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MORPHOLOGICAL AND MOLECULAR EXAMINATION OF RELATIONSHIPS AND EPITYPE ESTABLISHMENT OF *PHACUS PLEURONECTES*, *PHACUS ORBICULARIS*, AND *PHACUS HAMELII*[1]

Sylwia Kosmala, Magdalena Bereza, Rafał Milanowski, Jan Kwiatowski, and Bożena Zakryś[2]

Department of Plant Systematics and Geography, Warsaw University, Al. Ujazdowskie 4, PL-00-478 Warszawa, Poland

Verification of morphological diagnostic features and the establishment of three epitypes for three species of *Phacus* Dujardin—*Phacus pleuronectes* (O. F. Müll.) Dujardin, *Phacus orbicularis* Hübner, and *Phacus hamelii* Allorge et Lefèvre—was performed based on literature studies and analysis of morphological (cell shape, cell size, and periplast ornamentation) as well as molecular (18S rDNA) characters. Periplast ornamentation was recognized as a main diagnostic character, distinguishing *P. orbicularis* from *P. pleuronectes* and *P. hamelii*. *Phacus orbicularis* has struts running perpendicular to the longitudinal axis of the strips, while *P. pleuronectes* and *P. hamelii* do not. On the SSU rDNA tree, obtained by the Bayesian method, *P. orbicularis*, *P. pleuronectes*, and *P. hamelii* belong to three distinct clades. Some of the phylogenetic relationships are not resolved, but there are at least three *Phacus* species (*P. hamatus*, *P. platyaulax*, *P. longicauda*; for taxonomic authors, see Introduction) that are more closely related to *P. orbicularis* than is *P. pleuronectes*. *Phacus hamelii* is more closely related to *P. ranula* and the assemblage of several species of *Phacus*, which have small cells, than to *P. orbicularis* or *P. pleuronectes*.

Key index words: **Euglenida; Euglenophyta; molecular phylogeny; morphology; rDNA**

Abbreviations: **BA, Bayesian analysis; bs, nonparametric bootstrap; ML, maximum likelihood; nt, nucleotide; pp, posterior probability**

For years, the study of the critical species of Euglenales (i.e., those that are difficult to distinguish from one another owing to their morphological similarities) was hampered by the lack of appropriate tools. Currently, due to the progress in acquisition of molecular as well as morphological characters, it is possible to revisit many outstanding questions in the classification of these relatively simple and not overly diversified organisms. The work presented herein concerns taxa similar morphologically to *Phacus pleuronectes* and is the next step to untangle difficulties in euglenoid classification. It follows papers dealing with other euglenoid species, such as *Euglena agilis* Carter (Zakryś and Kucharski 1996, Zakryś et al. 1996, Zakryś 1997, Zakryś et al. 2004), *E. geniculata* Dujardin (Zakryś et al. 2002), *E. viridis* Ehrenb. (Shin and Triemer 2004), *Lepocinclis spirogyroides* (Ehrenb.) Marin et Melkonian, *L. fusca* (Klebs) Kosmala et Zakryś (Kosmala et al. 2005), and *Monomorphina pyrum* (Ehrenb.) Mereschkowsky (Kosmala et al. 2007).

In this work, we attempt to verify a large group of taxa—similar in morphology to *Phacus pleuronectes*—that has been extensively studied and described in the literature. The group includes the following taxa, in chronological order by description:

P. pleuronectes var. *pleuronectes* (Ehrenb.) Dujardin 1841; *P. pleuronectes* var. *brevicaudata* Klebs 1883; *P. pleuronectes* var. *triquetra* Klebs 1883; *P. orbicularis* var. *orbicularis* Hübner 1886; *P. gigas* Cunha 1913; *P. pleuronectes* var. *insecta* Koczwara 1915; *P. orbicularis* var. *undulatus* Skvortzov 1917; *P. pleuronectes* var. *australis* Playfair 1921; *P. pleuronectes* var. *rothertii* Namysłowski 1921; *P. pleuronectes* var. *zmudae* Namysłowski 1921; *P. pleuronectes* var. *citriformis* Dreżepolski 1921/1922; *P. granulatus* Roll 1925; *P. hamelii* Allorge et Lefèvre 1925; *P. megapyrenoidea* Roll 1925; *P. ovoidea* Roll 1925; *P. platalea* Dreżepolski 1925; *P. prunoideus* Roll 1925; *P. pulcher* Roll 1925; *P. zingeri* Roll 1925; *P. platalea* fo. *minor* Deflandre 1928; *P. orbicularis* var. *caudatus* Skvortzov 1928; *P. pleuronectes* var. *marginatus* Skvortzov 1928; *P. orbicularis* var. *cingeri* (Y. V. Roll) Swirenko 1938; *P. acuminata* var. *granulata* (Y. V. Roll) Pochmann 1942; *P. acuminata* var. *megapyrenoidea* (Y. V. Roll) Pochmann 1942; *P. brachykentron* Pochmann 1942; *P. hamatus* Pochmann 1942; *P. undulatus* (Skvortzov) Pochmann 1942; *P. pseudoplatalea* Pochmann 1942; *P. orbicularis* fo. *communis* Popova 1947; *P. orbicularis* fo. *gigas* (Cunha) Popova 1947; *P. pleuronectes* var. *hamelii* (Allorge et Lefévre) Popova 1947; *P. orbicularis* var. *citriformis* (Dreżep.) Popova 1951; *P. pleuronectes* var. *prunoideus* (Y. V. Roll) Popova 1955; *P. orbicularis* fo. *cingeri* (Y. V. Roll) Safonova in Popova and Safonova 1976; *P. hamelii* var. *ovatus* Shi 1994; and *P. granulatus* var. *laevis* Shi 1996.

Criteria for distinguishing these taxa are vague and are based on differences in cell morphology

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[2]Author for correspondence: e-mail zakrys@biol.uw.edu.pl.

(i.e., the size and shape of cells, the length and the degree of tail curvature, the number and morphology of the large paramylon grains, and the length of the fold, which is called a ''comb''). The discussion regarding the need to distinguish particular taxa and their taxonomical ranks has been taking place for over 170 years. As a consequence, there are a large number of taxa of different ranks—species, varieties, forms—as well as numerous reclassifications. However, there is a lack of diagnostic features for proper identification, even of species as common as *P. pleuronectes* or *P. orbicularis*. This situation makes interpretation of phylogenetic trees difficult. The main cause of this situation is the supposed close relationship of *P. pleuronectes* to *P. orbicularis* and their enormous morphological variability. Consequently, it has been impossible to achieve a successful taxonomical revision by means of classical euglenoid taxonomy (Dreżepolski 1925, Swirenko 1938, Pochmann 1942, Popova 1951, Popova and Safonova 1976). Contemporary methods of molecular phylogenetics afford an objective tool for assessing relationships between organisms, irrespective of their morphology; therefore, it has become possible to (i) perform comparative studies that take into account molecular and morphological characters of the species included within the group, (ii) reconstruct their phylogenetic relationships, (iii) verify morphological diagnostic features for particular taxa, and (iv) perform taxonomic verification, emended diagnoses, and designation of epitypes for well-distinguished taxa.

MATERIALS AND METHODS

Euglenoid strains and culture conditions. The strains used in this study are described in Table 1. All strains were cultivated in a liquid soil-water medium, enriched with a small piece of a garden pea (medium 3c, Schlösser 1994), under identical conditions in a growth chamber maintained at 17°C and 16:8 light:dark (L:D) with ~27 μmol photons $\cdot m^{-2} \cdot s^{-1}$ provided by cool-white fluorescent tubes.

Light microscopy observations. Observation of morphological features was performed using a light microscope (Nikon Eclipse E-600 with Nomarski contrast; Nikon, Tokyo, Japan), equipped with the software for image recording and processing. Photographic documentation was performed using a Nikon DX-1200 digital camera. Cultures were sampled every 2 weeks, for periods of 3–4 months. Such sampling enabled us to observe all the cells during their developmental stages, from the young cells (immediately after division) to older ones.

Biometric studies. Biometric studies were performed using the LUCIA Measurement program (Laboratory Imaging s. r. o., Prague, Czech Republic). One hundred randomly chosen actively swimming cells from each of the strains were analyzed. All observations were made on material preserved with a 5% solution of glutaraldehyde by adding one drop of a fixative to the fresh material placed on the slide. Three parameters were measured for each strain: length, width, and length of the tail. The data were analyzed using Statistica software (StatSoft Inc., Tulsa, OK, USA).

DNA isolation, amplification, and sequencing. Isolation of total DNA, amplification of 18S rDNA regions, purification, and sequencing of PCR products were performed as previously described (Milanowski et al. 2001, Zakryś et al. 2002, Kosmala et al. 2005).

Sequence accession numbers, alignment, and phylogenetic analysis. The GenBank accession numbers for the SSU rDNA sequences reported here and used for the phylogenetic analyses are shown in Table 1. The alignment of sequences, obtained by using Clustal X 1.83 (Thompson et al. 1997) with default options, was manually checked and edited according to the secondary structure of *Euglena gracilis* G. A. Klebs as suggested by Wuyts et al. (2002), using GenDoc 2.6 (Nicholas et al. 1997). The alignment used for analyses is available in the EMBL (European Molecular Biology Laboratory) Nucleotide Sequence Database (ALIGN_000990). Regions that could not be unambiguously aligned were omitted from the analyses. Maximum-likelihood (ML) tree calculations and base frequency test (χ^2) of nucleotide distribution were performed using PAUP*, Version 4.0b10 for Microsoft Windows (Swofford 1998). To find the best tree, the heuristic search option was used with MULTREES (in PAUP), tree-bisection-reconnection (TBR) branch swapping, and random addition, with 10 replications. Bootstrap support for specific nodes (Felsenstein 1985) was estimated by the default options using 100 replications for ML analyses, as implemented in PAUP*. Models of sequence evolution and their parameters for the ML method were chosen by Modeltest 3.7 software (Posada and Crandall 1998). The Bayesian analyses were performed, and their model parameters were calculated by MrBayes 3.1 software (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Four Markov chains were run, with 1,000,000 generations per chain, the first 2000 trees being discarded. Trees were drawn using TreeView, Version 1.6.1 for Microsoft Windows (Page 1996).

RESULTS

Light microscope observations: In all 19 strains investigated, there was certain morphological variability, both within as well as between strains. This differentiation concerned such diagnostic features as cell size and shape, number and shape of large paramylon grains, size and degree of tail curvature, length of the fold (also called the comb), and periplast ornamentation.

Cell size: According to this feature, the 19 strains were divided into three distinct groups: (i) strains with cell length <35 μm and tail length <5 μm (ACOI 1088 and ACOI 2434); (ii) strains with cell length 35–55 μm and tail length 5–8 μm (AICB 525, ACOI 614, ACOI 996, ACOI 1142, ACOI 1143, ACOI 1144, ACOI 1145, ACOI 2955, ACOI 2996, SAG 1261-1, SAG 1261-2b, and SAG 1261-3b); and (iii) strains with cell length >55 μm and tail length >10 μm (AICB 502, ACOI 2349, ACOI 2376, ACOI 2423, ACOI 2437; Table 2).

Substantial intrastrain diversification of the cell size—dependent mostly on the development stage—was observed, in addition to the cell-size differentiation between strains. In young and not overcrowded cultures, small, vigorously dividing cells dominated, as expected. As the populations aged and growth conditions deteriorated, the rate of cell divisions subsided, and consequently, the cells were able to grow to a larger size, sometimes twice that of the young cells (Table 2).

TABLE 1. The euglenoid strains and the corresponding 18S rDNA GenBank accession numbers for the taxa used in this study.

Taxon	Strain	Accession number
Lepocinclis acus (Müller) Marin et Melkonian	ASW 08037	AJ532458
Lepocinclis fusca (Klebs) Kosmala et Zakryś	ACOI 1032	AY935690
Lepocinclis fusiformis (Carter) Lemmerm.	ACOI 1025	AY935697
Lepocinclis ovum (Ehrenb.) Minkevich	AICB 278	AJ532455
	SAG 1244-8	AF110419
Lepocinclis spirogyroides (Ehrenb.) Marin et Melkonian	ACOI 1227	AY935693
Lepocinclis tripteris (Dujard.) Marin et Melkonian	UW-OB/99 (=CCAP 1224/45)	AY935696
Phacus acuminatus Stokes	ASW 08004	AF283311
	ASW 08056	AF283312
	SAG 1261-7 (=UTEX 1317) (as *P. brachykentron*)	AJ532481
Phacus caudatus Hübner	ASW 08020	AJ532482
	M-1127	AJ532483
Phacus granum Drežep.	AICB 349	DQ249880
Phacus hamatus Pochmann	ASW 08032 (as *P. pleuronectes*)	AJ532473
Phacus hamelii Allorge et Lefèvre	ACOI 1088	(identical with DQ397673—R. Triemer, personal communication)
	ACOI 2434	**DQ397673**
Phacus longicauda var. *torta* Lemmerm.	ACOI 1108	AJ532480
Phacus orbicularis Hübner	ACOI 614 (as fo. *communis*)	**DQ397664**
	ACOI 996 (as fo. *cingeri*)	**DQ397670**
	ACOI 1145	**DQ397666**
	ACOI 2349	**DQ397665**
	ACOI 2376	**DQ397667**
	ACOI 2437 (as fo. *gigas*)	**DQ397672**
	ACOI 2955 (as *P. communis*)	**DQ397671**
	ACOI 2996	**DQ397668**
	AICB 502	AY935698
	AICB 525	AY935699
	ASW 08054 (as *P. pleuronectes*)	AF283315
	M-1424	AJ532479
Phacus oscillans Klebs	UTEX 1285	AF181968
	M-1682 (= CCAC-0089)	AJ532468
Phacus parvulus Klebs	ASW 08060	AF283314
Phacus platyaulax Pochm.	ASW 08027	AJ532474
Phacus pleuronectes (O. F. Müller.) Dujard.	SAG 1261-3b	**DQ397669**
	M (strain from Korea)	AF081591
	SAG 1261-2b (as *P. alatus* or *P. pleuronectes* var. *triquetra* in Brosnan et al. 2003)	AJ532476
	SAG 1261-1 (=UTEX 1288) (as *P. acuminatus*)	AJ532477
Phacus pusillus Lemmerm.	SAG 1261-5	AJ532471
	ACOI 1093	AJ532472
Phacus ranula Pochm.	M-1307	AJ532484
Phacus similis Christen	ACOI 1226	AF119118
	SAG 58.81	AJ532467
Phacus skujae Skvortzov	AICB 323	DQ249882

Accession numbers of new sequences are in boldface. Strains used in biometric studies are underlined. ACOI, Culture Collection of Algae at the Department of Botany, University of Coimbra, Portugal; AICB, Culture Collection of Algae at the Institute of Biological Research Cluj-Napoca, Romania; ASW, Culture Collection of Algae at the University of Vienna, now available from CCAC; CCAC, Culture Collection of Algae at the University of Cologne, Germany; CCAP, Culture Collection of Algae and Protozoa at Center for Ecology and Hydrology, Cumbria, UK; M, Research Culture Collection Melkonian at the University of Cologne, Germany; SAG, Sammlung von Algenkulturen Pflanzenphysiologisches Institut der Universität Göttingen, Germany; UTEX, Culture Collection of Algae at the University of Texas at Austin, USA; UW, Department of Plant Systematics and Geography of Warsaw University, Poland.

Tail length: The tail length was proportional to the length of the cell (Table 2; Fig. 1, a–m, p, r, and s) but did not influence the degree of its curvature.

Cell shape: According to this feature, the 19 strains were divided into two distinct groups, characterized by either (i) cells flat, longitudinally oval or longitudinally ovoid, ending with a rather inconspicuous tail and including strains ACOI 1088, ACOI 2434, SAG 1261-1, SAG 1261-2b, and SAG 1261-3b (Fig. 1, a–e); or (ii) cells flat, widely oval, ending with a more or less conspicuous tail curved to a certain, varying degree and comprising the remaining 14 strains (Fig. 1, f–m, p, r, and s), of which only

TABLE 2. Comparison of cell morphology of *Phacus hamelii*, *P. orbicularis*, and *P. pleuronectes* (length of the cell with the tail included).

Taxa	Strain	Cell length (μm)		Cell width (μm)		Tail (μm)	
		Mean ± SD	Min.–max.	Mean ± SD	Min.–max.	Mean ± SD	Min.–max.
Phacus hamelii	ACOI 1088	32.6 ± 1.4	28.9–36.6	21.5 ± 1.5	17.1–24.4	4.9 ± 0.7	3.3–7.4
	ACOI 2434	31.0 ± 1.5	27.0–34.5	21.6 ± 1.7	18.5–28.9	5.0 ± 0.8	3.0–7.0
Phacus orbicularis	AICB 502	64.3 ± 3.7	54.0–70.8	42.6 ± 2.5	37.4–46.9	12.0 ± 1.5	8.5–15.5
	AICB 525	40.6 ± 1.9	36.0–44.8	29.3 ± 1.9	21.8–33.1	7.0 ± 0,9	5.0–10.0
	ACOI 614	37.2 ± 1.5	31.6–41.0	28.9 ± 1.2	26.0–31.2	5.9 ± 0.8	4.0–8.5
	ACOI 996	44.6 ± 1.9	40.0–49.6	31.3 ± 1.5	25.0–35.0	8.0 ± 1.0	5.0–10.0
	ACOI 1142	40.1 ± 1.8	33.2–44.5	28.8 ± 1.6	23.8–32.7	5.9 ± 0.8	4.0–9.0
	ACOI 1143	38.4 ± 2.3	32.4–46.6	28.1 ± 1.7	23.3–31.7	6.0 ± 0.7	4.0–8.0
	ACOI 1144	42.0 ± 1.6	37.0–45.8	32.0 ± 1.2	29.3–35.2	6.0 ± 0.8	4.0–8.0
	ACOI 1145	36.4 ± 2.6	32.3–42.0	27.5 ± 2.0	24.1–31.9	5.0 ± 0.7	3.0–6.5
	ACOI 2349	66.5 ± 2.7	57.5–73.7	42.5 ± 1.6	38.7–47.0	13 ± 2.2	7.5–17.0
	ACOI 2376	64.0 ± 3.2	54.0–74.0	40.7 ± 3.3	29.0–48.5	11.0 ± 1.6	7.0–16.0
	ACOI 2423	63.8 ± 2.5	57.3–69.2	41.0 ± 2.8	33.8–49.4	11.0 ± 1.7	7.7–16.0
	ACOI 2437	69.5 ± 2.6	62.2–75.0	42.9 ± 2.3	36.8–48.7	12.5 ± 1.6	8.0–16.7
	ACOI 2955	36.9 ± 1.3	34.0–40.0	27.6 ± 0.9	24.8–30.0	5.4 ± 0.6	4.0–7.0
	ACOI 2996	33.0 ± 1.9	29.0–37.5	27.3 ± 1.5	23.2–30.5	5.7 ± 0.7	4.0–7.5
Phacus pleuronectes	SAG 1261-3b	43.6 ± 1.6	39.1–48.9	27.7 ± 1.7	22.4–33.3	5.4 ± 0.9	3.5–8.5
	SAG 1261-2b	51.7 ± 4.0	39.4–55.0	25.8 ± 2.5	22.4–32.0	5.0 ± 0.7	4.1–6.9
	SAG 1261-1	44.8 ± 1.8	38.7–50.2	26.5 ± 2.0	21.7–31.3	5.2 ± 0.5	3.8–6.5

the strain ACOI 2996 (Fig. 1m) had distinctively asymmetrical cells.

Nevertheless, there was a relatively substantial intrastrain variation of the cell shape, caused by accumulation of paramylon grains. Such paramylon-loaded cells were subject to various deformations, becoming rounder and less flat (Fig. 1, i and j). Cells with collapsing or indented edges were observed very rarely in populations that were young, but frequently in ones that were aging. This characteristic was related to the overall degradation of the cell (and affected cells in bad physiological shape) and not to the accumulation of paramylon.

Number and shape of large paramylon grains: This characteristic changed in relation to deteriorating growth conditions. In all young (several days old) cultures growing in fresh media, the cells were dividing vigorously and possessed one or two paramylon grains that were larger than the rest and were usually platelike (Fig. 1, a, c, d, and h–l), though sometimes ringlike (Fig. 1, b, m, p, and s). As the population aged, becoming more overcrowded, one (but sometimes two or more) of the paramylon grains began to increase in size, eventually nearly occupying the entire volume of the cell (Fig. 1, i–l). Thus, in the 2- to 3-month-old cultures, most of the cells had one or two (sometimes more) rather large paramylon grains (Fig. 1, i and j). Their shape seemed to depend on the physiological condition of the cell and differed between individual cells.

Fold size and length: The fold, running along the upper (convex) side of the cell, was the characteristic feature of individual cells, changeable within populations and dependent, to some extent, on the number and size of the paramylon grains accumulated in the cytoplasm (Fig. 1, d–g, m, r, and s). In the cells full of the storage material, and thus more oblate, the fold was shorter and less protruding. Its size was also correlated with the size of the cell. The most conspicuous and lengthy folds were observed in strains with large cells, such as AICB 502, ACOI 2349, ACOI 2376, ACOI 2423, and ACOI 2437 (Fig. 1r).

Periplast ornamentation: According to this feature, the 19 strains were divided into two distinct groups. Numerous perpendicular struts were present between longitudinal periplast strips in 14 strains (AICB 502, AICB 525, ACOI 614, ACOI 996, ACOI 1142, ACOI 1143, ACOI 1144, ACOI 1145, ACOI 2349, ACOI 2376, ACOI 2423, ACOI 2437, ACOI 2955, ACOI 2996; Fig. 1, n and u), while in the five remaining strains (ACOI 1088, ACOI 2434, SAG 1261-1, SAG 1261-2b, and SAG 1261-3b), there was only a longitudinal pattern of periplast ornamentation, devoid of perpendicular struts (Fig. 1, o and t).

Phylogenetic analysis: The 18S rDNA data set of 2646 characters was generated for phylogenetic analysis. After the removal of sites of an uncertain homology, which could not be unambiguously aligned, 1617 positions were left in the 18S rDNA alignment of 42 sequences (966 of which were constant). The chi square tests showed the homogenous nucleotide distributions ($P = 0.98$), permitting reliable phylogenetic analyses.

The likelihood ratio test (hLRTs) of the Modeltest program (Posada and Crandall 1998) suggested a SYM + I + G model (Zharkikh 1994) with a fraction of unchangeable nucleotides (I) and a gamma (G) distribution of nucleotide substitution rates, while the Akaike test (AIC) used a general time reversible GTR + I + G model (Lanave et al. 1984, Tavare 1986, Rodriguez et al. 1990) for phylogenetic analyses. These models were applied to calculate the ML tree, with parameters drawn from Modeltest

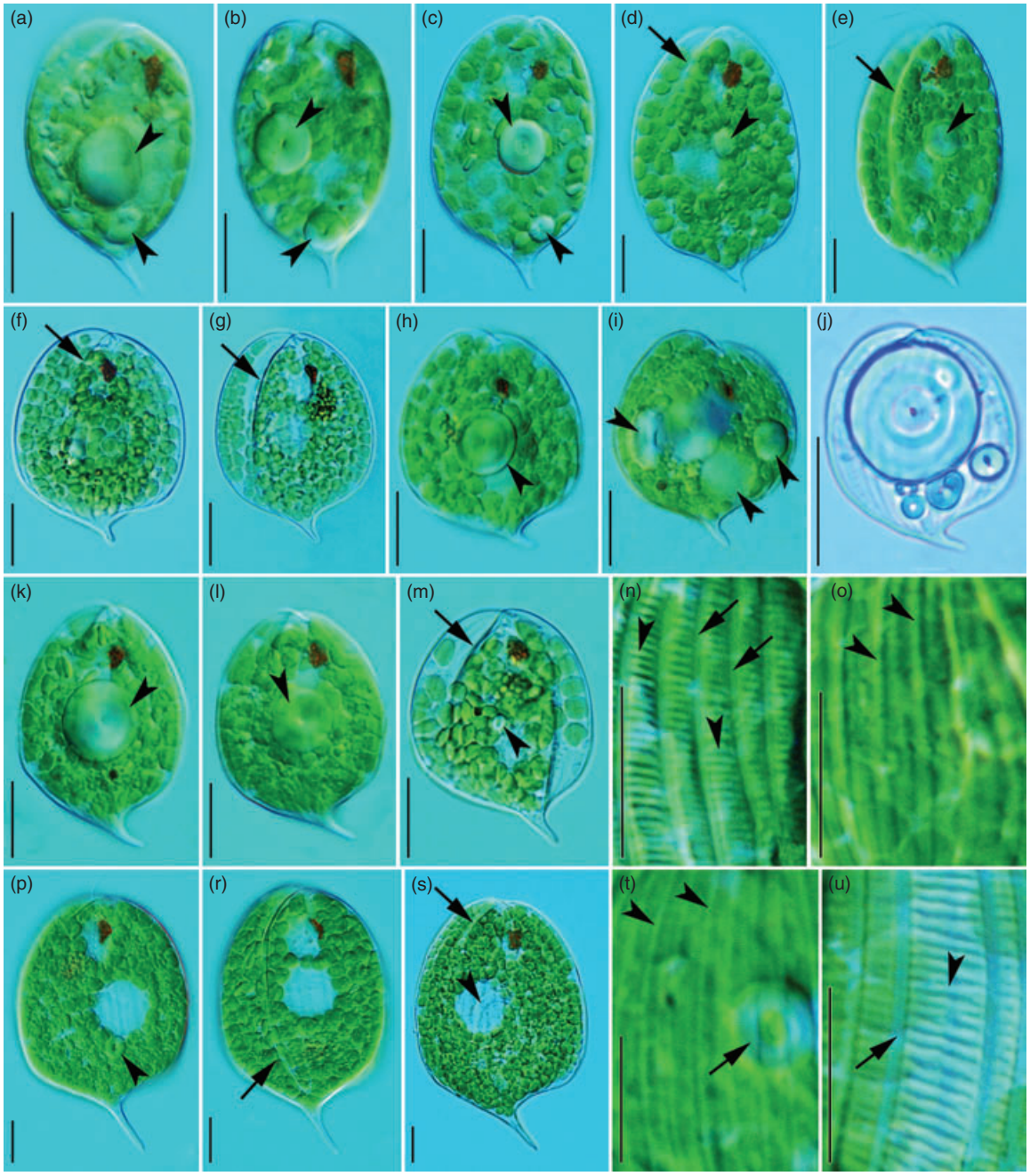

FIG. 1. Light microscope photographs showing an overview of the living cells of *Phacus hamelii*, *Phacus pleuronectes*, and *Phacus orbicularis*. (a, b) Flat, longitudinally ovoid cells of *P. hamelii* (strain ACOI 2434), ending with thin, short, and markedly refluxed tail. Two considerably large, platelike/ringlike paramylon grains (arrowheads) are visible. (c–e) Flat, longitudinally ovoid (egglike) cells of *P. pleuronectes* (strain SAG 1224-3b), ending with a short and refluxed tail. One or two large paramylon grains (arrowheads) and folds of varying lengths (arrows) are visible. (f–m) Flat, widely ovoid cells of *P. orbicularis*, ending with a short, more or less refluxed tail. (f, g) Cells of the strain ACOI 996 with visible folds of varying lengths. (h, i) One or several large paramylon grains visible in the cells of the strain ACOI 2955. (j) A cell of the strain ACOI 614, devoid of chloroplasts, with one large platelike paramylon grain and numerous small, ring-shaped paramylon grains. (k, l) Cells of the strain ACOI 1145, with single, large, platelike paramylon grains (arrowheads). (m) The conspicuously asymmetrical cell of the strain ACOI 2996, with a fold measuring half of the cell's length (arrow). One small, ring-shaped paramylon grain (arrowhead) is visible in the center. (n) Periplast ornamentation of the *P. orbicularis* (strain ACOI 2955) with numerous struts visible (arrowheads), oriented perpendicular to the longitudinal axis of the strips (arrows). (o) Fragment of the *P. hamelii* (strain ACOI 2434) cell surface, with visible longitudinal periplast strips. (p, r, and s) Large, widely ovoid cells of *P. orbicularis*, ending with a relatively long tail. (p, r) One ring-shaped paramylon grain (arrowhead) and a long fold reaching the end of the cell (arrow) are visible in the cells of the strain AICB 502. (s) The cell of the strain ACOI 2437, with a short fold (arrow) and a single, large, ring-shaped paramylon grain (arrowhead). (t) Longitudinal periplast ornamentation (arrowheads) of the cell of *P. pleuronectes* (strain SAG 1224-3b) with visible ring-shaped paramylon grain (arrow). (u) Fragment of *P. orbicularis* (strain ACOI 2437) cell surface. Numerous perpendicular struts (arrowhead) are located between longitudinal periplast strips (arrow). Scale bars, 10 μm.

results or estimated by the phylogeny inferring program (BA), and the calculations produced virtually identical trees with respect to the *Phacus* branch.

Figure 2 shows an 18S rDNA-BA-phylogenetic tree of the *Phacus* genus with the *Lepocinclis* genus as an outgroup. The ML tree had essentially the same topology as the BA tree, but lower branch supports (bs, bootstrap). The Ln likelihood score of the ML best tree was −11157.6. The genus *Phacus* on this tree is divided into four main clades. The first clade consists of four species: *P. orbicularis*, *P. hamatus*, *P. longicauda*, and *P. platyaulax*. This clade is well supported (posterior probability [pp] = 0.99) and may be divided into two sister groups, each in turn divided into two sister groups (*orbicularis* + *hamatus*, pp = 0.88; *longicauda* + *platyaulax*, pp = 0.95). *Phacus orbicularis* is represented by several strains, all having struts (AICB 502, AICB 525, ACOI 614, ACOI 996, ACOI 1145, ACOI 2349, ACOI 2376, ACOI 2437, ACOI 2955, ACOI 2996, M-1424, and ASW 08054). The last strain is apparently misidentified as *P. pleuronectes*. Its placement in the *P. orbicularis* clade is confirmed by its morphology (the presence of struts between longitudinal periplast strips). The clade of *P. orbicularis* is divided into four well-distinguished clades. No morphologically well-defined feature could be ascribed to any of the four individual clades (see above; Fig. 1, Table 2). *Phacus hamatus* (ASW 08032) may be a sister group to *P. orbicularis* and, again, is apparently misidentified as *P. pleuronectes*. *Phacus hamatus* is distinguished from *P. pleuronectes* by lemon-like cells with a long and considerably curved tail, and from *P. orbicularis* by not having struts (see Discussion).

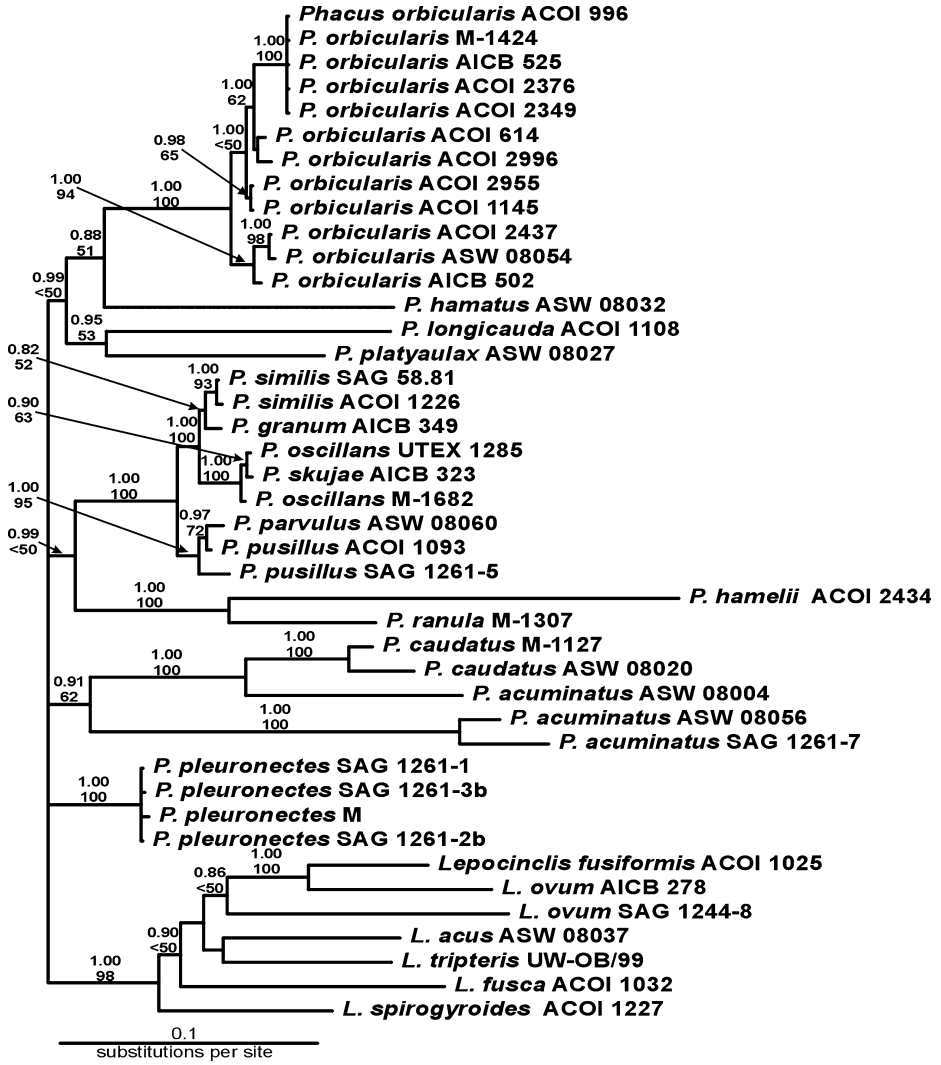

FIG. 2. The phylogenetic tree of the 18S rDNA sequence obtained by Bayesian inference (model GTR + I + G with the following parameters: base frequencies A = 0.219, C = 0.264, G = 0.287, T = 0.231; proportion of invariable sites I = 0.336, shape G = 0.587; substitution rates A–C = 1.057, A–G = 2.935, A–T = 1.223, C–G = 0.337, C–T = 4.474, G–T = 1.000). Numbers at the nodes show posterior probabilities of the tree bipartitions for the Bayesian analysis (upper values) and bootstrap support for maximum-likelihood analysis (lower values). Probabilities of <75% are not shown. Branches leading to nodes with support of <0.5 are collapsed.

The second well-defined and resolved clade is composed of two monophyletic groups. The first group is composed of two very genetically diverged species (*P. hamelii* and *P. ranula*), each represented on the tree by only a single sequence and forming a sturdy clade (pp = 1.00; the 18S rDNA sequences of *P. hamelii* strains ACOI 1088 and ACOI 2434 are identical, R. Triemer, personal communication). The second clade is an assemblage of closely related species (*P. granum, P. oscillans, P. pusillus, P. parvulus,* and *P. similis*), all having cells 30–35 μm long (Pochmann 1942, Popova and Safonova 1976). Resolving their relationships, and those of additional closely related strains, is in progress (our unpublished results).

The third clade is not very well supported (pp=0.91), but its topology is well resolved. It consists of two species (*P. acuminatus* and *P. caudatus*), which are not the scope of this work. The apparently erroneous classification of the strain SAG 1261-7 as *P. brachykentron* (see Discussion) is noteworthy.

The last well-discerned clade is composed of four very closely related sequences of *P. pleuronectes* strains. This clade includes a sequence of the strain SAG 1261-3b, the strain ''M'' from Korea, the strain SAG 1261-2b (erroneously identified as *P. alatus,* but recently verified as *P. pleuronectes* var. *triquetra* by Brosnan et al. [2003]), and a sequence of the strain SAG 1261-1. Our observations confirm that the strain kept presently at SAG under the number 1261-2b is indeed *P. pleuronectes*. The strain SAG 1261-1 is nominally *P. acuminatus*, which is confirmed by its LSU rDNA sequence (Brosnan et al. 2003) and the 18S rDNA sequence of the equivalent UTEX 1288 strain (R. Triemer, personal communication). Its 18S rDNA sequence, however, deposited in GenBank under accession no. AJ532477, is obviously not that of *P. acuminatus*, but of *P. pleuronectes*.

TAXONOMIC REVISION

Phacus pleuronectes (O. F. Müller) Dujard., Hist. Nat. Zoophyt.-Infus.: 336, pl. 5, fig. 5a, b, 1841. Emend. Zakryś et Kosmala.

Emended diagnosis: Cells flat, 39–55 μm long and 22–33 μm wide, ovoid, ending with an inconspicuous tail. Periplast longitudinally striped without struts. With the five SSU rDNA signature sequences:
P1: 5′ ATG GCA CCA CCT GCC AGG TGC CCC T 3′ (helix E 8_1)
P2: 5′ CCA TGC ATC GAT CAG CCA TGA TGG GAC TGC TCG AGG T 3′ (helix 24)
P3: 5′ TGT TGG TGG TGC AAG CTA TCC GTA CGC CAT CAG CAC C 3′ (helix 29)
P4: 5′ GCC CCA GTC CCG CAT TCT GTA GGG CCG GCA CGG TGT T 3′ (helix 43)
P5: 5′ CAC TAC TCC CTC GCG GAG TCC TGC CCG GAA GTG GGT 3′ (helices E 45_1 and 46)

Basionym: Cercaria pleuronectes Müller O. F., Vermium Terrestrium et Fluviatilium, seu Animalium Infusoriorum, Helminthicorum et Testaceorum, non Marinorum, Succincta Historia, 1773: 1(1), p. 70.

Neotype: Euglena pleuronectes Ehrenberg, Abh. Königl. Akad. Wiss. Berlin Phys. Kl. 1830:83, pl. VI, fig. V (1–6). 1832.

Epitype: Permanently preserved material of strain SAG 1261-3b (cells in resin, for electron microscopy), deposited at the herbarium of the Department of Plant Systematic and Geography at Warsaw University, Al. Ujazdowskie 4 PL-00478 Warszawa, Poland. The culture from which the epitype was described has been deposited in the Sammlung von Algenkulturen Pflanzenphysiologisches Institut der Universität Göttingen, Germany. Figure 1, c, d, e, and t are illustrations of the epitype.

Synonyms: P. pleuronectes var. *triquetra* Klebs, Unters. Bot. Inst. Tübingen 1:310, 1883; *P. pleuronectes* var. *insecta* Koczw., Kosmos 40:261, pl. 1, fig. 14, 1915; *P. prunoideus* Roll, Russk. Arch. Protistol. 4:141, 148, pl. 5, fig. 21, 1925; *P. pleuronectes* var. *prunoideus* (Roll) Popova, Opred. Precnov. Vodor. CCCP 7:224, pl. 93, figs. 5–9, 1955; *P. megapyrenoidea* Roll, op. cit. 140, pl. 5, fig. 16, 1925; *P. acuminata* var. *megapyrenoidea* (Roll) Pochmann, Arch. Protistenk. 95:144, fig. 32 o, 1942; *P. pulcher* Roll, op. cit. 141, 148, pl. 5, fig. 20, 1925; *P. granulatus* Roll op. cit. 140, pl. 5, fig. 18, 1925; *P. acuminata* var. *granulata* (Roll) Pochmann op. cit. 144, fig. 32 l, m, 1942; *P. granulatus* var. *laevis* Shi, Acta Phytotax. Sin. 34:107, fig. 1 (1–3), 1996.

Phacus orbicularis Hübner, Programm d. Realgymnasiums Stransund: 5, fig. 1, 1886. Emend. Zakryś et Kosmala.

Emended diagnosis: Cells flat, 29–75 μm long and 22–49 μm wide, widely ovoid, ending with a more or less prominent and curved tail. Fine, numerous struts—perpendicular to the longitudinal axis—located between periplast strips. With the three SSU rDNA signature sequences:
O1: 5′ TTG GCA CCA CCC CTG CCA GGT GCC CTC 3′ (helix E 8_1)
O2: 5′ CCT GTC GGC CAC GAT GGG ACT GCT CGG GGT 3′ (helix 24)
O3: 5′ TCC CTC GCT CGT CGA GCC CTG CCC TGA AGG AGG GT 3′ (helices E 45_1 and 46)

Lectotype: Here designated fig. 1 in Hübner, d. c.

Epitype: Permanently preserved material of strain ACOI 2955, (cells in resin for electron microscopy), deposited at the herbarium of the Department of Plant Systematic and Geography at Warsaw University, Al. Ujazdowskie 4 PL-00478 Warszawa, Poland. The culture from which the epitype was described has been deposited in the Algae Culture Collection of the Department of Botany, University of Coimbra, Portugal, as number

ACOI 2955. Figures 1, h, i, and n are illustrations of the epitype.

Synonyms: P. orbicularis var. *undulatus* Skvortzov, J. Microbiol. 4, 1–2:65, pl. 4, fig. 1, 1917; *Phacus undulatus* (Skvortzov) Pochmann, op. cit. 191, figs. 95, 96, 1942; *P. pleuronectes* var. *australis* Playfair, Proc. Linn. Soc. New South Wales 46:123, 124, pl. 5, fig. 3, 1921; *P. ovoidea* Roll, op. cit. 141, pl. 5, fig. 19, 1925; *P. platalea* Dreżepolski, Kosmos 50:232, fig. 110, 1925; *Phacus zingeri* Roll, op. cit. 142, pl. 5, fig. 24, 1925; *P. orbicularis* var. *cingeri* (Roll) Swirenko, Vizn. prisnov. vodor. URSR 2:69, fig. 80, 1938; *P. orbicularis* fo. *cingeri* (Roll) Safonova in Popova and Safonova, Fl. Spor. Rast. SSSR, 9 (2): 74, pl. 18, figs. 10, 13, 14, 1976; *P. pleuronectes* var. *marginatus* Skvortzov, Ber. Deutsch. Bot. Gesellsch. 46 (2): 115, pl. 2, fig. 33, 1928; *P. orbicularis* var. *caudatus* Skvortzov, op. cit. 109, pl. 2, figs. 4, 5, 1928; *P. orbicularis* fo. *communis* Popova, Izv. Zapadno.-Sybirsk. Fil. Akad. Nauk SSSR, Ser. Biol. 2:59, 1947.

Phacus hamelii Allorge et Lefèvre, Bull. Soc. Bot. France 27:128, figs. 55–57, 1925. Emend. Zakryś et Kosmala.

Emended diagnosis: Cells flat, 24–37 μm long and 12–21 μm wide, longitudinally ovoid, ending with a thin, short, and nearly straight tail, bent off at the end. Periplast longitudinally striped, devoid of perpendicular struts. With the five SSU rDNA signature sequences:
H1: 5′ ATG GCA ACC TCC TTC TGA CCA GTT GCC CGC 3′ (helix E 8_1)
H2: 5′ AGG CGC CGT CCC GGC CGC GGA GGG GAC CGC TCG GGG T 3′ (helix 24)
H3: 5′ CGC CGA GGG CAC ATC ATC ATC CCA TGC CCC CGG CAC CCG 3′ (helix 29)
H4: 5′ GCC TGG GCC TCG CAT CCG GTA GGG TCC GGC ACG GCC G 3′ (helix 43)
H5: 5′ AGT ATA TCT GAC TGT GTC TTG AGC GCG GCC GTG CCC CGC AGG GGG T 3′ (helices E 45_1 and 46)

Lectotype: Here designated fig. 56 in Allorge et Lefèvre, d. c.

Epitype: Permanently preserved material of strain ACOI 2434, (cells in resin, for electron microscopy), deposited at the herbarium of the Department of Plant Systematic and Geography at Warsaw University, Al. Ujazdowskie 4, PL-00478 Warszawa, Poland. The culture from which the epitype was described has been deposited in the Algae Culture Collection of the Department of Botany, University of Coimbra, Portugal, as number ACOI 2434. Figure 1, a, b, and o are illustrations of the epitype.

Synonyms: P. pleuronectes var. *rothertii* Namysłowski, Études Hydrobiologiques: 21, 22, fig. IIIA a–d. 1921; *P. brachykentron* Pochmann op. cit. 145, fig. 33. 1942; *P. pleuronectes* var. *hamelii* (Allorge et Lefèvre) Popova, op. cit. 68, pl. 16, figs. 17, 18, 1947; *P. hameliis* var. *ovatus* Shi, Compil. rep. survey algal resour. South-West. China: 268, pl. 1, figs. 9–11, 1994.

Phacus hamatus Pochmann, op. cit. 182-184, fig. 86, a–f, 1942.

Synonym: P. pleuronectes var. *citriformis* Dreżepolski, Rozpr. Wiadom. Muz. Dzieduszyckich, 7/8:4, pl. 1, fig. 5, 1922.

Commentary for taxonomic revision: We have not considered the varieties *P. pleuronectes* var. *minutus* Playfair (1921) (= *P. minutus* [Playfair] Pochmann 1942), *P. platalea* fo. *minor* Deflandre 1928 (= *P. pseudoplatalea* Pochmann 1942), and *P. pleuronectes* var. *żmudae* Namysłowski 1921 (= *P. circulatus* Pochmann [1942]), distinguished on the basis of the cell size (20–28 × 11–22 μm and 28 × 21 μm, respectively), since we have not observed such small cells in the populations studied. We have not considered the variety *P. pleuronectes* var. *brevicaudata* Klebs 1883 either, since we were unable to observe cells without tails.

DISCUSSION

Phacus pleuronectes *and* Phacus orbicularis. In 1830, Ehrenberg described a new genus *Euglena* and moved into it the species *C. pleuronectes* Müller, which he called *E. pleuronectes* (O. F. Müll.) Ehrenb. He illustrated his work by six figures, which show significantly flattened cells, ending with a short tail. These pictures also show considerable morphological diversification of *E. pleuronectes'* cells. The cells differ with regard to shape—being widely oval (Ehrenberg's figs. 3, 4), oval to ovoid (Ehrenberg's fig. 1), and elliptical (Ehrenberg's fig. 6)—as well as with regard to size (Ehrenberg's figs. 1, 3, 4, and 6) (Ehrenberg 1830 [1832], pl. VI, fig. V [1–6]; also available on the Web page of the Ehrenberg Collection – Institut für Paläontologie, Museum für Naturkunde, Humboldtät Universität zu Berlin, Germany (see link 1 in the supplementary material). A year later, Ehrenberg provided a succinct description of *E. pleuronectes* (Ehrenberg 1831), accompanied by an account of cell size: ''cells 1/48'' (= 45.4 μm) long, elliptic, front outfitted with a lip, tail very short'' (Physik. Abh. Akad. Wiss. Berlin 1831 [1832], pp. 72 and 73; also available on the Web page of the Ehrenberg Collection – Institut für Paläontologie, Museum für Naturkunde, Humboldtät Universität zu Berlin, Germany (see links 2 and 3 in the supplementary material).

Ten years later, paying little attention to its morphology and calling it a type species (*P. pleuronectes*), Dujardin (1841) moved *E. pleuronectes* to a new genus, which he described himself. Only later did Klebs (1883, p. 311) point out the distinctive characteristic of *P. pleuronectes*, namely, the fold protruding from the dorsal convex side of the cell (also known in the literature as the comb), as well as its interpopulation morphological diversification. He consequently distinguished three varieties: var. *brevicaudata* (cell size 31 × 23 μm, without a tail but ending sharply), colorless var. *hyalina* (cell size 36 × 26 μm), and var. *triquetra* (with a fold along the entire length of the cell).

He thus questioned the idea of giving this variety the rank of a species (= *P. triquetrus* [Ehrenb.] Dujard.). Unfortunately, no figures were provided. The length of the tail is not considered to be a diagnostic characteristic (although it is taken into account in the descriptions) by the authors of critical treatises (Namysłowski 1921, Swirenko 1938, Pochmann 1942, Popova 1955, Popova and Safonova 1976). We concur with this opinion since we have observed extensive variability of this feature among individual cells of the same species.

Hübner (1886) described a new species, *P. orbicularis*, which is very similar to *P. pleuronectes*. According to Hübner, it differs from *P. pleuronectes* by having oval-like cells, which are smaller and have a shorter tail. However, the features Hübner considered as diagnostic are in fact rather subjective and not very precise. Therefore, subsequent authors tried to complement descriptions of both taxa, recounting very precisely the morphology of their cells (Lemmermann 1910, Playfair 1921, Roll 1925, Swirenko 1938, Pochmann 1942, Popova and Safonova 1976, and others). Although the results remained unsatisfactory and did not help with identification, they produced numerous reclassifications and descriptions of new taxa of different ranks (species, varieties, and forms), with morphology similar to *P. pleuronectes* and *P. orbicularis*. Thus, considering various characteristics, a multitude of taxa were described.

1. *Phacus pleuronectes* var. *insecta* Koczw. 1915, *P. orbicularis* var. *undulatus* Skvortzov 1917, *P. pleuronectes* var. *marginatus* Skvortzov 1928, and *P. undulatus* (Skvortzov) Pochmann 1942 were described taking into account the degree of indentation of cell ridges.
2. The shape of the cell has allowed the following to be distinguished: *P. pleuronectes* var. *triquetra* Klebs 1883; *P. pleuronectes* var. *citriformis* Drežepolski 1921/1922; *P. pleuronectes* var. *rothertii* Namysłowski 1921; *P. platalea* Drežepolski 1925; *P. prunoideus* Roll 1925; *P. hamelii* Allorge et Lefèvre 1925; *Phacus hamatus* Pochmann 1942; *P. circulatus* Pochmann 1942; *P. pleuronectes* var. *hamelii* (Allorge et Lefèvre) Popova 1947; *P. hamelii* var. *ovatus* Shi 1994; *P. orbicularis* fo. *communis* Popova 1947; *P. orbicularis* var. *citriformis* (Drežep.) Popova 1951; and *P. pleuronectes* var. *prunoideus* (Y. V. Roll) Popova 1955.
3. The shape and the number of large paramylon grains were used to distinguish *P. megapyrenoidea* Roll 1925; *P. pulcher* Roll 1925; *P. granulatus* Roll 1925; *P. acuminata* var. *granulata* (Y. V. Roll) Pochmann 1942; *P. acuminata* var. *megapyrenoidea* (Y. V. Roll) Pochmann 1942; and *P. granulatus* var. *laevis* Shi 1996.
4. The size and the degree of tail curvature were relevant in the creation of *P. pleuronectes* var. *brevicaudata* Klebs 1883; *P. zingeri* Roll 1925; *P. orbicularis* var. *caudatus* Skvortzov 1928; *P. orbicularis* var. *cingeri* (Y. V. Roll) Swirenko 1938; and *P. orbicularis* fo. *cingeri* (Y. V. Roll) Safonova in Popova and Safonova 1976.
5. The size of the cell was involved in the description of *P. gigas* Cunha 1913; *P. pleuronectes* var. *australis* Playfair 1921; *P. pleuronectes* var. *minutus* Playfair 1921; *P. pleuronectes* var. *żmudae* Namysłowski 1921; *P. platalea* Drežepolski 1925; *P. zingeri* Roll 1925; *P. pleuronectes* var. *marginatus* Skvortzov 1928; *P. platalea* fo. *minor* Deflandre 1928; *P. orbicularis* var. *cingeri* (Y. V. Roll) Swirenko 1938; *P. minutus* (Playfair) Pochmann 1942; *P. circulatus* Pochmann 1942; *P. pseudoplatalea* Pochmann 1942; *P. orbicularis* fo. *cingeri* (Roll) Safonova in Popova and Safonova 1976; *P. orbicularis* fo. *communis* Popova 1947; and *P. orbicularis* fo. *gigas* (Cunha) Popova 1947.

Collapsing cell ridges (sometimes described as indentations) were questioned as far as their usefulness as a diagnostic feature by Czosnowski (1948), who described their creation as a consequence of changes in the cytoplasm under adverse conditions (e.g., in the presence of formaldehyde). Popova and Safonova (1976) agreed with these findings and consequently included *P. undulatus* (Skvortzov) Pochm. as synonyms of *P. orbicularis*. Our observations are also consistent with the above interpretation and additionally provide arguments against using other morphological features—such as the number, size, and shape of large paramylon grains; the length of the fold; or the size and degree of tail curvature—as diagnostic characters. The existence of these characteristics may merely be the consequence of variation between individual cells, which is reflected in the stage of development, the physiological shape of the cells, or varying environmental conditions. Consequently, we do not see the justification for the existence of taxa (*P. megapyrenoidea* Y. V. Roll; *P. acuminata* var. *megapyrenoidea* [Y. V. Roll] Pochmann; *P. pulcher* Y. V. Roll; *P. granulatus* Y. V. Roll; *P. acuminata* var. *granulata* [Y. V. Roll] Pochmann; *P. granulatus* var. *laevis* Shi; *P. orbicularis* var. *caudatus* Skvortzov; *P. orbicularis* var. *cingeri* [Y. V. Roll] Swirenko; *P. orbicularis* fo. *cingeri* [Y. V. Roll] Safonova) described by means of these features, with cells similar in shape to *P. pleuronectes* or *P. orbicularis*. We therefore consider all of these taxa to be synonyms of either *P. pleuronectes* or *P. orbicularis*.

There is considerable disagreement in the literature regarding the *P. pleuronectes* cell size (45.4 μm [Ehrenberg 1831]; 49 × 33 μm [Namysłowski 1921]; 45–80 × 30–50 μm [Drežepolski 1925]; 40–80 × 30–50 μm [Pochmann 1942]; 40–100 × 29–70 μm [Allorge et Jahn 1943]; 21–47.5 × 12–34 μm [Popova and Safonova 1976]) and that of *P. orbicularis* (smaller than *P. pleuronectes* [Hübner 1886]; 50–100 × 30–60 μm [Pochmann 1942]; 31–124 × 23–95 μm [Popova and Safonova 1976]). Our biometrical studies revealed a number of *P. orbicu-*

laris strains with large cells—approaching 75 μm in length—and differing considerably from the rest, which did not exceed 56 μm in length (Table 2). However, phylogenetic analysis did not justify the use of cell size as a diagnostic feature. Three strains with large cells, >60 μm long (AICB 502, ACOI 2437, and ASW 08054), do form a well-supported clade, branching off first among the *P. orbicularis* strains; while the other two strains (ACOI 2349, ACOI 2376) belong to another clade, which contains the strains not exceeding 45 μm in length as well (AICB 525, ACOI 996). The results of phylogenetic analysis thus suggest that *P. gigas* Cunha should be included with the *P. orbicularis* Hübner synonyms, or at least the strain ACOI 2437 should be renamed as *P. orbicularis*. However, a population of large cells (>100 μm long) possessing a periplast with longitudinal strips, but not perpendicular struts, was identified in North America (R. Triemer, personal communication). This finding suggests that *P. gigas* Cunha does exist, and ensuing molecular studies should establish its status and relationship to *P. pleuronectes* and *P. orbicularis*.

Our studies also point out periplast ornamentation, described as ''struts'' by Leedale (1985), which are positioned perpendicular to the longitudinal axis of the strips. The struts were present in all *P. orbicularis* strains surveyed by us (Fig. 1, n and u)—all SSU sequences for the *P. orbicularis* strains clade together on the 18S rDNA tree (Fig. 2)—but struts were not observed in *P. pleuronectes* or *P. hamelii* (Fig. 1, o and t). Lefévre (1931) was the first to notice ''perpendicular stripes'' in *P. orbicularis* and take them into account in his drawings. Lefévre's drawings were used later by Pochmann (1942, fig. 78, k and n), albeit without any commentary. This action suggests that both Lefévre and Pochmann, as well as others, did not consider struts to be a diagnostic feature distinguishing *P. orbicularis* from *P. pleuronectes*. This also might be the reason for not considering struts as diagnostic in spite of their appearing on drawings of several other species (*P. rostafiński* [Dreżepolski 1921/1922, pl. 1, fig. 3]; *P. platalea* [Dreżepolski 1925, pl. 3, fig. 110]; *P. caudata* var. *minor* and var. *ovalis* [Dreżepolski 1925, pl. 3, figs. 107 and 111]; *P. longicauda* [Lefévre 1931, pl. 3, fig. 32]; *Phacus triqueter* [Leander and Farmer 2001, fig. 3a]). The presence of perpendicular struts on the iconotype of *P. platalea* Dreżepolski 1925 (fig. 110), as well as its other features, such as the size and shape of the cell and the relatively long (12–15 μm) tail, prompted us to consider *P. platalea* as a synonym of *P. orbicularis*.

In our view, the presence of struts is a good diagnostic feature for distinguishing *P. orbicularis* from *P. pleuronectes* since it is not susceptible to individual, developmental, and environmental variability. Moreover, struts are clearly visible under the light microscope, even in very small cells (in which case, a brief drying out of the material facilitates observations; Fig. 1, n and u).

Our molecular studies show that *P. pleuronectes* and *P. orbicularis* are not as closely related as has been suggested by Dreżepolski (1925), Swirenko (1938), Pochmann (1942), Popova (1951), Popova and Safonova (1976), and many others. Some of the phylogenetic relationships are not resolved on the SSU rDNA tree (Fig. 2), but there are at least three *Phacus* species (*P. hamatus*, *P. platyaulax*, *P. longicauda*) that are more closely related to *P. orbicularis* than is *P. pleuronectes*. Nevertheless, our morphological studies confirm the most prevalent opinion, promoted in monographic and floristic treatises, concerning morphological similarity of the two species, which is nevertheless difficult to assess because of the high variability within the species (Dreżepolski 1925, Swirenko 1938, Pochmann 1942, Popova 1951, Popova and Safonova 1976, and many others).

Phacus hamelii *and* Phacus hamatus. *Phacus hamelii* was described by Allorge and Lèfevre in 1925. Its diagnostic description and iconotype (figs. 55–57) accurately fit *P. pleuronectes* var. *rothertii*, described by Namysłowski in 1921 (fig. IIIA, a–d). While the latter description existed only in Polish, the former included a Latin diagnosis and became accepted in English, French, and German literature. Outside of Poland, Namysłowski's (1921) work was known to Skvortzov (1928), and it was apparently through him that it reached Pochmann (1942), who raised the rank of *P. pleuronectes* var. *rothertii* to the species level, giving it the name *P. brachykentron*. However, it appears that he did this without consulting the original description in Polish, made by Namysłowski (1921), which is manifested by his use of two rather schematic *P. brachykentron* drawings made by Skvortzov (pl. 33, fig. a and b only remotely resembling the original ones made by Namysłowski) and one drawing by Huzel (pl. 33, fig. d in Pochmann 1942), which is more likely an illustration of *P. acuminatus*, instead of Namysłowski's iconotype. Consequently, *P. brachykentron* and *P. hamelii* are practically indiscernible and would have been to Pochmann, who includes both of them in the same monograph (1942), had he realized that they are the same morphological form, merely based on two different basionyms. This error has been repeated by Huber-Pestalozzi (1955), Starmach (1983), Shi (1994), Wołowski (1998), Kusel-Fetzmann (2002), and others. Some (e.g., Németh 1997) consider *P. brachykentron* as a synonym of *P. acuminatus*. In a similar way, Pochmann (1942) had raised other varieties to the rank of species, giving them new names. For example, he gives the names *P. circulatus* Pochmann (1942) to *P. pleuronectes* var. *żmudae* Namysłowski (1921), *P. pseudoplatalea* Pochmann (1942) to *P. platalea* fo. *minor* Deflandre 1928, and *P. hamatus* Pochmann (1942) to *P. pleuronectes* var. *citriformis* Dreżepolski (1925). As a result, there are multitudes of synonyms of *Phacus* species, contributing to the overall taxonomic confusion of the genus.

On the 18S rDNA phylogenetic tree (Fig. 2), *P. hamatus* strain ASW 08032 is a sister group with respect to *P. orbicularis*. *Phacus hamatus* differs from *P. orbicularis* in periplast ornamentation and in cell shape. *Phacus hamatus* has no struts between the longitudinal periplast ribs and has cells resembling a lemon, substantially narrowed at the front, which is reflected in the variety name coined by Drežepolski (*P. pleuronectes* var. *citriformis*). We are not defining the epitype for *P. hamatus*, since we believe that this would require thorough measurements of at least several strains in conditions enabling the assessment of the range of diversity of morphological features.

In the case of *P. hamelii*, we have data on two strains (all available), and the literature is consistent with our findings. Therefore, we estimated the size range for this species at 24–37 × 12–21 μm (24–33 × 15–21 μm [Namysłowski 1921]; 25–37 × 12–20 μm [Allorge and Lefèvre 1925]; 24–33 × 15–21 μm [Pochmann 1942]; 25.7–37 × 12–17.4 μm [Popova and Safonova 1976]; 23–35 × 12–20 μm [Shi 1994]).

Five short SSU rDNA signature sequences (Ekelund et al. 2004, Kosmala et al. 2007) were chosen to distinguish *P. orbicularis* from *P. pleuronectes* and *P. hamelii*. All of them are in the conserved region of rDNA and can be easily compared to homologous sequences from other taxa. The sequences of P1, O1, and H1 correspond to helix E 8_1 in the SSU rRNA secondary structure; sequences P2, O2, and H2 to helix 24; sequences P3 and H3 to helix 29; sequences P4 and H4 to helix 43; and sequences P5, O3, and H5 to helices E 45_1 and 46.

Cell shape would be a good diagnostic feature (when disregarding deformations of any kind), if it could be assessed in an absolute manner. Therefore, cell shape must not be the main diagnostic character. We denominate the cell shape of *P. pleuronectes* as ovoid (Fig. 1, c–e), of *P. orbicularis* as widely ovoid (Fig. 1, f–m, p, r, and s), and of *P. hamelii* as longitudinally ovoid (Fig. 1, a and b).

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Supplementary Material

The following supplementary material is available for this article:

Links to the Web page of the Ehrenberg Collection – Institut für Paläontologie, Museum für Naturkunde, Humboldtät Universität zu Berlin, Germany

Link 1: http://bibliothek.bbaw.de/bibliothek-digital/digitalequellen/schriften/anzeige/index_html?band=07-abh/1830&seite:int=124

Link 2: http://bibliothek.bbaw.de/bibliothek-digital/digitalequellen/schriften/anzeige/index_html?band=07-abh/1831&seite:int=89

Link 3: http://bibliothek.bbaw.de/bibliothek-digital/digitalequellen/schriften/anzeige/index_html?band=07-abh/1831&aufloesung:int=2&seite:int=90

Link to alignment data: The alignment used for analyses is available in EMBL (ALIGN_000990), at ftp://ftp.ebi.ac.uk/pub/databases/embl/align/.

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