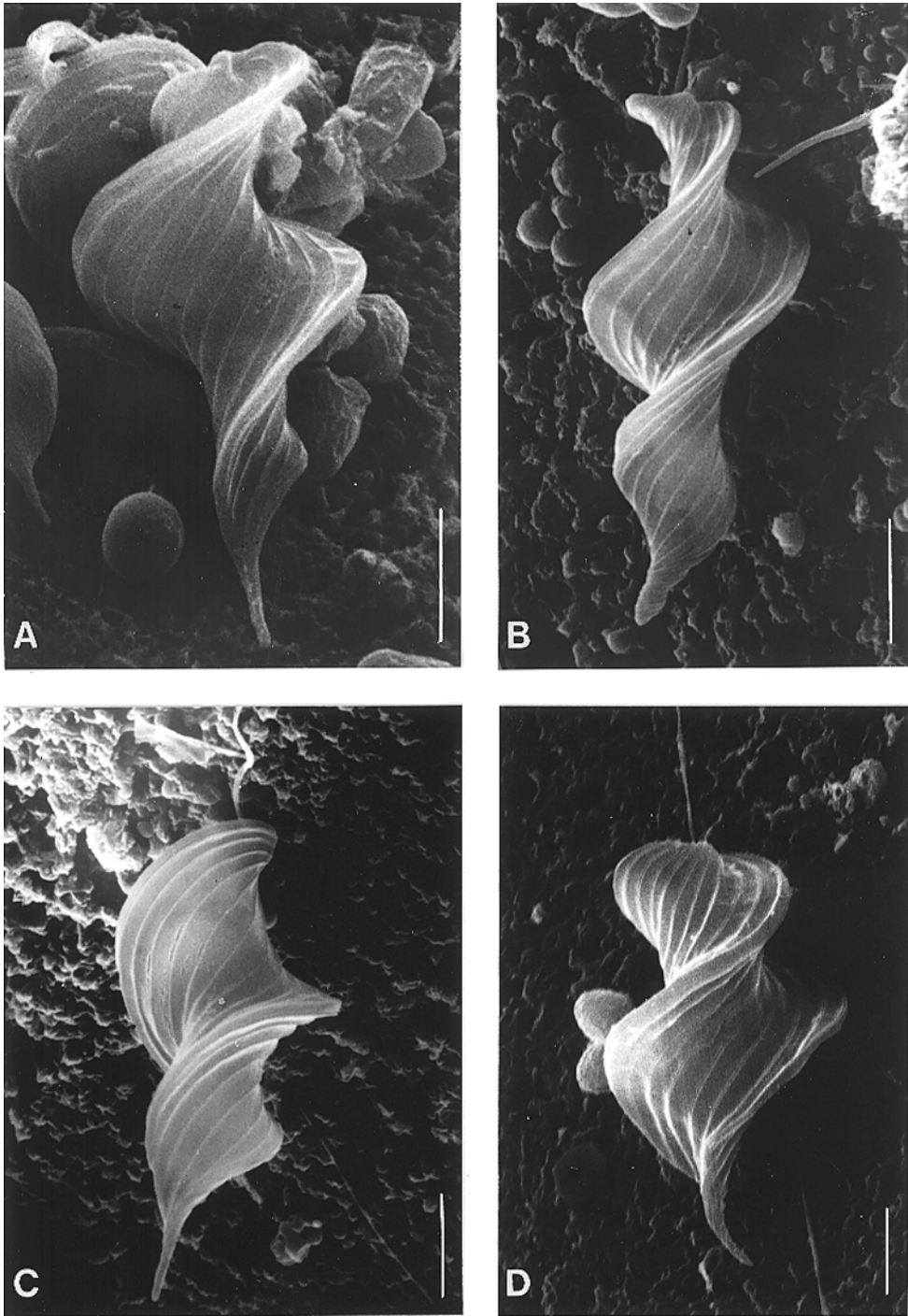


Fig. 1. SEM micrographs of *Phacus smulkowskianus* – A-C: from ‘Teich Lichterfelde’; D: from a shallow eutrophic lake in Klein-Machnow near Berlin. – Scale bar: 5  $\mu$ m.

Tab. 1. Size of *Phacus smulkowskianus* from 'Teich Lichterfelde'.

|                               | Range       | Mean | n = |
|-------------------------------|-------------|------|-----|
| Cell length (µm)              | 24.4 - 41.4 | 32.4 | 371 |
| Posterior spine (µm)          | 2.2 - 9.6   | 5.8  | 371 |
| Cell breadth (µm)             | 7.3 - 21.6  | 14.4 | 371 |
| Paramylon 1 length (µm)       | 3.6 - 11.6  | 8.3  | 306 |
| Paramylon 1 breadth (µm)      | 2.6 - 10.8  | 6.4  | 306 |
| Paramylon 2 length (µm)       | 0.7 - 9.5   | 5.6  | 296 |
| Paramylon 2 breadth (µm)      | 0.7 - 8.4   | 3.8  | 296 |
| Flagellum (µm)                | 8.8 - 31.2  | 19.3 | 62  |
| Flagellum : cell-length ratio | 0.26 - 0.86 | 0.6  | 62  |

flagellum emerges from an apical canal opening. The cell margins, similar to keels, arise from a small anterior papilla. Pellicular striations passing helically along the cell lead to a whorl along the posterior spine. This whorl is visible only in SEM (see broad posterior spine in Fig. 1B). The cell contains numerous discoid chloroplasts of 2.2-2.4 µm in diameter, lacking pyrenoids. The paramylon size of individuals from 'Teich Lichterfelde' is given in Tab. 1. The intracellular arrangement is the following: paramylon grain 1 anterior with respect to the nucleus, grain 2 posterior (as shown by Zakryś 1986 for *Euglena smulkowskiana* Zakryś). Rarely the two paramylon grains are shifted to a lateral position, as shown by Christen (1962 for *Phacus similis*). In most cases the anterior paramylon grain is slightly larger than the posterior one (Tab. 1). Cells with one or three paramylon grains were rarely found. In 'Teich Lichterfelde', this flagellate is associated with other flattened, twisted, and rounded euglenoids.

### Ecology

'Teich Lichterfelde', located in the America section of the Botanic Garden Berlin-Dahlem, is a highly eutrophic, shallow water body. The range of nutrient contents in the pond was 86-175 µg l<sup>-1</sup> total phosphorus and 28-65 µg l<sup>-1</sup> dissolved reactive phosphorus (Didwiszus unpubl. thesis 1992, Kusber 1998a). The phytoplankton was rich in species (175 taxa, of which 51 belong to the *Chlorophyceae* and 42 to the *Euglenophyceae*). Total biovolumes were high (more than 20 mm<sup>3</sup> l<sup>-1</sup> in the vegetation period, see Didwiszus & Kusber 1998).

The temperature range of the studied flagellate from 'Teich Lichterfelde', as shown in Tab. 2, ranges from 0.0-26.4 °C. The pH is basic to neutral. Fig. 3 shows high cell densities in autumn, winter and spring. Cell numbers decreased in winter, when 'Teich Lichterfelde' was covered with ice (as indicated by increasing conductivity in Fig. 3) and increased again with ice break. Cells tolerate a range of conductivity of 126-971 µS cm<sup>-1</sup>, the highest value being limited to a short period in winter (Fig. 3).

### Discussion

#### Taxonomy

The described *Phacus* species from 'Teich Lichterfelde' in the Botanic Garden Berlin-Dahlem agrees in all characters with the descriptions of *Euglena smulkowskiana* (Zakryś 1986) and *Phacus similis* (Christen 1962). Since many samples were studied, a wide range of cell (Fig. 2: item 7; Tab. 1) and subcellular dimensions (Tab. 1) was documented. Small cells are in the size class of *Ph. similis* f. *minor* Bourr. & Couté (Bourrelly & Couté 1978: 296, pl. 1, fig. 3), which is therefore considered a synonym. In comparison with the drawings of Christen (1962), the occurrence of more (Bourrelly 1963) or less twisted cells (Wawrik 1979, 1981) was observed. Fig. 1 documents the wide range of morphological variation in this *Phacus* species.

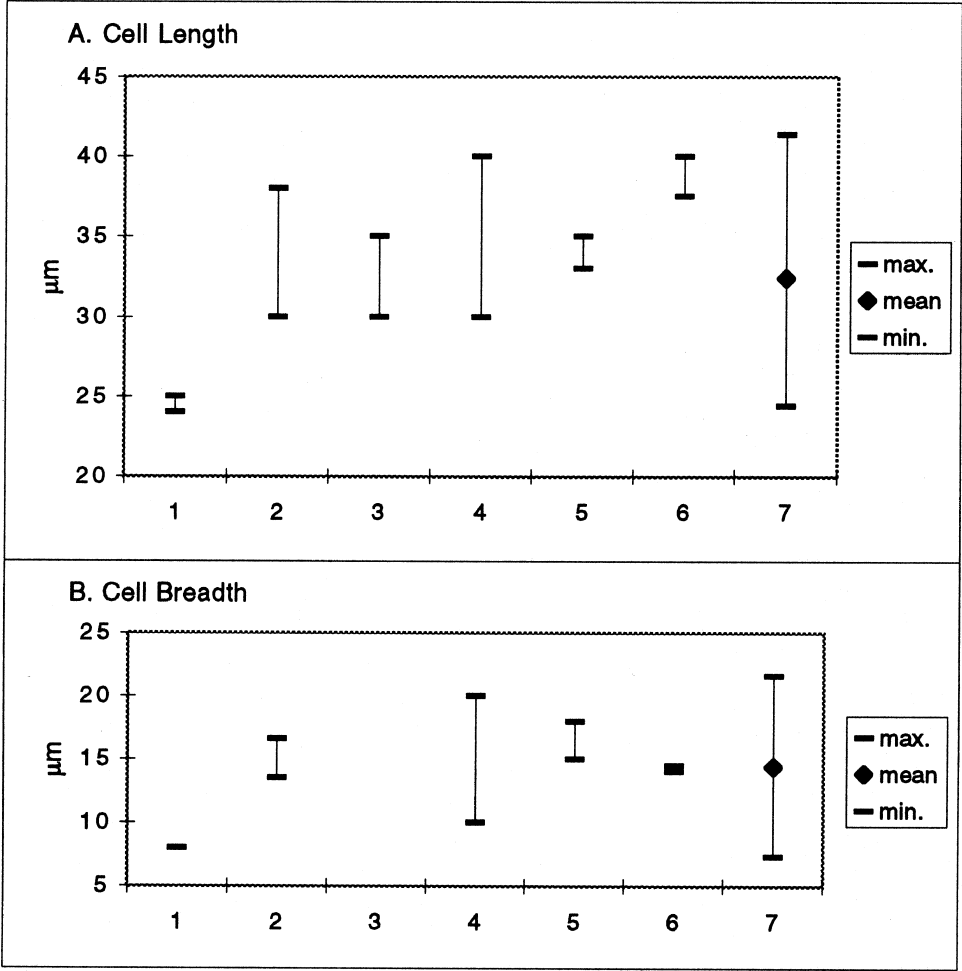


Fig. 2. Size of *Phacus smulkowskianus* as given by different authors – 1: Bourrelly & Couté (1978), 2: Zakryś (1986), 3: Christen (1962), 4: Wawrik (1979), 5: Bourrelly (1963), 6: Tracanna (1985), 7: this study; 1 & 3-6 as *Phacus similis*, 2 as *Euglena smulkowskiana*.

*Phacus* is distinguished from *Euglena*, among others, by a rigid cell, flattened in cross section (Huber-Pestalozzi 1955). *Phacus longicauda* (Ehrenb.) Dujardin is the accepted type of *Phacus* (see Greuter & al. 1994: 130). Silva (1960) designated this type because the “identity as a species of *Phacus* is beyond reasonable doubt”. In Starmach (1983), a footnote in his discussion of *Ph. longicauda* (Ehrenb.) Dujardin hints at its similarity with *Ph. similis*, whose morphology strongly resembles the flagellate discussed here.

Christen (1962) described *Ph. similis* from ponds and bogs in Switzerland. Since then, euglenoids very similar to Christen’s findings were found occasionally in different water bodies throughout the world: in ponds of ‘Waldviertel’ and in lake ‘Neusiedler See’ in Austria (Wawrik 1979, 1981, 1982, Kusel-Fetzmann & Schagerl 1992), in Lake Trummen in Sweden (Cronberg 1982), as well as in bogs and a lake in Argentina (Tracanna 1985, Tell & Conforti 1986, Conforti & Tell 1988).

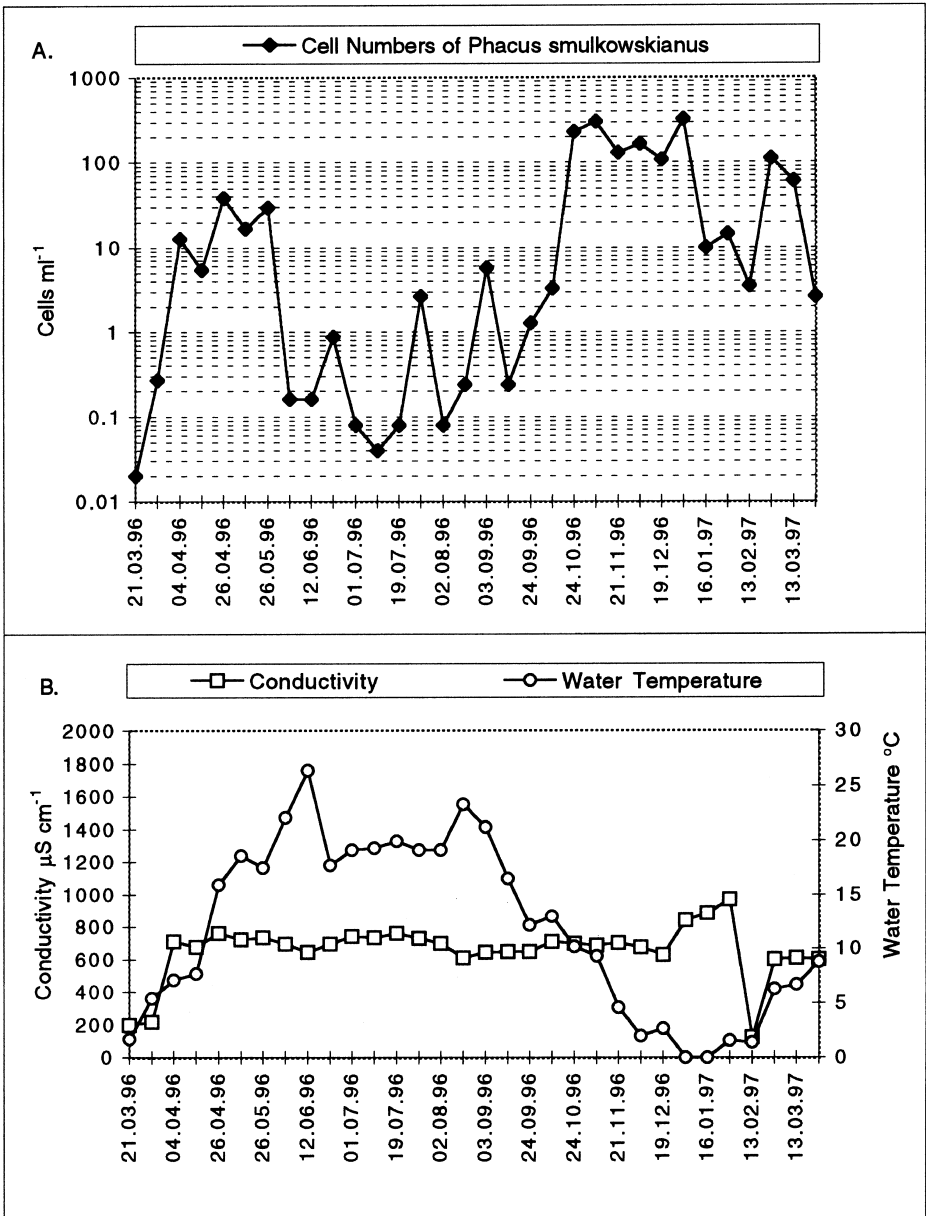


Fig. 3. Cell numbers of *Phacus smulkowskianus*, water conductivity and water temperature in 'Teich Lichterfelde' Botanic Garden Berlin-Dahlem, data from 1996/97.

Morphological deviations from Christen's taxon were noticed in material from ponds in Austria (Wawrik 1979, 1981) and France (Bourrelly 1963), and were formally recognized at infraspecific rank for specimens from a French swamp (Bourrelly & Couté 1978).

Christen (1962) compares *Phacus similis* with *Ph. helicoides* Pochm. and *Ph. tortus* (Lemm.) Skvortzov. Diagnostic features are the latter taxon's large size (at least 70  $\mu\text{m}$ , accor-

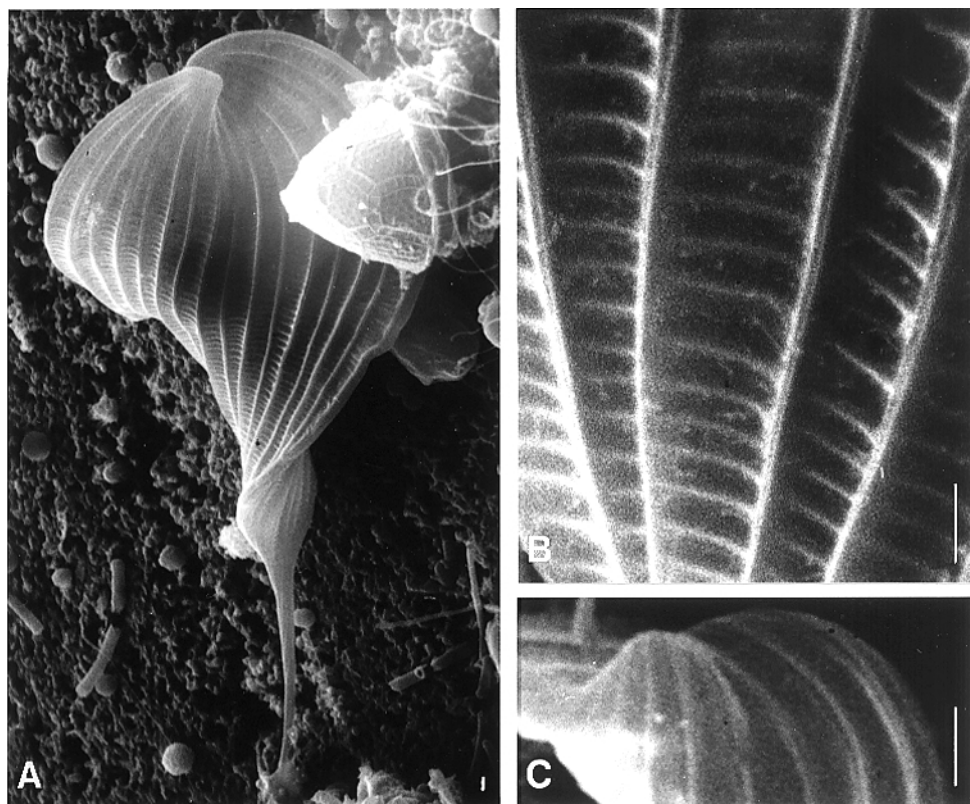


Fig. 4. SEM micrographs of *Phacus* spp. – A: cell of *Phacus tortus*; B: *Phacus tortus*, detail: pellicular striation with transversal striae; C: *Phacus smulkowskianus*, detail: pellicular striation; note the absence of transversal striae. – Scale bar: 1  $\mu$ m.

ding to Christen 1962) and the disk-shaped paramylon. I agree with Christen (1962), with two additions. The disk-shaped paramylon in *Ph. tortus* is only one view of a complex bobbin-shaped body consisting of two disks connected by an axis. A second differentiating character is pellicular striation (see Fig. 4). *Ph. tortus* (Fig. 4A-B) and *Ph. helicoides* have small transversal striae in addition to longitudinal striation documented by Huber-Pestalozzi (1955) for *Ph. helicoides*. The studied *Phacus* lacks such transversal striae (Fig. 1, 4C).

In the protologue of *Phacus similis* (Christen 1962: 164, 195, pl. 1, fig. 3-4) diagnoses in Latin and German are given as well as two figures. However, the type is not indicated, hence *Ph. similis* is not validly published. Christen (1962) cites at least five collections from three sampling sites without sampling dates. The published figures lack a link to a concrete collection or locality. Christen (1962) referred *Ph. similis* to the genus *Phacus* because of its flattened cell and its similarity to *Ph. tortus* and *Ph. helicoides*. The rank of the latter taxa is still under discussion. Some authors accept them as distinct species (Pochmann 1942, Huber-Pestalozzi 1955, Tell & Conforti 1986). Others accept twisted deviations from *Ph. longicauda* on an infraspecific level as var. *tortus* Lemmerm. (Lemmermann 1913, Popova & Safonova 1976, Starmach 1983); the latter author, in an appendix, referred *Ph. helicoides* as well as *Ph. similis* to *Ph. longicauda*.

In 1986 *Euglena smulkowskiana* Zakryś was described from a polluted pond in Poland (Zakryś 1986). Zakryś (1986) described *E. smulkowskiana* as having a rigid cell, but she never

Tab. 2. Reported occurrence and ecology of *Phacus smulkowskianus*.

| Reference                                                           | Country     | Season (month)         | pH        | Temperature<br>(° C) |
|---------------------------------------------------------------------|-------------|------------------------|-----------|----------------------|
| Christen (1962, as <i>Ph. similis</i> )                             | Switzerland | –                      | 5.5 - 7.0 | -                    |
| Bourrelly (1963, as <i>Ph. similis</i> )                            | France      | late spring (V)        | –         | -                    |
| Bourrelly & Couté (1978, as<br><i>Ph. similis</i> f. <i>minor</i> ) | France      | –                      | -         | -                    |
| Wawrik (1979, 1981, 1982, as<br><i>Ph. similis</i> )                | Austria     | (I, IV, VIII, X, XII)  | 6.7 - 7.6 | 0.9 - 22.0           |
| Cronberg (1982, as <i>Ph. similis</i> )                             | Sweden      | winter, under ice (II) | –         | -                    |
| Tracanna (1985, as <i>Ph. similis</i> )                             | Argentina   | late spring (IX)       | 6.2 - 7.5 | 22.0                 |
| Zakryś (1986, as <i>Euglena smul-</i><br><i>kowskiana</i> )         | Poland      | late summer (IX)       | –         | -                    |
| Tell & Conforti (1986, as <i>Ph. similis</i> )                      | Argentina   | –                      | 5.5 - 6.0 | -                    |
| Didwizsus (unpubl. thesis 1992,<br>as <i>Ph. similis</i> )          | Germany     | (IV-VI, IX)            | 8.0 - 8.8 | 10.8 - 20.8          |
| this study                                                          | Germany     | all seasons            | 6.9 - 8.6 | 0.0 - 26.4           |

considered the genus *Phacus* in her protologue. Christen (1962) described *Ph. similis* as flat-tened, while Zakryś (1986) described *E. smulkowskiana* as being cylindrical with two flat wings. There is no real conflict between these two descriptions of cell cross-section. Young cells of *Phacus* are often very flat, whereas the cross section of older cells is modified by their higher paramylon content. In contrast to the occurrence of long, rod-shaped paramylon grains in the genus *Euglena*, our taxon of *Phacus* has small, ring-shaped grains, and cells with high paramylon content bear large granules but never long rods.

Ecology

The temperature range of the studied flagellate from ‘Teich Lichterfelde’ is wider than reported in the literature (see Tab. 2), whereas pH is basic to neutral and falls within the range of literature data, which also include slightly acidic water bodies (Tab. 2).

High abundance in winter is known from Sweden (up to 0.34 mg l<sup>-1</sup> biomass in Lake Trummen, Cronberg 1982) and Austria (up to 1000 cells ml<sup>-1</sup>, Wawrik 1981). In spring the studied *Phacus* taxon appears with moderate and in summer with low cell numbers in ‘Teich Lichterfelde’. High cell numbers at low temperatures and low cell numbers at high temperatures are known from *Trachelomonas volvocina* Ehrenb. (*Euglenophyceae*) from this same water body (Kusber 1998b). Almost all other euglenoid taxa have their highest abundances in summer at high temperatures (Kusber 1998a).

Conclusions

The organism from ‘Teich Lichterfelde’ can be referred to *Phacus similis* Christen and *Euglena smulkowskiana* Zakryś (Zakryś 1986), which are synonyms (Kusber 1992, Kusber & Kasten 1997, Kusel-Fetzmann, pers. comm.). Since the name *Ph. similis* is not validly published, *Euglena smulkowskiana* is the only available binomial, but, as explained above, its epithet has not so far been combined under *Phacus*.

*Phacus smulkowskianus* (Zakryś) Kusber, **comb. nova**  $\equiv$  *Euglena smulkowskiana* Zakryś in Nova Hedwigia 42: 524, pl. 4, fig. 6. 1986.

[– *Phacus similis* Christen in Rev. Algol. 6: 164, 195, pl.1, fig. 3-4. 1962, nom. inval. (Greuter & al. 1994, Art. 37.1)]

[– *Phacus similis* f. *minor* Bourr. & Couté in Rev. Algol. 4: 296, pl. 1, fig. 3. 1978, nom. inval. (Greuter & al. 1994, Art. 43.1)].

Comparing my own observations to literature data, it can be concluded that *Phacus smulkowskianus* is a pH tolerant species like *Ph. platyaulax* Pochm. or *Ph. orbicularis* K. Hübner (Kusber 1998a). The occurrence of *Ph. smulkowskianus* in ‘Teich Lichterfelde’ indicates a wide tolerance range concerning abiotic factors. Further studies are needed to clarify whether it is indeed a rare species or has been overlooked or misdetermined.

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