European Journal of Phycology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tejp20

Generic and species concepts in Microglena (previously the Chlamydomonas monadina group) revised using an integrative approach

Eduard Demchenko [a], Tatiana Mikhailyuk [b], Annette W. Coleman [c] & Thomas Pröschold [c d e]

[a] Department of Botany, Taras Shevchenko National University of Kyiv, Institute of Biology, Volodymyrska Str. 64, Kyiv 01001, Ukraine

[b] M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Tereschenkivska Str. 2, Kyiv 01601, Ukraine

[c] Department of Biology and Medicine, Brown University, Providence, RI-02912, USA

[d] Department of Limnology, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria

[e] Department of Applied Ecology, University of Rostock, Albert-Einstein-Str. 3, D-18059 Rostock, Germany

Version of record first published: 09 Aug 2012

To cite this article: Eduard Demchenko, Tatiana Mikhailyuk, Annette W. Coleman & Thomas Pröschold (2012): Generic and species concepts in Microglena (previously the Chlamydomonas monadina group) revised using an integrative approach, European Journal of Phycology, 47:3, 264-290

To link to this article: http://dx.doi.org/10.1080/09670262.2012.678388

Eur. J. Phycol., (2012), **47(3)**: 264–290

Taylor & Francis
Taylor & Francis Group

Generic and species concepts in *Microglena* (previously the *Chlamydomonas monadina* group) revised using an integrative approach†

EDUARD DEMCHENKO[1], TATIANA MIKHAILYUK[2], ANNETTE W. COLEMAN[3] AND THOMAS PRÖSCHOLD[3,4,5]

[1]*Department of Botany, Taras Shevchenko National University of Kyiv, Institute of Biology, Volodymyrska Str. 64, Kyiv 01001, Ukraine*
[2]*M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Tereschenkivska Str. 2, Kyiv 01601, Ukraine*
[3]*Department of Biology and Medicine, Brown University, Providence, RI-02912, USA*
[4]*Department of Limnology, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria*
[5]*Department of Applied Ecology, University of Rostock, Albert-Einstein-Str. 3, D-18059 Rostock, Germany*

(Received 8 August 2011; revised 1 January 2012; accepted 18 January 2012)

Traditionally the genus *Microglena* Ehrenberg has been used to contain species that belong to the Chrysophyceae; however, the type species of *Microglena*, *M. monadina*, represents a green alga, which was later transferred to the genus *Chlamydomonas*. The taxonomic status of the genus has therefore remained unclear. We investigated 15 strains previously assigned to *C. monadina* and two marine species (*C. reginae* and *C. uva-maris*) using an integrative approach. Phylogenetic analyses of SSU and ITS rDNA sequences revealed that all strains form a monophyletic lineage within the Chlorophyceae containing species from different habitats. The strains studied showed similar morphology with respect to cell shape and size, but showed differences in chloroplast and pyrenoid structures. Some representatives of this group have the same type of sexual reproduction (homothallic advanced anisogamy). Three different morphotypes could be recognized. Strains belonging to type I have a cup-shaped chloroplast with a massive basal part, in which a large, single, ellipsoidal pyrenoid is located. The members of type II also have a cup-shaped chloroplast, which is partly lobed and has a thinner basal part than type I; here the pyrenoid is half-ring or horseshoe-shaped and occupies different positions in the chloroplast depending on the strain. The strains of type III have multiple pyrenoids, which appear to have developed from the subdivision of a single ring-shaped pyrenoid into several parts. We compared the results of our morphological investigations with the literature and found that 15 strains could be identified with existing species. Two strains did not fit with any described species. As a result of our study, we transfer all strains to the genus *Microglena*, propose 11 new combinations, and describe two new species. Comparison of the ITS-1 and ITS-2 secondary structures confirmed the species delineations. All species have characteristic compensatory base changes in their ITS secondary structures and are supported by ITS-2 DNA barcodes.

Key words: *Chlamydomonas*, Chlorophyceae, DNA barcode, ITS-2 DNA Barcode, *Microglena*, molecular phylogeny, *Monadina*-clade, phenotypic plasticity, species concept, systematics

Introduction

The genus *Chlamydomonas* Ehrenberg comprises biflagellate unicellular green algae, which are distributed in almost all habitats. More than 800 species have been described, many of them so incompletely that it is difficult or impossible to determine what they are. Therefore, Ettl (1976, 1983) recognized only around 400 species. Phylogenetic analyses have clearly demonstrated that the traditional genus *Chlamydomonas* is polyphyletic (Buchheim *et al.*, 1990, 1996; Hepperle *et al.*, 1998) and can be split into eight independent, well-supported lineages within the so-called clockwise group of the Chlorophyceae (Pröschold *et al.*, 2001; Pröschold & Leliaert, 2007). Taxonomic revision of the genus has been initiated by the typification of *Chlamydomonas reinhardtii* Dangeard as the conserved type (Pröschold & Silva, 2007) and by the description of two new genera *Oogamochlamys* and *Lobochlamys* (Pröschold *et al.*, 2001). Another of the eight well-supported lineages is the '*Monadina*'-clade, which is not related to *C. reinhardtii* and until

Correspondence to: Thomas Pröschold. E-mail: thomas.proeschold@uni-rostock.de

†Molecular phylogeny and taxonomic revision of *Chlamydomonas* (Chlorophyta). II.

ISSN 0967-0262 print/ISSN 1469-4433 online/12/0300264–290 © 2012 British Phycological Society
http://dx.doi.org/10.1080/09670262.2012.678388

now has contained only a few strains, the only named species *C. monadina* (Ehrenberg) Stein (SAG 31.72) and different unidentified strains isolated from polar regions. However, the taxonomic status of the *Monadina*-clade has remained unclear and is the focus of this study.

Chlamydomonas monadina was originally described by Ehrenberg (1832) as *Microglena monadina*. The second species of this genus described by Ehrenberg (1832) was *Microglena volvocina* Ehrenberg, which was later transferred to the genus *Trachelomonas* (Euglenophyta) as *T. volvocina* (Ehrenberg) Ehrenberg (1834). Since Ehrenberg, six species of Chrysophyceae have been described under the name *Microglena* (see Ettl, 1978), e.g. *M. arenicola* Droop. Stein (1878) transferred *Microglena monadina* to *Chlamydomonas*, with the consequence that the only algal species referred to *Microglena* during the last century have been chrysophycean algae. In addition, the generic names *Microglena* Lönnroth and *Microglaena* Körber have also been used and refer to a genus of lichen, described independently twice. According to the International Code for Botanical Nomenclature (ICBN) the name *Microglena* Ehrenberg has priority against both *Microglena* Lönnroth and *Microglaena*. This was recognized by Mayrhofer and Poelt (1985), who made both lichen generic names synonyms of *Thelenella* Nylander. However, *Microglena* remains a valid name for chlorophycean algae related to the type species *M. monadina*.

As mentioned above, recent studies have shown that *Chlamydomonas*-like species isolated from snowfields and ice of different regions belong to the *Monadina*-clade (Leya, 2004; Liu *et al.*, 2006; Eddie *et al.*, 2008). We compared these data with our investigations of 15 strains previously designated as *C. monadina* from public culture collections and two marine species (*C. reginae* Ettl & J.C. Green and *C. uva-maris* Butcher), using the integrative approach proposed by Pröschold & Leliaert (2007). Phylogenetic analyses of SSU and ITS rDNA sequences were made to establish the relationships of the strains and to establish an appropriate generic classification. Comparisons of morphology and studies of the ITS secondary structure were made to determine species limits and the newly developed, unique ITS-2 DNA Barcode was used to provide a basis for unambiguous future identifications.

Materials and methods

Cultures and light microscopy

Strains were obtained from the Sammlung von Algenkulturen, University of Göttingen, Germany (SAG: Schlösser, 1994; www.epsag.uni-goettingen.de), Culture Collection of Algae, University of Cologne, Germany (CCAC; www.ccac.uni-koeln.de) and Algal Collection of Kyiv University, Ukraine (ACKU; Kostikov *et al.*, 2009); they are listed in Table S1 (Supplementary material). The freshwater strains were grown in modified Bold's Basal Medium (3N-BBM + V: medium 26a in Schlösser, 1997), both marine strains in modified artificial seawater medium (MASM: www.ccap.ac.uk/media/documents/MASM_000.pdf). The cultures were grown in Erlenmeyer flasks at 20°C, 50 $\mu E\,m^{-2}\,s^{-1}$ light intensity and a light : dark-cycle of 14 : 10 h. Sexual reproduction was induced using a similar method to that described for *Oogamochlamys* in Pröschold *et al.* (2001): dense cell suspensions from 14-day-old cultures in Erlenmeyer flasks were centrifuged at low speed and the pellets were resuspended in either 2 ml 3N-BBM + V or in 2 ml BBM–N + V (the same medium without sodium nitrate). These suspensions were transferred into the shallow centre of special cell culture dishes (Fa. Corning, NY, USA, 60 mm × 15 mm, Costar No. 3260). In the surrounding part of these dishes 2–3 ml of distilled water were added to reduce evaporation. Observations were made with ZEISS Axio Imager (Zeiss, Oberkochen, Germany) and Olympus BX60 microscopes (Olympus, Tokyo, Japan), using the ZEISS and Cell^D imaging software respectively, and with an XSP-XY microscope (Ningbo Shengheng Optics & Electronics, Ningbo, China).

DNA isolation, PCR and sequencing

Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen GmbH). The SSU and ITS rDNA were PCR amplified according to Luo *et al.* (2006), using the Taq PCR Mastermix Kit (Qiagen, Hilden, Germany) with the primers EAF3 and ITS055R (Pröschold *et al.*, 2001). The PCR products were purified using the Qiagen PCR purification following the instructions provided by the manufacturer. Then the purified PCR products were sequenced with an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA) using the primers (EAF3, E528F, 920F, BR, N920R, 536R, GF and GR; Marin *et al.* [2003], Pröschold *et al.* [2005]). The nucleotide sequences are available in the EMBL, GenBank and DDBJ sequence databases under the accession numbers given in Fig. 1 and Table S1.

Phylogenetic analyses

The SSU rDNA sequences were aligned according to their secondary structure by comparison of the structure presented for *Chlamydomonas reinhardtii* M32703 (Wuyts *et al.*, 2000) and included in a dataset (1524 base-pairs [bp]) containing 85 taxa of all representative clades belonging to the clockwise (CW-) group of Chlorophyceae (Gerloff-Elias *et al.*, 2005; Pröschold & Leliaert, 2007). The sister clade of the CW-group, the *Chaetophora*-clade *sensu* Pröschold *et al.* (2001) was used as the outgroup. The ITS-1 and ITS-2 sequences of all strains were folded by using the program mfold

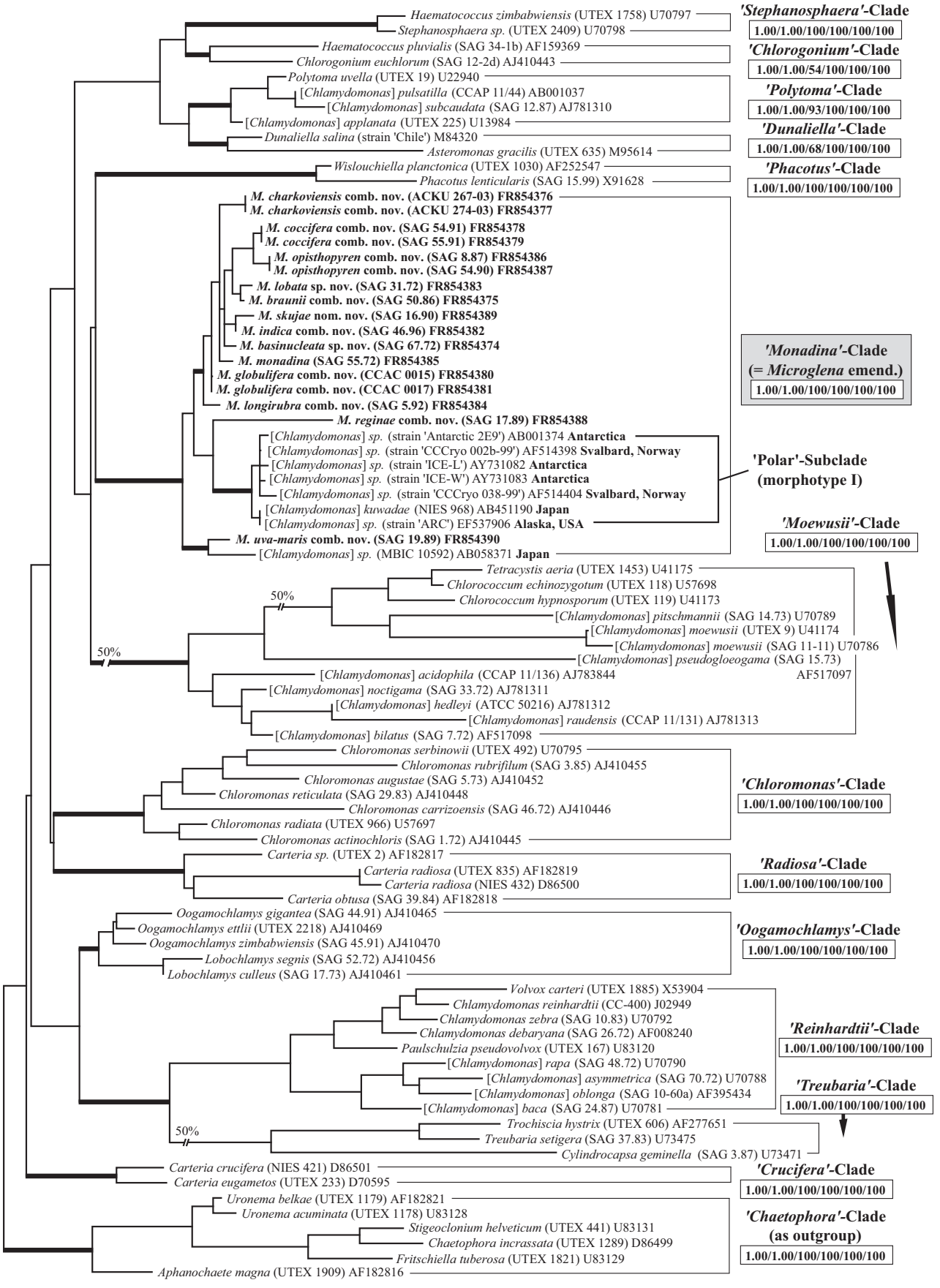


Fig. 1. Molecular phylogeny of the clockwise group of the Chlorophyceae, based on SSU rDNA sequence comparisons. The phylogenetic tree shown was inferred by maximum likelihood method based on a dataset of 1524 aligned positions of 85 taxa using PAUP 4.0b10. For the analysis, the GTR model (base frequencies: A 0.2465, C 0.2107, G 0.2819, T 0.2609; rate

(*continued*)

(Mathews *et al.*, 1999; Zuker, 2003; mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form) and their structures are summarized in Fig. S1 (Supplementary material), produced using the programs LoopDloop (Gilbert, 1992) and Adobe Illustrator CS2 (Adobe, San Jose, California). Based on these structures, ITS-1 and ITS-2 were separately aligned manually and using the program MARNA (Siebert & Backofen, 2005: www.bioinf.uni-freiburg.de/Software/MARNA/index.html) to avoid any bias in the alignments. The resulting alignments of the 17 strains were included in a concatenated dataset (2866 bp) of SSU (1775 bp), ITS-1 (435 bp), 5.8S (159 bp), ITS-2 (477 bp) and LSU (20 bp) rDNA sequences. The alignments are available via TreeBASE (http://www.treebase.org) under the number S12530.

To determine the evolutionary model that fits best for both datasets the program Modeltest 3.7 (Posada, 2008) was used. Based on the results of these tests, the best models were selected by the Akaike Information Criterion (Akaike, 1974). For both datasets the GTR model with a proportion of invariable sites (I), and a gamma shape parameter (G) was used for the phylogenetic analyses. The phylogenetic trees (Figs 1 and S2) were inferred by distance (neighbour-joining [NJ] using the GTR + I + G model), parsimony (MP), and maximum likelihood (ML; using GTR + I + G) criteria using PAUP version 4.0b10 (Swofford, 2002), by randomized accelerated maximum likelihood using RAxML version 7.0.3 (Stamatakis, 2006), and by Bayesian inference (BI) using MrBayes version 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) and the PHASE package 2.0 (Jow *et al.*, 2002; Higgs *et al.*, 2003; Hudelot *et al.*, 2003; Gibson *et al.*, 2005; Telford *et al.*, 2005). The RAxML analyses of the concatenated dataset were performed partitioned according to their genes. For PHASE analyses, two models were used according to their secondary structure, the THREESTATE model (Tavare, 1986) being used for the unpaired regions and the RNA7D model (Tillier & Collins, 1998) for the paired regions.

To find molecular signatures (DNA barcodes) for all species of *Microglena*, the conserved region of ITS-2 was extracted manually from the alignment created by MARNA. According to the recommendation of Coleman (2009), the conserved region, using the 15 bp of the 5.8S-LSU stem, the first 5 bp of Helix I, the first 11 bp of Helix II (including the pyrimidine–pyrimidine mismatch), and all base-pairs of Helix III were selected for the DNA barcode (highlighted in grey boxes in Fig. 2). The resulting sequences were manually aligned again and proven automatically using MARNA (Fig. 2). This alignment was used to find compensatory base changes (CBCs; see Table S2) using the program CBCAnalyzer version 1.1 (Wolf *et al.*, 2005). The uncorrected p-distances were calculated using PAUP. The differences (CBCs and distances) among the species are summarized in Table 1. This sequence alignment was translated into the base-pair alignment by replacing each base-pair by a number (A–U = 1; U–A = 2, G–C = 3, C–G = 4, G•U = 5, U•G = 6, mismatch = 7, deletion/unpaired or single bases = 8; Fig. 3). From this base-pair alignment a NEXUS file was created for the maximum parsimony analysis calculated in PAUP (Fig. 4).

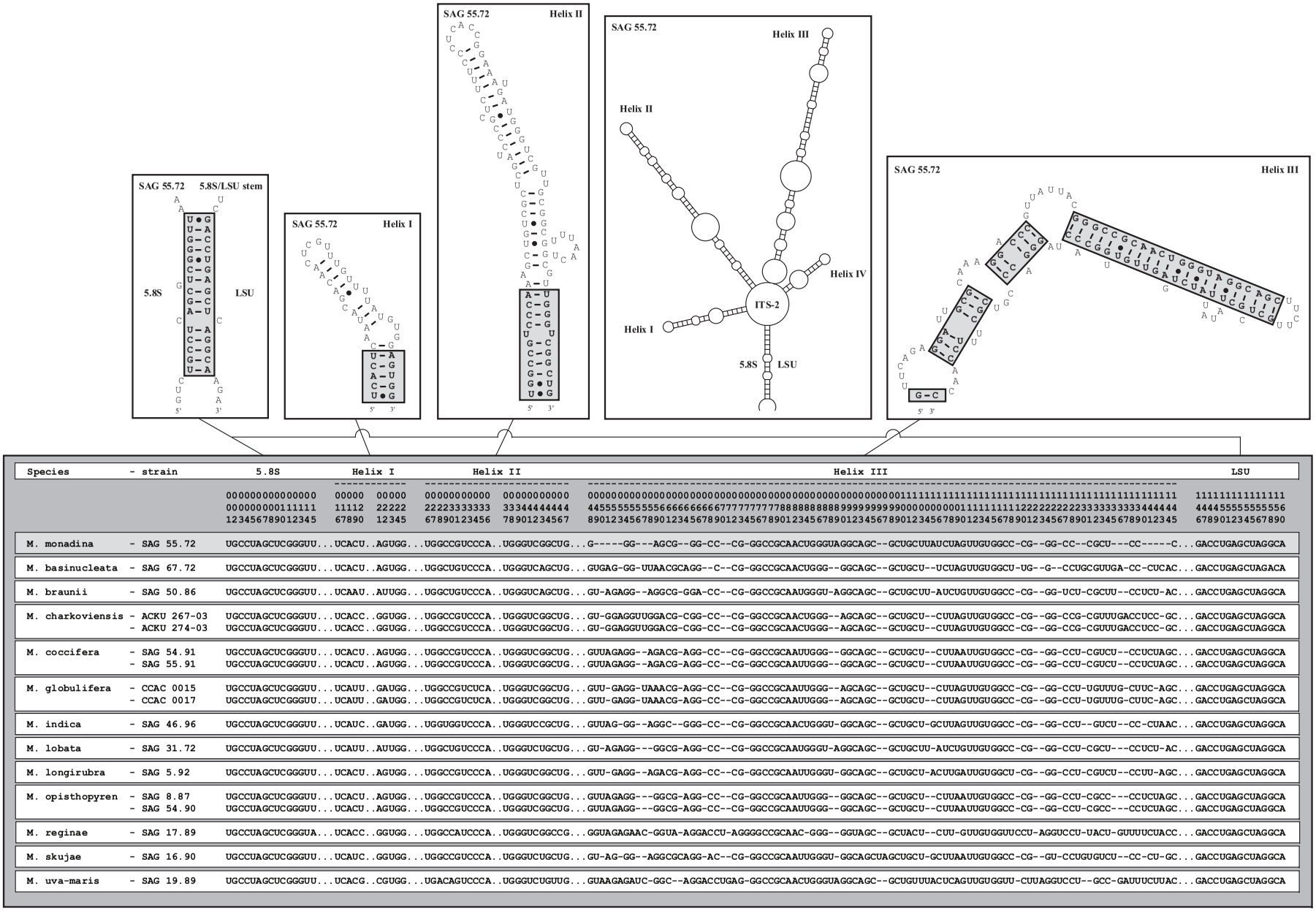
Results

Molecular phylogeny, secondary structures and DNA barcoding

To examine whether all strains studied belonged to a monophyletic lineage, a SSU rDNA dataset was established including representatives of the clockwise group among the Chlorophyceae, with the *Chaetophora*-clade *sensu* Pröschold *et al.* (2001) as outgroup. The phylogenetic analyses presented in Fig. 1 showed that all strains investigated in this study belonged to the *Monadina*-clade (=*Microglena*; see below), together with several strains previously isolated and sequenced from snowfields or glaciers of polar regions or from marine habitats in Japan (the 'Polar-subclade' *sensu* Eddie *et al.*, 2008, in Fig. 1). The principal clades of clockwise Chlorophyceae, including the *Monadina*-clade, have a high posterior probability (Bayesian: PHASE and MrBayes) and bootstrap proportion (ML, NJ, MP) in all analyses, with the exception of the *Chlorogonium* and *Dunaliella* clades, which have high Bayesian support but no or weak bootstrap support in the ML analysis.

In order to get better resolution within the *Monadina*-clade, a concatenated dataset of SSU and ITS rDNA sequences was aligned according to their secondary structures and analysed using different phylogenetic methods with the two marine strains (SAG 17.89 and SAG 19.89) as outgroup. Figure S2 shows that the 15 strains

Fig. 1. Continued
matrix: A-C 1.1409, A-G 2.6738, A-T 1.4865, C-G 0.9087, C-T 5.3353, G-T 1.0000) with the proportion of invariable sites (I = 0.5213) and gamma distribution shape parameter (G = 0.5923) was chosen, which was calculated as best model by Modeltest 3.7. Bayesian values (>0.95) were calculated by MrBayes 3.1 (first value in boxes) using the covarion settings (5 million generations) and PHASE 2.0 (second value) using THREESTATE and RNA7D models for unpaired and paired nucleotides respectively. Bootstrap values (>50%) of the maximum likelihood (using the GTR + I + G model, 100 replicates; third value) using PAUP, the randomized accelerated maximum likelihood using the RAxML 7.0.3 (using the GTR + I + G model, 1000 replicates; fourth value), neighbour-joining (using the GTR + I + G model, 100 replicates; fifth value), and maximum parsimony (1000 replicates; sixth value) were marked in boxes and only given for the clades. The strains marked in bold are new sequences in this study. Strain and accession numbers are given after the species name. The clade designation follows Pröschold & Leliaert (2007). The *Chaetophora*-clade was used as outgroup.



Species	- strain	5.8S	Helix I	Helix II	Helix III	LSU
		000000000000000	00000 00000	00000000000 00000000000	000000000000000000000000000000000000000000000000000000000000011111111111111111111111111111111111111111111111111111	111111111111111
		000000000111111	11112 22222	22223333333 33344444444	445555555555666666666677777777778888888888999999990000000000011111111112222222223333333333444444	444455555555556
		123456789012345	67890 12345	67890123456 78901234567	890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345	678901234567890
M. monadina	- SAG 55.72	UGCCUAGCUCGGGUU...UCACU..AGUGG...UGGCCGUCCCA..UGGGUCGGCUG...G-----GG---AGCG--GG-CC--CG-GGCCGCAACUGGGUAGGCAGC--GCUGCUUAUCUAGUUGUGGCC-CG--GG-CC--CGCU---CC-----C...GACCUGAGCUAGGCA				
M. basinucleata	- SAG 67.72	UGCCUAGCUCGGGUU...UCACU..AGUGG...UGGCUGUCCCA..UGGGUCAGCUG...GUGAG-GG-UUAACGCAGG--C--CG-GGCCGCAACUGGG--GGCAGC--GCUGCU--UCUAGUUGUGGCU-UG--G--CCUGCGUUGA-CC-CUCAC...GACCUGAGCUAGACA				
M. braunii	- SAG 50.86	UGCCUAGCUCGGGUU...UCAAU..AUUGG...UGGCUGUCCCA..UGGGUCAGCUG...GU-AGAGG--AGGCG-GGA-CC--CG-GGCCGCAAUGGGU-AGGCAGC--GCUGCUU-AUCUGUUGUGGCC-CG--GG-UCU-CGCUU--CCUCU-AC...GACCUGAGCUAGGCA				
M. charkoviensis	- ACKU 267-03	UGCCUAGCUCGGGUU...UCACC..GGUGG...UGGCCGUCCCA..UGGGUCGGCUG...GU-GGAGGUUGGACG-CGG-CC--CG-GGCCGCAACUGGG--AGCAGC--GCUGCU--CUUAGUUGUGGCC-CG--GG-CCG-CGUUUGACCUCC-GC...GACCUGAGCUAGGCA				
	- ACKU 274-03	UGCCUAGCUCGGGUU...UCACC..GGUGG...UGGCCGUCCCA..UGGGUCGGCUG...GU-GGAGGUUGGACG-CGG-CC--CG-GGCCGCAACUGGG--AGCAGC--GCUGCU--CUUAGUUGUGGCC-CG--GG-CCG-CGUUUGACCUCC-GC...GACCUGAGCUAGGCA				
M. coccifera	- SAG 54.91	UGCCUAGCUCGGGUU...UCACU..AGUGG...UGGCCGUCCCA..UGGGUCGGCUG...GUUAGAGG--AGACG-AGG-CC--CG-GGCCGCAAUUGGG--GGCAGC--GCUGCU--CUUAAUUGUGGCC-CG--GG-CCU-CGUCU--CCUCUAGC...GACCUGAGCUAGGCA				
	- SAG 55.91	UGCCUAGCUCGGGUU...UCACU..AGUGG...UGGCCGUCCCA..UGGGUCGGCUG...GUUAGAGG--AGACG-AGG-CC--CG-GGCCGCAAUUGGG--GGCAGC--GCUGCU--CUUAAUUGUGGCC-CG--GG-CCU-CGUCU--CCUCUAGC...GACCUGAGCUAGGCA				
M. globulifera	- CCAC 0015	UGCCUAGCUCGGGUU...UCAUU..GAUGG...UGGCCGUCUCA..UGGGUCGGCUG...GUU-GAGG-UAAACG-AGG-CC--CG-GGCCGCAAUUGGG--AGCAGC--GCUGCU--CUUAGUUGUGGCC-CG--GG-CCU-UGUUUG-CUUC-AGC...GACCUGAGCUAGGCA				
	- CCAC 0017	UGCCUAGCUCGGGUU...UCAUU..GAUGG...UGGCCGUCUCA..UGGGUCGGCUG...GUU-GAGG-UAAACG-AGG-CC--CG-GGCCGCAAUUGGG--AGCAGC--GCUGCU--CUUAGUUGUGGCC-CG--GG-CCU-UGUUUG-CUUC-AGC...GACCUGAGCUAGGCA				
M. indica	- SAG 46.96	UGCCUAGCUCGGGUU...UCAUC..GAUGG...UGGUGGUCCCA..UGGGUCCGCUG...GUUAG-GG--AGGC--GGG-CC--CG-GGCCGCAACUGGGU-GGCAGC--GCUGCU-GCUUAGUUGUGGCC-CG--GG-CCU--GUCU--CC-CUAAC...GACCUGAGCUAGGCA				
M. lobata	- SAG 31.72	UGCCUAGCUCGGGUU...UCAUU..AUUGG...UGGCUGUCCCA..UGGGUCUGCUG...GU-AGAGG---GGCG-AGG-CC--CG-GGCCGCAAUGGGU-AGGCAGC--GCUGCUU-AUCUGUUGUGGCC-CG--GG-CCU-CGCU---CCUCU-AC...GACCUGAGCUAGGCA				
M. longirubra	- SAG 5.92	UGCCUAGCUCGGGUU...UCACU..AGUGG...UGGCCGUCCCA..UGGGUCGGCUG...GUU-GAGG--AGACG-AGG-CC--CG-GGCCGCAAUUGGGU-GGCAGC--GCUGCU-ACUUGAUUGUGGCU-CG--GG-CCU-CGUCU--CCUU-AGC...GACCUGAGCUAGGCA				
M. opisthopyren	- SAG 8.87	UGCCUAGCUCGGGUU...UCACU..AGUGG...UGGCCGUCCCA..UGGGUCGGCUG...GUUAGAGG---GGCG-AGG-CC--CG-GGCCGCAAUUGGG--GGCAGC--GCUGCU--CUUAAUUGUGGCC-CG--GG-CCU-CGCC---CCUCUAGC...GACCUGAGCUAGGCA				
	- SAG 54.90	UGCCUAGCUCGGGUU...UCACU..AGUGG...UGGCCGUCCCA..UGGGUCGGCUG...GUUAGAGG---GGCG-AGG-CC--CG-GGCCGCAAUUGGG--GGCAGC--GCUGCU--CUUAAUUGUGGCC-CG--GG-CCU-CGCC---CCUCUAGC...GACCUGAGCUAGGCA				
M. reginae	- SAG 17.89	UGCCUAGCUCGGGUA...UCACC..GGUGG...UGGCCAUCCCA..UGGGUCGGCCG...GGUAGAGAAC-GGUA-AGGACCU-AGGGGCCGCAAC-GGG--GGUAGC--GCUACU--CUU-GUUGUGGUUCCU-AGGUCCU-UACU-GUUUUCUACC...GACCUGAGCUAGGCA				
M. skujae	- SAG 16.90	UGCCUAGCUCGGGUU...UCAUC..GGUGG...UGGCCGUCCCA..UGGGUCUGCUG...GU-AG-GG--AGGCGCAGG-AC--CG-GGCCGCAAUUGGGU-GGCAGCUAGCUGCU-GCUUAAUUGUGGCC-CG--GU-CCUGUGUCU--CC-CU-GC...GACCUGAGCUAGGCA				
M. uva-maris	- SAG 19.89	UGCCUAGCUCGGGUU...UCACG..CGUGG...UGACAGUCCCA..UGGGUCUGUUG...GUAAGAGAUC-GGC--AGGACCUGAG-GGCCGCAACUGGGUAGGCAGC--GCUGUUUACUCAGUUGUGGUU-CUUAGGUCCU--GCC-GAUUUCUUAC...GACCUGAGCUAGGCA				

Fig. 2. Comparison of the conserved DNA Barcode region of ITS-2 among species of *Microglena*. The upper part of the figure shows the ITS-2 secondary structure of SAG 55.72 *M. monadina*, including details of the stem of 5.8S and LSU rDNA, and helices I–III. The conserved regions are highlighted in grey boxes. The bottom part of the figure shows the conserved region of ITS-2 aligned manually or automatically using MARNA. Helices I-IV of ITS-2 are drawn with straight lines for practical reasons.

Table 1. Compensatory base changes (CBCs) and uncorrected *p*-distances among the ITS-2 DNA barcodes of the *Microglena* species. The upper-right half of the table shows the total number of compensatory changes (CBC/Hemi-CBCs), while the lower-left half gives the uncorrected *p*-distances calculated in PAUP.

Species	CBC	*M. monadina*	*M. basinucleata*	*M. braunii*	*M. charkoviensis*	*M. coccifera*	*M. globulifera*	*M. indica*	*M. lobata*	*M. longirubra*	*M. opisthopyren*	*M. reginae*	*M. skujae*	*M. uva-maris*
uncorrected p-distance	Strains	SAG 55.72	SAG 67.72	SAG 50.86	ACKU 267-03 ACKU 274-03	SAG 54.91 SAG 55.91	CCAC 0015 CCAC 0017	SAG 46.96	SAG 31.72	SAG 5.92	SAG 8.87 SAG 54.90	SAG 17.89	SAG 16.90	SAG 19.89
M. monadina	SAG 55.72	0	4 (2/2)	8 (4/4)	6 (4/2)	5 (2/3)	10 (8/2)	9 (5/4)	5 (4/1)	7 (4/3)	4 (2/2)	13 (7/6)	10 (6/4)	13 (7/6)
M. basinucleata	SAG 67.72	0.05911	0	12 (7/5)	13 (8/5)	10 (5/5)	16 (12/4)	13 (7/6)	8 (6/2)	11 (6/5)	10 (5/5)	18 (7/11)	11 (8/3)	16 (7/9)
M. braunii	SAG 50.86	0.11678	0.15049	0	14 (5/9)	11 (5/6)	15 (9/6)	11 (5/6)	2 (1/1)	13 (7/6)	10 (5/5)	18 (7/11)	12 (7/5)	16 (7/9)
M. charkoviensis	ACKU 267-03 ACKU 274-03	0.06571	0.13545	0.16873	0	7 (3/4)	9 (7/2)	9 (6/3)	9 (4/5)	9 (6/3)	7 (2/5)	16 (6/10)	10 (6/4)	17 (9/8)
M. coccifera	SAG 54.91 SAG 55.91	0.06839	0.11841	0.12512	0.08090	0	8 (6/2)	8 (4/4)	6 (4/2)	3 (3/0)	1 (0/1)	14 (5/9)	5 (3/2)	14 (6/8)
M. globulifera	CCAC 0015 CCAC 0017	0.10091	0.15529	0.15852	0.08099	0.07250	0	11 (9/2)	10 (8/2)	11 (9/2)	9 (6/3)	17 (10/7)	9 (7/2)	18 (9/9)
M. indica	SAG 46.96	0.10473	0.14895	0.12997	0.10541	0.08867	0.09718	0	8 (6/2)	12 (8/4)	8 (4/4)	15 (8/7)	6 (4/2)	16 (9/7)
M. lobata	SAG 31.72	0.09790	0.12130	0.03706	0.14889	0.10491	0.13144	0.11663	0	9 (6/3)	6 (4/2)	14 (6/8)	9 (6/3)	12 (6/6)
M. longirubra	SAG 5.92	0.08285	0.12982	0.14325	0.09116	0.02196	0.09411	0.11864	0.12225	0	4 (3/1)	14 (5/9)	9 (7/2)	15 (7/8)
M. opisthopyren	SAG 8.87 SAG 54.90	0.05025	0.12013	0.11401	0.08970	0.01492	0.08973	0.09041	0.08953	0.03789	0	13 (5/8)	5 (3/2)	13 (6/7)
M. reginae	SAG 17.89	0.15905	0.19780	0.18983	0.19015	0.16900	0.20536	0.17675	0.15924	0.17639	0.15499	0	15 (8/7)	12 (4/8)
M. skujae	SAG 16.90	0.12154	0.14130	0.15838	0.11075	0.05975	0.09203	0.07413	0.12277	0.09057	0.06090	0.17029	0	15 (9/6)
M. uva-maris	SAG 19.89	0.18214	0.24338	0.23779	0.20971	0.18706	0.19663	0.18883	0.21400	0.19178	0.17228	0.15803	0.19657	0

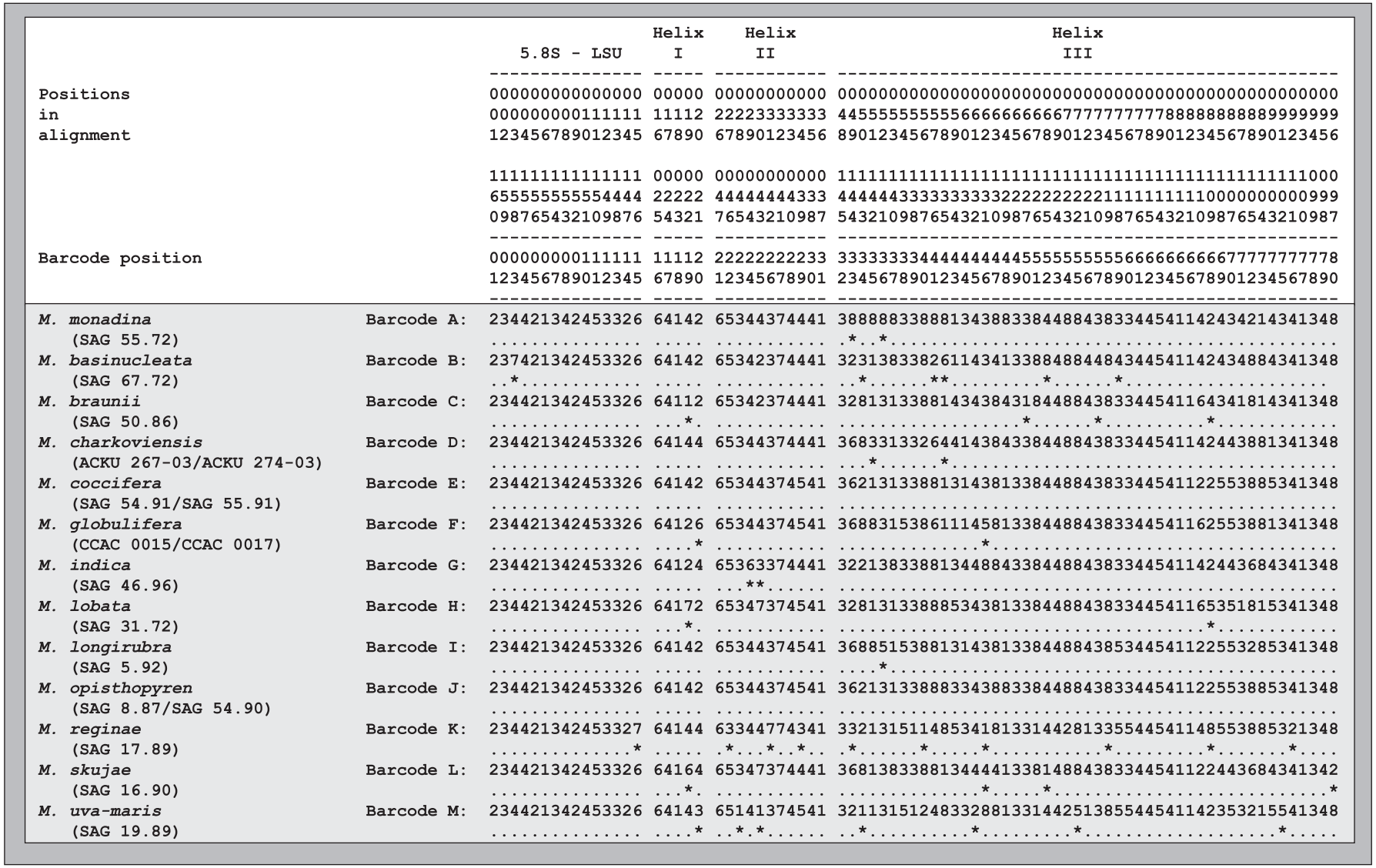


Fig. 3. ITS-2 DNA Barcode of the *Microglena* species. The sequence alignment presented in Fig. 3 was translated into a base-pair alignment using a number coding for each base-pair. The base positions in the sequence alignment are given for comparison. The barcode for each species is named by a letter (A–M). Unique compensatory base changes and non-homoplasious synapomorphies for *Microglena* species are marked by asterisks.

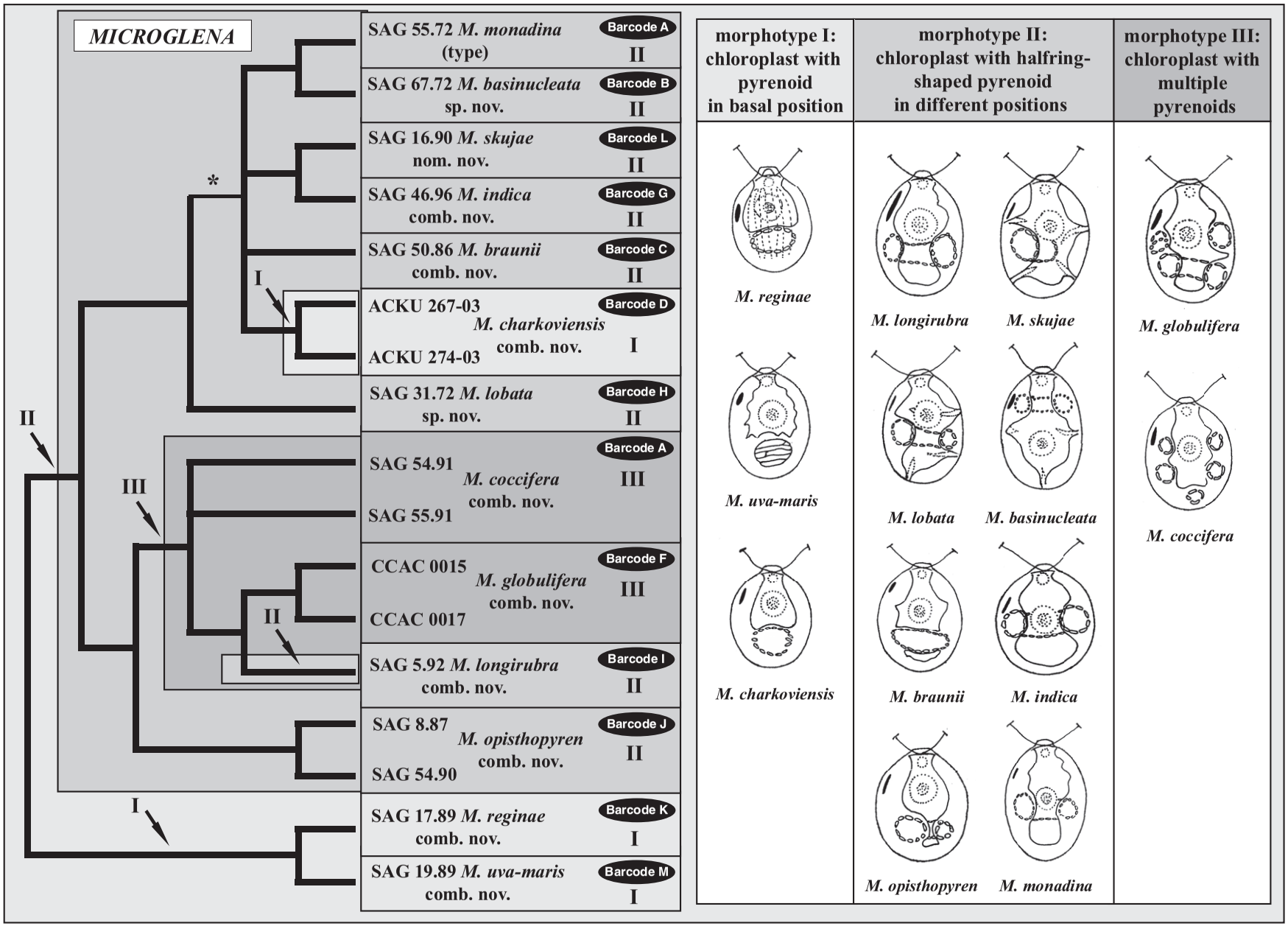


Fig. 4. Molecular phylogeny of the ITS-2 DNA Barcode in comparison to the morphotypes in *Microglena*. The phylogenetic tree represents a majority-rule consensus of five equal trees (120 steps) calculated by maximum parsimony in PAUP. The asterisk marks the only branch that varies among the five trees. Morphotypes I–III are characterized by chloroplast shape and the shape and position of the pyrenoid. Each species is documented by a line drawing and grouped according to morphotype.

previously assigned to *Chlamydomonas monadina* represent 11 lineages. However, the relationships among these lineages were unresolved. To gain further information about relationships and to decide if the 13 lineages in Fig. S2 represent different species, we studied the secondary structures of ITS-1 and ITS-2 (see Fig. S1; Supplementary material); we also used the conserved region of ITS-2 as a DNA barcode, in accordance with the proposal for biological species presented by Coleman (2009). From each ITS-2 sequence, the conserved base-pairs of the helices I–III were selected for the DNA barcode (highlighted in grey boxes in Fig. 2), as described in the Materials and methods. The resulting sequences were manually aligned and checked automatically using MARNA to avoid any bias (Fig. 2). Both alignments were identical. This sequence alignment (160 bp) was translated into a base-pair alignment (80 base-pairs) by replacing each base-pair by a number. Each of the 13 lineages had a characteristic barcode sequence (unique changes are highlighted with an asterisk in Fig. 3) and were also supported by compensatory base changes (summarized in the upper corner in Table 1; a comprehensive analysis of CBCs is summarized in Table S2). Each of the 13 can therefore be interpreted as a separate species. Uncorrected p-distances between them are given in Table 1 (lower corner).

In contrast to the unresolved relationships from the sequence-based SSU–ITS alignment (Fig. S2), phylogenetic analysis (by maximum parsimony) of the base-pair alignment presented in Fig. 3 showed that species with similar morphologies (morphotypes I–III; described in detail below) are more closely related (Fig. 4). Only the relationship of six species belonging to the subclade marked with an asterisk in Fig. 4 remained unresolved.

Morphology of vegetative stages

The strains investigated were not only phylogenetically closely related, they also had similar morphology of the vegetative cells. The vegetative cells of all strains had a broadly ellipsoidal to spherical cell shape; a wide truncate papilla (excluding the marine strain SAG 17.89); two apical contractile vacuoles; two flagella that were as long as the cell length or slightly longer or shorter; an anterior or central, ellipsoidal to rod-like or fusiform stigma; a cup-shaped chloroplast; and a large pyrenoid surrounded by several to many starch grains, which were orientated in parallel rows. The nucleus was always central (excluding SAG 67.72).

However, there were also some morphological differences among the strains studied, which could be allocated to three different morphological groups, based mainly on chloroplast shape. We refer to these as morphotypes I–III (Fig. 4).

The strains belonging to morphotype I had a cup-shaped chloroplast with a thick basal part, in which a single broadly ellipsoidal pyrenoid was located. Algae from morphotype II also had a cup-shaped chloroplast but this was without a thick basal part; the single pyrenoid had a characteristic shape (horseshoe-, sausage-like or half- to quarter-ring shaped) and was located in a lateral ring-like thickening of the chloroplast. Morphotype III comprised algae with a cup-shaped chloroplast lacking a thicker basal part, as in morphotype II, but with several spherical or ellipsoidal pyrenoids located in different places in the lateral part of the chloroplast.

Comparison of the three morphotypes with original species descriptions and several monographs (Pascher, 1927; Korshikov, 1938; Huber-Pestalozzi, 1961; Ettl, 1976, 1983) showed similarities to existing species. Details of the morphological features are summarized in Table 2 and documented in Figs 5–7.

- Morphotype I contains four strains: the authentic strains of both species, *C. reginae* (SAG 17.89 = *Microglena reginae*, *comb. nov.*; see below) and *C. uva-maris* (SAG 19.89 = *M. uva-maris*, *comb. nov.*), isolated from marine habitats, and two strains (ACKU 267-03 and ACKU 274-03) isolated at different times from the same freshwater body in the Ukraine. The first two strains fit the original descriptions (Butcher, 1959; Ettl & Green, 1973), while the two Ukrainian strains could be identified as *Chlamydomonas monadina* var. *charkoviensis* (Korshikov) Korshikov (= *M. charkoviensis*, *comb. nov.*: Korshikov, 1938).
- Nine strains belong to morphotype II. Seven of these could be clearly identified as *Chlamydomonas monadina* var. *monadina* (SAG 55.72 = *M. monadina.*; see below: Ehrenberg, 1832; Stein, 1878), *C. monadina* var. *longirubra* Ettl (SAG 5.92 = *M. longirubra*, *comb. nov.*: Ettl, 1976), *C. opisthopyren* Skuja (SAG 54.90 and SAG 8.87 = *M. opisthopyren*, *comb. nov.*: Skuja, 1956), *C. monadina* var. *indica* Iyengar (SAG 46.96 = *M. indica*, *comb. nov.*: Iyengar & Desikachary, 1981), *C. nova* Skuja (SAG 16.90 = *M. skujae*, *nom. nov.*: Skuja, 1956), and *C. braunii* Goroschankin (SAG 50.86 = *M. braunii*, *comb. nov.*: Goroschankin, 1890). In contrast, two strains did not fit with any diagnoses of described species and are therefore described below as new species (SAG 31.72 = *M. lobata*, *sp. nov.* and SAG 67.72 = *M. basinucleata*, *sp. nov.*: see below).

Table 2. Morphological characters and reproduction of investigated species.

		Morphology of the vegetative cells							Reproduction	
Species	Strains	Cell shape, size in µm	Cell wall, papilla	Chloroplast (morphotype)	Pyrenoid	Stigma	Nucleus	Flagellar length, contractile vacuoles	asexual	sexual
M. monadina	SAG 55.72	ellipsoid to wide ellipsoid, 19–23 × 12–18	thick with large, broad, trapezoid papilla	cup-shaped (II)	halfring-shaped surrounded by many small starch grains	bright, short rod-like in anterior-medial position	central position	as long as the cell; two apical	2–4 zoospores	advanced anisogamy
M. basinucleata	SAG 67.72	ellipsoid to wide ellipsoid, 15–22 × 12–20	thick with large, broad, trapezoid papilla	cup-shaped (II)	halfring-shaped without starch grains	bright, elongated in anterior-medial position	basal position	as long as the cell; two apical	2–4 zoospores	not observed
M. braunii	SAG 50.86	ellipsoid to wide ellipsoid, almost spherical, 17–25 × 11–23	thick with large, broad, trapezoid papilla	cup-shaped (II)	horseshoe-like, halfring-shaped or widely ellipsoid surrounded by many small starch grains	bright, short rod-like in anterior-medial position	central position	as long as the cell; two apical	2–4 zoospores	advanced anisogamy
M. charkoviensis	ACKU 267-03 ACKU 274-03	ellipsoid to wide ellipsoid, 10–16.5 × 8–14.5	thick with large, broad, trapezoid papilla	cup-shaped (I)	widely ellipsoid surrounded by many small starch grains	bright, rod-like to fusiform in anterior-medial position	central position	as long as the cell; two apical	2–4 zoospores	not observed
M. coccifera	SAG 54.91 SAG 55.91	wide ellipsoid, (16)22–24(27) × 19–23(27)	thick with large, broad, trapezoid papilla	cup-shaped (III)	1 to 4–5 (8); widely ellipsoid or spherical surrounded by many small starch grains	bright, rod-like to fusiform in anterior-medial position	central position	as long as the cell; two apical	2–4 zoospores	advanced anisogamy
M. globulifera	CCAC 0015 CCAC 0017	wide ellipsoid to spherical, 16–22 × 10–19	thick with large, broad, trapezoid papilla	cup-shaped perforated with small fissures (III)	horseshoe-like or halfring-shaped surrounded by many small starch grains, fragmented into 3–5 spherical	bright, rod-like to fusiform in anterior-medial position	central position	as long as the cell; two apical	2–4 zoospores	not observed
M. indica	SAG 46.96	spherical to wide ellipsoid, (9)14–18 × (7)13–17	thick with large, broad, trapezoid papilla	cup-shaped (II)	halfring-to almost ring-shaped surrounded by many small starch grains	bright, rod-like in anterior-medial position	central position	as long as the cell; two apical	2–4 zoospores	not observed

(*continued*)

Table 2. Continued.

		Morphology of the vegetative cells							Reproduction	
Species	Strains	Cell shape, size in μm	Cell wall, papilla	Chloroplast (morphotype)	Pyrenoid	Stigma	Nucleus	Flagellar length, contractile vacuoles	asexual	sexual
M. lobata	SAG 31.72	ellipsoid to wide ellipsoid, (12)17–23 × (9)13–20	thick with large, broad, trapezoid papilla	cup-shaped (II)	halfring-shaped surrounded by many small starch grains	small, rod-like in anterior-medial position	central position	as long as the cell; two apical	2–4 zoospores	not observed
M. longirubra	SAG 5.92	ellipsoid to wide ellipsoid, (16)20–26 × (10)17–20	thick with large, broad, trapezoid papilla	cup-shaped (II)	halfring-shaped surrounded by many small starch grains	bright, large, elongated to fusiform in anterior-medial position	central position	as long as the cell; two apical	2–4 zoospores	advanced anisogamy
M. opisthopyren	SAG 8.87 SAG 54.90	ellipsoid to wide ellipsoid, 10–20 × (8)11–17	thick with large, broad, trapezoid papilla	cup-shaped (II)	horseshoe-like, halfring-shaped or widely ellipsoid surrounded by many small starch grains	bright, elongated in anterior-medial position	central position	as long as the cell; two apical	2–4 zoospores	not observed
M. reginae	SAG 17.89	ellipsoid to ovoid, wide ovoid to almost spherical, 15–22 × 13–18	thick with large, broad, trapezoid papilla	cup-shaped (I)	widely ellipsoid surrounded by many small starch grains	bright, elongated in anterior-medial position	central position	as long as the cell; two apical	2–4 zoospores	not observed
M. skujae	SAG 16.90	ellipsoid to wide ellipsoid, (12) 18–23(25) × (8)14–20	thick with large, broad, trapezoid papilla	cup-shaped dissected in several lobes (II)	widely ellipsoid to quarterring-shaped surrounded by many small starch grains	small, rod-like in anterior-medial position	central position	as long as the cell; two apical	2–4 zoospores	not observed
M. uva-maris	SAG 19.89	ellipsoid to wide ellipsoid, 8–17 × 6–13	thick with large, broad, trapezoid papilla	cup-shaped (I)	widely ellipsoid surrounded by several large starch grains	bright, ellipsoid to elongated in anterior-medial position	central position	as long as the cell; two apical, do not pulse in seawater	2–4 zoospores	not observed

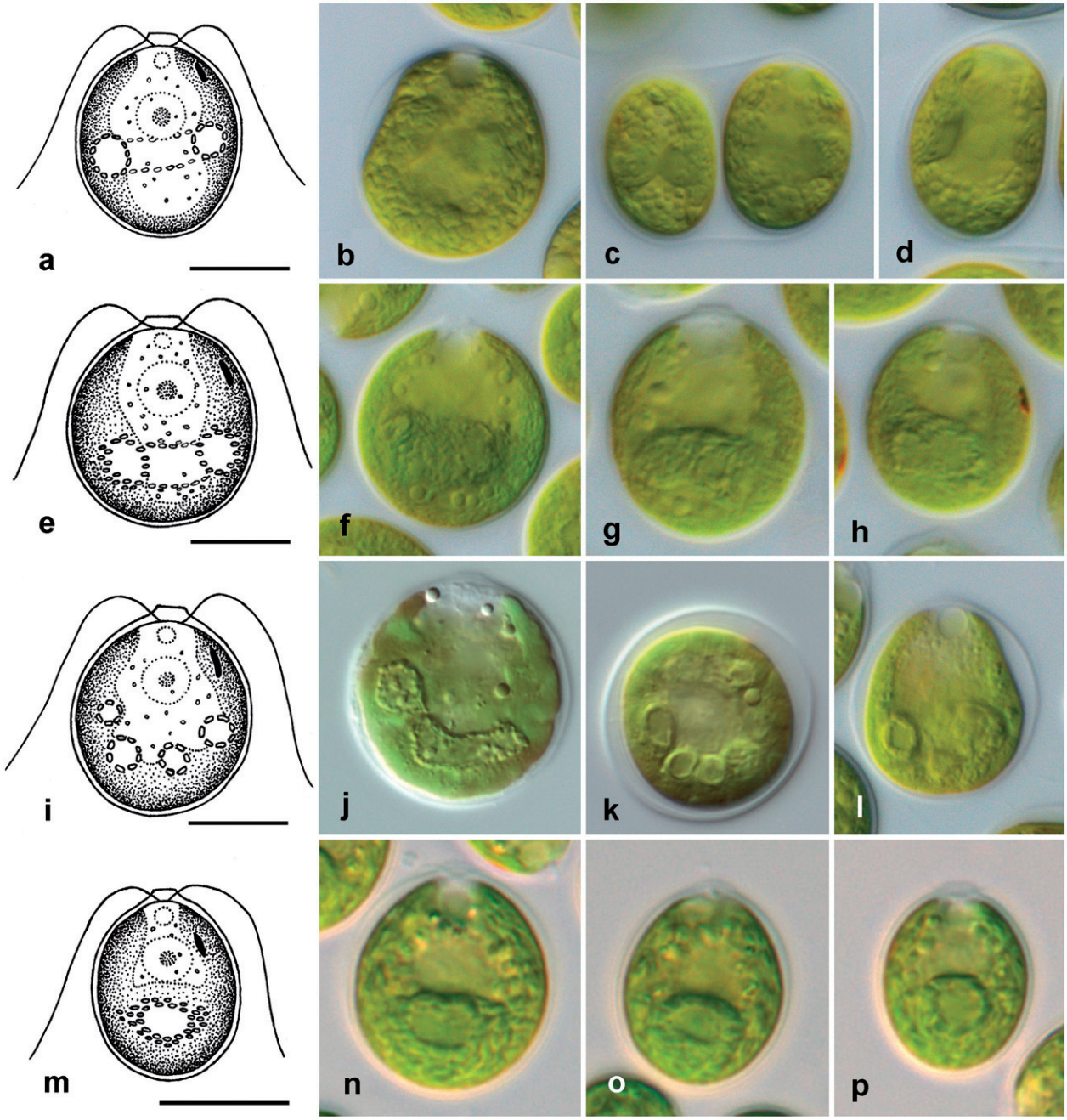


Fig. 5. Morphology of vegetative cells in *Microglena*. **a–d.** *Microglena monadina* (SAG 55.72). **e–h.** *Microglena braunii* (SAG 50.86). **i–l.** *Microglena coccifera* (SAG 55.91). **m–p.** *Microglena charkoviensis* (ACKU 267-03). **a, b, d–j** and **l–p** show general views of vegetative cells; **c**, sporangium, **k**, cell in apical view (optical section). Scale bars = 10 µm.

- The morphology of the four strains belonging to morphotype III fits with the original descriptions of *C. monadina* var. *globulifera* Korshikov (CCAC 0015 and CCAC 0017 = *M. globulifera*, *comb. nov.*: Korshikov, 1938) and *C. coccifera* Goroschankin (SAG 54.91 and SAG 55.91 = *M. coccifera*, *comb. nov.*: Goroschankin, 1905).

Morphology of reproductive stages

Asexual reproduction by sporulation was observed in all of the strains studied. During sporulation, the protoplast of the mother cell divided by longitudinal division, with or without rotation (Fig. 8a, b; see Ettl, 1979, 1988). Two to four zoospores were usually formed per sporangium. Unusual motile aggregates of cells were observed in all strains (Fig. 8c–f). These cells were extremely similar to stages in a type of asexual reproduction described by Massjuk & Demchenko (2001) and referred to as 'protocytotomy'. This type of cell division is characterized by formation of a new elastic cell wall within the old mother cell wall, which enlarges and dissolves with the production of mucilage. The new internal cell wall divides together with the protoplast and forms a new cell wall surrounding each

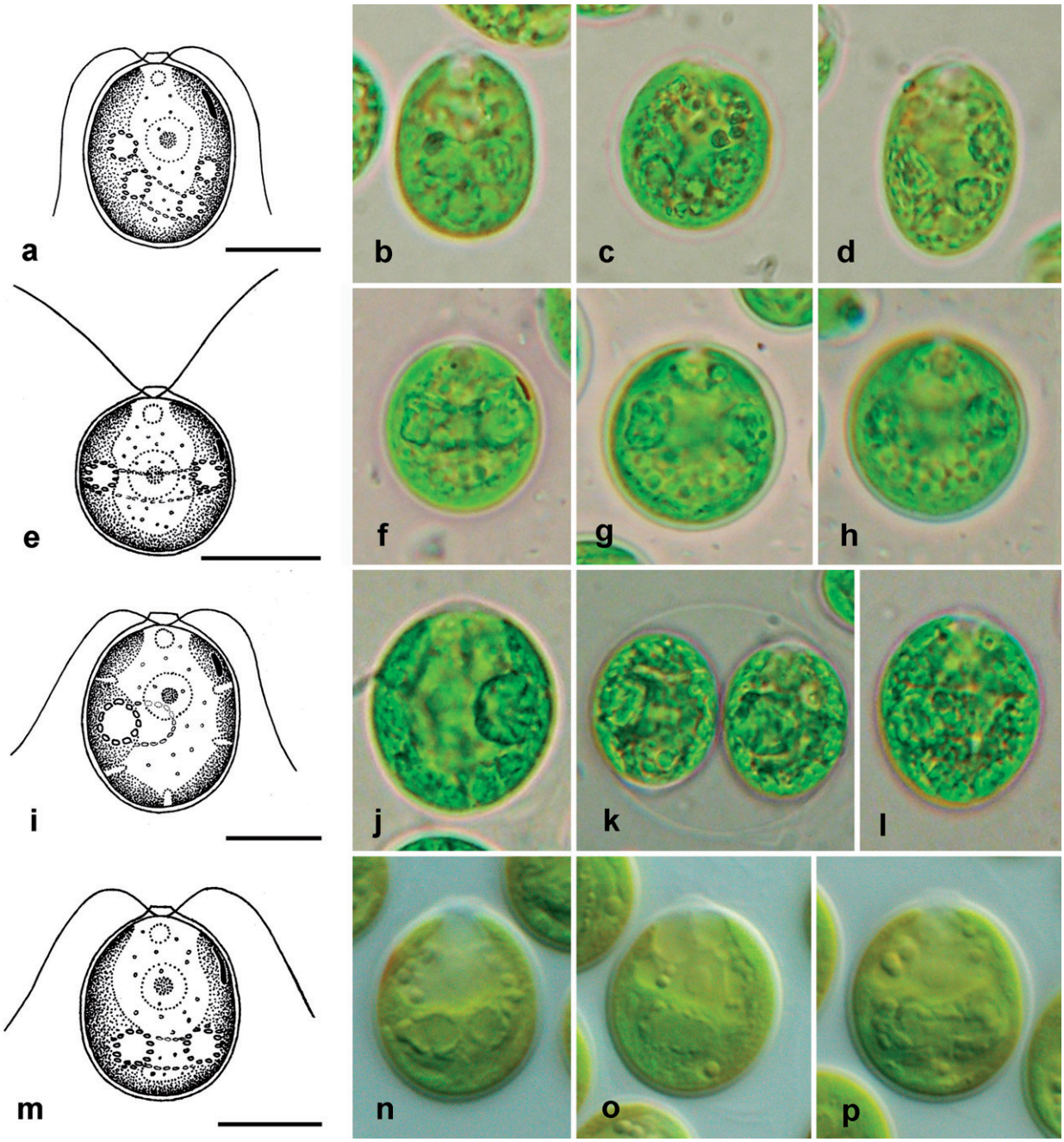


Fig. 6. Morphology of vegetative cells in *Microglena*. **a–d.** *Microglena globulifera* (CCAC 0017). **e–h.** *Microglena indica* (SAG 46.96). **i–l.** *Microglena skujae* (SAG 16.90). **m–p.** *Microglena opisthopyren* (SAG 8.87). **a–j** and **l–p** show general views of vegetative cells; **k**, sporangium. Scale bars = 10 μm.

product of the cell division. Unfortunately we did not observe the full course of development and cannot confirm if cell division was protocytotomy or some other process.

Palmelloid stages were not observed in any strains. Akinetes were observed in one strain only (SAG 55.72 *M. monadina*) and were similar in morphology to zygotes (data not shown).

Sexual reproduction was an advanced form of anisogamy, which is illustrated here for strain SAG 55.91 *M. coccifera* (Fig. 8g–i) and was observed also in strains SAG 55.72, SAG 50.86, SAG 54.91 and SAG 5.92. In all cases the female gamete (macrogamete) developed directly from a vegetative cell by rounding up of the protoplast and slight expansion. The macrogamete was usually surrounded by the gametangium cell wall, but was sometimes released. In contrast, each male gametangium usually contained four to eight, rarely 16 male gametes (microgametes). After release the microgametes were small (in SAG 55.91; 8.3–14.4 μm long), ovate or drop-shaped, and surrounded by a cell wall; they possessed two long flagella, which were twice as long as the cell. After attachment of the microgamete to the macrogamete, mostly at the macrogamete's anterior

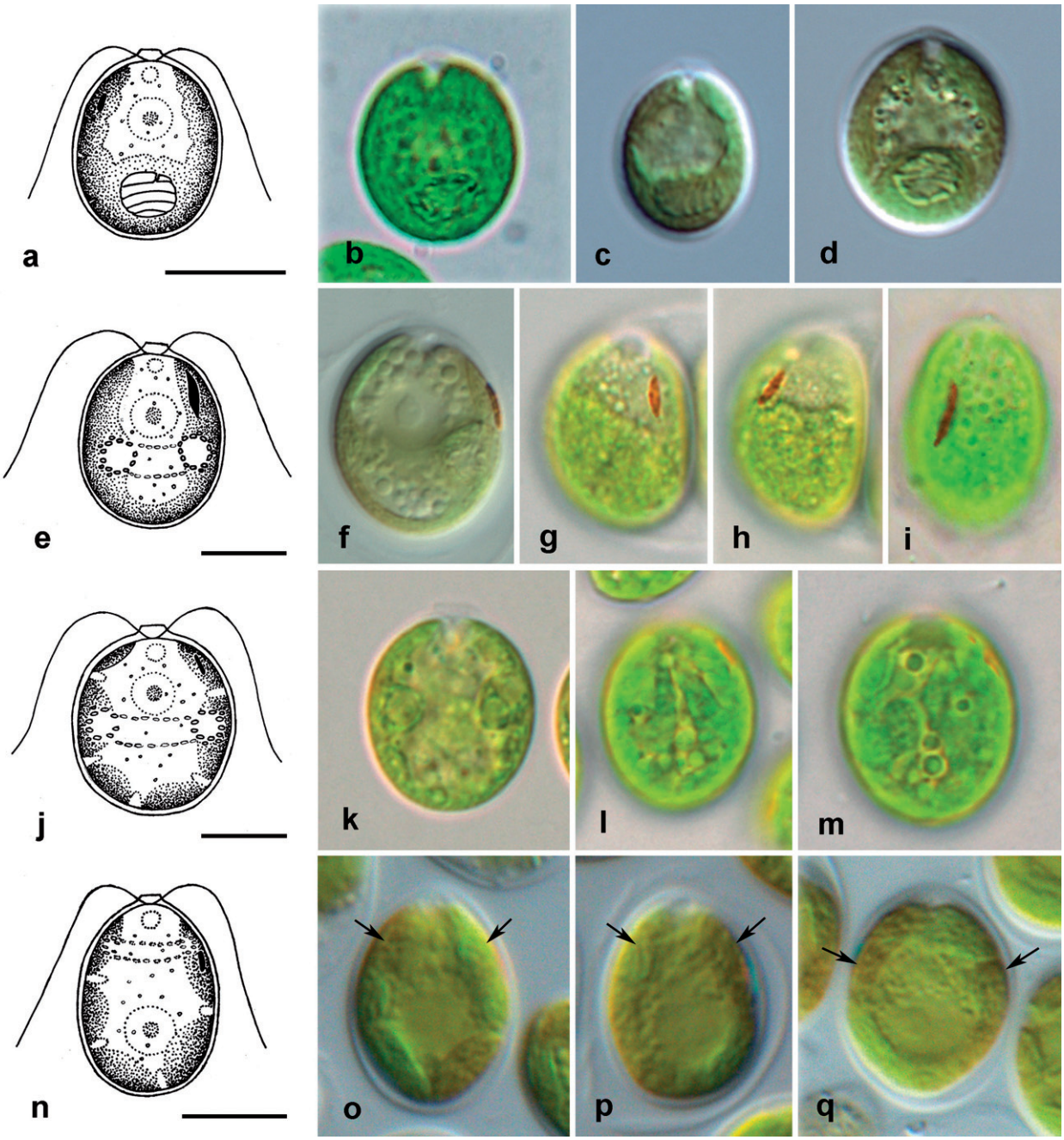


Fig. 7. Morphology of vegetative cells in *Microglena*. **a–d.** *Microglena uva-maris* (SAG 19.89). **e–i.** *Microglena longirubra* (SAG 5.92). **j–m.** *Microglena lobata* (SAG 31.72). **n–q.** *Microglena basinucleata* (SAG 67.72; arrows indicate the position of the pyrenoid, which is unclear). **a–f, j, k** and **n–q** show general views of vegetative cells; **g–i, l** and **m**, surface views. Scale bars = 10 μm.

end, the cell walls of both gametes dissolved at the point of connection. Then the protoplast of microgamete moved towards the protoplast of the macrogamete and fused with it (Fig. 8g). Sometimes the protoplasts of both gametes were released from their cell walls and fused, whereas the inner part of the microgamete cell wall was always visible (Fig. 8h). Rarely, several microgametes fused with a single macrogamete (polyspermy); however, this event was clearly recognizable by the presence of the inner parts of the empty microgamete cell walls, which are always absent in macrogametes (see also Goroschankin, 1890). During sexual reproduction, the flagella of the gametes were sometimes lost, sometimes retained, but in the latter case they were always without function. After fusion, the resulting zygote was always without flagella. Young zygotes were surrounded by a primary zygote wall, but often bore the empty cell wall of the microgamete (sometimes also of the macrogamete) (Fig. 8h). The secondary zygote walls were smooth without ornamentation. Mature zygotes contained many transparent or yellowish globules (Fig. 8i).

Discussion

Genetic diversity and DNA barcoding in Microglena

Phylogenetic analyses of SSU rDNA sequences (Fig. 1) show that there is a monophyletic lineage within the Chlorophyceae that includes all of our *C. monadina* strains, together with *C. reginae*, *C. uva-maris* and various strains isolated from marine rock pools and snow/ice fields, indicating a high genetic diversity. This clade has previously been called the *Monadina*-clade (Pröschold *et al.*, 2001) or *Monadinia* (Nakada *et al.*, 2008). However, the correct name for a genus corresponding to the *Monadina*-clade is *Microglena*, dating from 1832. The monophyly of *Microglena* is supported by all Bayesian and bootstrap analyses and we therefore make formal transfers of all of the *Monadina* clade to *Microglena* below. As shown in Fig. 1, we also confirmed that the psychrophilic strains isolated by Eddie *et al.* (2008), Liu *et al.* (2006) and Leya (2004) form a monophyletic lineage within *Microglena,* called the Polar-subclade.

To get better resolution, we analysed a concatenated dataset of SSU and ITS rDNA sequences, aligned according to their secondary structure. Figure S2 shows that our 17 strains belong to 13 independent lineages within *Microglena*. However, the relationship among these lineages is unresolved by the concatenated SSU and ITS data (no Bayesian and bootstrap support). Therefore, to decide if the 13 lineages represent different species, the conserved region of the ITS-2 secondary structure was analysed using the CBC approach proposed by Coleman (2000, 2009), Moniz & Kaczmarska (2009) and Bock *et al.* (2011). Figures 3 and 4 and Table 1 show that all 13 lineages differ by at least one CBC, mostly by more (up to 18). This indicates that these lineages represent different species in accordance with Müller *et al.* (2007), who showed that in 93% of the cases studied independent species differ in at least one CBC. As a consequence, we propose the use of the conserved region in ITS-2 presented in Figs 3 and 4 to provide diagnostic characters to distinguish microalgal isolates at species level.

Chloroplast morphology and phenotypic plasticity in Microglena

The most striking character of the species in *Microglena* is the structure of the chloroplast and its embedded pyrenoid(s). All representatives of this group have a cup-shaped chloroplast but the pyrenoid varies in shape and position. Summarizing, three morphotypes could be observed in *Microglena* (Fig. 4). Morphotype I seems to be the ancestral type of chloroplast in *Microglena*. This type, in which the chloroplast has a thick basal part, where the single large pyrenoid is located, is present in *M. charkoviensis*, *M. reginae* and *M. uva-maris*. Strains of the Polar-subclade were not the focus of our study, but comparisons with published pictures show that these strains also have a chloroplast of morphotype I (Leya, 2004; Liu *et al.*, 2006; Eddie *et al.*, 2008).

Morphotypes II and III are also characterized by a cup-shaped chloroplast, but without a thick basal part (Fig. 4). However, during ontogenesis intermediate stages can be observed among the different morphotypes. For example, mature cells of *Microglena braunii* (SAG 50.86) and *M. opisthopyren* (SAG 8.87 and SAG 54.90) have type II chloroplasts, but the young cells have a cup-shaped chloroplast with a thick basal part containing a horseshoe-like pyrenoid; it is only later that the basal part of the chloroplast becomes thinner and the pyrenoid moves into a parietal position, creating the type II morphology. This ontogenesis was not indicated in the accounts of *Microglena braunii* (as *Chlamydomonas braunii*) by Goroschankin (1890), Pascher (1927) and Ettl (1983). Such morphological variability makes it very difficult to give a clear species identification through comparison with the original description or using the identification keys presented in Pascher (1927), Korshikov (1938), Huber-Pestalozzi (1961) and Ettl (1976, 1983). In addition, most species have small dissections of the chloroplast (Figs 5–7), which are largely ignored in published descriptions and indeed, they are difficult to observe and may need long investigation to discover. In *Microglena lobata* (SAG 31.72), *M. skujae* (SAG 16.90) and *M. basinucleata* (SAG 67.72), however, the incisions are easily visible and create a clearly lobed chloroplast. As Ettl & Green (1973) have already noted, *M. reginae* (SAG 17.89) has a perforated chloroplast with regular longitudinal fissures.

The main character of the vegetative cells is the easily visible pyrenoid and its shape. Among *Microglena* species the pyrenoid is very variable in shape, but all strains have in common that the pyrenoid is surrounded by several or many starch grains arranged in parallel rows. This was also reported by Ettl & Green (1973) for *M. reginae*, by Rosowski & Hoshaw (1988) for *M. longirubra*, and Eddie *et al.* (2008) and Liu *et al.* (2006) for two unidentified psychrophilic *Chlamydomonas* strains, based on electron microscopical investigations. Pyrenoid shape varies according to morphotype (Fig. 4): spherical to widely ellipsoidal in morphotype I, elongate to horseshoe-like in morphotype II, and fragmented and nearly spherical in morphotype III. These different pyrenoid shapes have been recognized and used by many authors as diagnostic features to describe different species (see

details in Ettl, 1976, and below). However, like the chloroplast itself, pyrenoid shape undergoes several transformations during ontogenesis. For example, shortly after release of the sporangia, young cells of all strains mostly have an ellipsoidal pyrenoid in the base of the chloroplast, which is typical for morphotype I. On the other hand, old cells of *M. opisthopyren*, *M. braunii* and *M. skujae*, which belong to morphotype II, sometimes have fragmented pyrenoids, which are characteristic for morphotype III. The morphological transformation of the pyrenoid within single populations of *Chlamydomonas monadina sensu lato* has also been recorded by Ettl (1965, 1976), Gerloff (1940), Skuja (1949, 1956) and Pascher (1927). The pyrenoid of *M. basinucleata* (SAG 67.72) is difficult to distinguish because the pyrenoid has no starch layer around the pyrenoid. Therefore *M. basinucleata* has previously been identified wrongly as *Chloromonas subdivisa* (Pascher & Jahoda) Gerloff and Ettl *ex* Ettl (Schlösser, 1994). Interestingly, despite the absence of a pyrenoid, true *Chloromonas subdivisa* is similar in cell morphology to species of *Microglena* (Pascher & Jahoda, 1928), which may indicate that it too should be transferred.

The stigma has a similar shape (elongated or rod-like) in all strains and is located in the anterior half of the cell. A very elongated eyespot, occupying almost a third of the cell, has been recorded for *Chlamydomonas monadina* var. *longirubra* (Ettl, 1976), and we found a similar eyespot in strain SAG 5.92 (*M. longirubra*). However, elongated eyespots can also be observed sometimes in other strains (for example CCAC 0015 and CCAC 0017) and the stigmata of all strains can vary in size: even in *M. longirubra*, shorter eyespots like those typical for other species can be observed. Ettl & Green (1973), Rosowski & Hoshaw (1988) and Eddie *et al.* (2008) respectively have reported that the ultrastructure of the stigma is the same (with a single thylakoid between two layers of pigmented globules) in *M. reginae* (SAG 17.89), *M. longirubra* (SAG 5.92) and an unidentified *Chlamydomonas* strain (ARC).

In contrast to the chloroplast, other organelles show little variation among strains. For example, all freshwater strains have two anterior contractile vacuoles, which can be also observed in the marine strain SAG 19.89 of *M. uva-maris*, if cultivated in freshwater (3N-BBM + V) medium. *Microglena reginae* (SAG 17.89) is unable to grow in freshwater conditions and its contractile vacuoles do not function in seawater. The nucleus is located in a central position in the cell in all strains except SAG 67.72, where it is in basal position (hence its name, *M. basinucleata*). A papilla can be observed in all species and is mainly wide and trapezoidal. Only *M. reginae* has a special, crown-like papilla, which is mentioned in the description by Ettl and Green (1973). Ultrastructural studies have shown that strains SAG 17.89, SAG 5.92 and ARC have a branched mitochondrion, which is located only around the nucleus and not between cell wall and chloroplast (Ettl & Green, 1973; Rosowski & Hoshaw, 1988; Eddie *et al.*, 2008).

Asexual reproduction by sporulation has been observed in all strains of *Microglena* (Fig. 8a, b). The cell division during sporulation occurred longitudinally after protoplast rotation of 90° (false transverse division *sensu* Ettl 1988), which corresponds to Ettl's (1965) documentation for *Chlamydomonas monadina*. However, in our study we observed that the first cell division can occur also longitudinally without rotation of the protoplast; this was also shown in Ettl (1979). Interestingly, some stages in cell division similar to another type of reproduction (protocytotomy *sensu* Massjuk & Demchenko 2001) were found also by Ettl & Green (1973) in *M. reginae*. These authors referred to such cell aggregations as being somewhat similar to gamete fusion during isogamy, but they mentioned that this was only a superficial impression and that it was more realistic to suppose that the aggregations were a monstrosity resulting from incomplete division (Ettl & Green 1973). According to our data *Microglena* aggregates arise in a manner similar to protocytotomy, but unfortunately, the further development of the cell division and the further development of the daughter cells could not be observed.

The sexual reproduction of *Microglena* showed two variations, which are known under different terms in the literature: advanced anisogamy (also called heterogamy) and oogoniogamy. The main differences between these types lie in the presence or absence of flagella in both gametes and the behaviour of the macrogamete during fusion with the microgamete. If the protoplast of the macrogamete remains in its cell wall and has no flagella, sexual reproduction is termed oogoniogamous; otherwise, anisogamous. However, both types can be observed in the same species. Therefore, we designate the sexual reproduction of *Microglena* as homothallic, advanced anisogamy, because both gametes usually have flagella (though without function for the macrogametes), but sometimes the flagella are discarded before fusion. It seems that this mode is characteristic for the genus *Microglena* and has been documented by Goroschankin (1890) for *M. braunii*, Goroschankin (1905) and Skuja (1949) for *M. coccifera* and Rosowski & Hoshaw (1988) for *M. longirubra*. In addition, Korshikov (1938) mentioned that *Chlamydomonas monadina* and all its

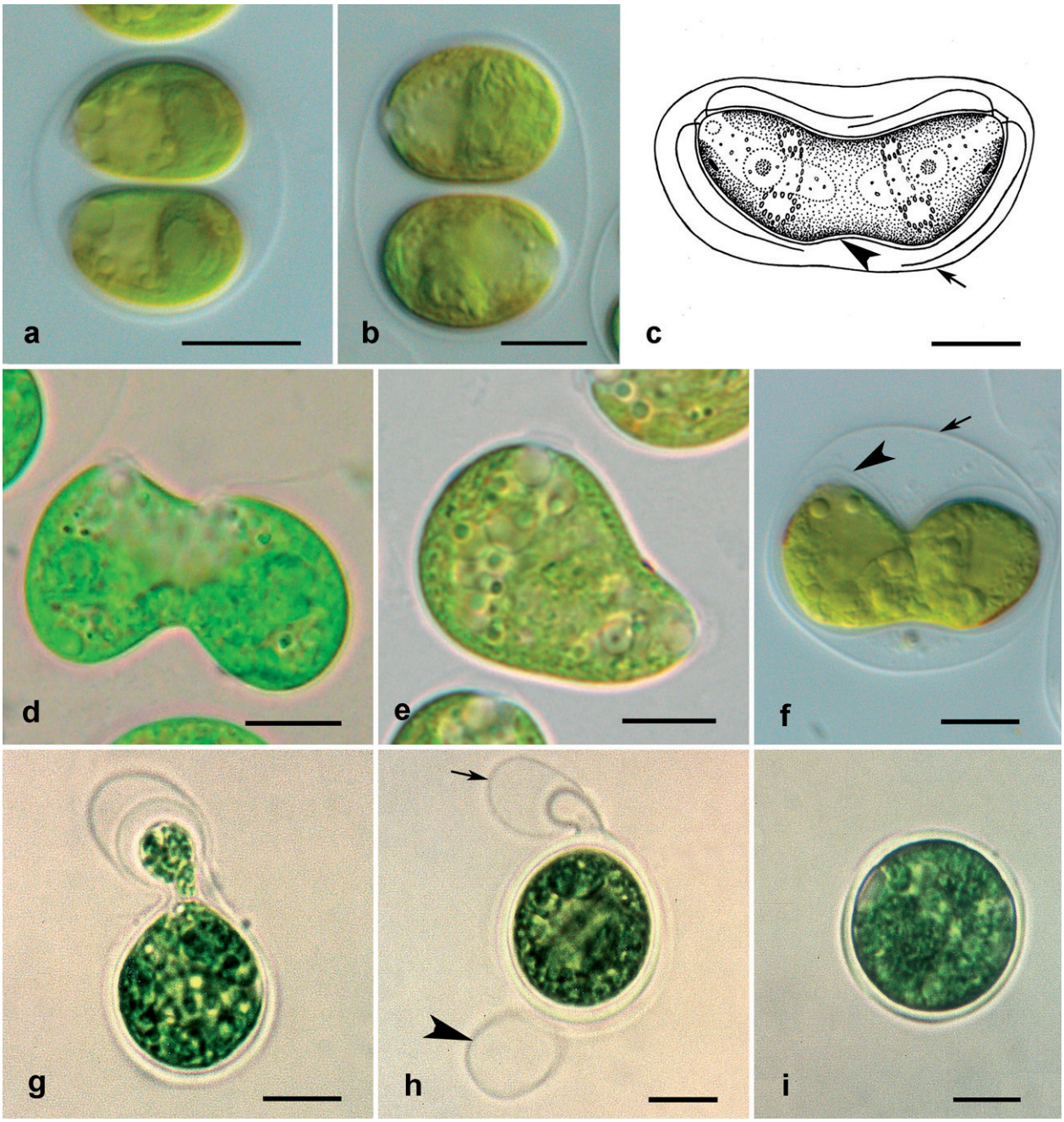


Fig. 8. Reproduction in *Microglena*. **a, b.** Asexual reproduction by zoosporulation (**a** without rotation of protoplast, **b** with rotation of protoplast by 90°). **c–f.** Incomplete cell division similar to asexual reproduction by protocytotomy; note the extended mother cell wall (arrows) and newly synthesized daughter cell walls (arrowheads). **g–i.** Sexual reproduction by advanced anisogamy: **g** shows fusing macro- and microgametes, **h** an empty microgametangium (arrow) and an empty macrogametangium (arrowhead) on the wall of the young zygote, and **i** a young zygote. **a** *Microglena opisthopyren* (SAG 8.87), **b, d** *Microglena longirubra* (SAG 5.92), **c** *Microglena monadina* (SAG 55.72), **e** *Microglena lobata* (SAG 31.72), **f–i** *Microglena coccifera* (SAG 55.91). Scale bars = 10 µm.

varieties, despite their variable morphology, have the same type of sexual reproduction and therefore belong to the same species. The same type of sexual reproduction was described by Pascher (1927) for *Chlamydomonas cingulata* (= *Microglena monadina*: see below), by Pascher (1943) for *C. praecox*, and by Gerloff (1940) for *C. heterogama*. Although *C. praecox* and *C. heterogama* have a different cell morphology, both probably belong to *Microglena* based on sexual reproduction. Unfortunately, no strains of these species are available for further studies.

Summarizing: *Microglena* species have a highly variable cell morphology but, so far as is known, the same type of sexual reproduction. However, the large, ellipsoidal to horseshoe-like pyrenoid and the homothallic advanced anisogamy are two characters that make it easy to recognize this genus

in water samples. The identification at species level in contrast needs comprehensive studies on cultured material.

Biodiversity and ecology of *Microglena*

The 13 known species of *Microglena* occur in three habitats: freshwater ponds, marine rock pools, and on snow and ice fields (Fig. 1). However, more species will have to be transferred to this genus, for example when the strains of the Polar-subclade *sensu* Eddie *et al*. (2008) have been investigated in a comparative study. In addition, several varieties of *Chlamydomonas monadina* and its relatives have been described that may represent independent species or may be synonyms of present species. These include *Chlamydomonas anulata* Nygaard (1949), *C. scutula* Pascher (1932 [= *C. braunii* var. *scutula* (Pascher) Gerloff]), *C. cingulata* var. *perforata* Vlk (1939/40) [= *C. monadina* var. *perforata* (Vlk) Ettl], *C. cingulata* var. *seligeriana* Korshikov *ex* Pascher (1927 [= *C. monadina* var. *seligeriensis* (Korshikov) Korshikov: Korshikov, 1938]), *C. monadina* var. *separatus* H.J. Hu (Hu & Wei, 2006), *C. nova* var. *minor* L.S. Péterfi (1968) and *C. sichuanensis* H.J. Hu & S. Chen (Hu & Wei, 2006): all have similar vegetative cell morphology, but differ in shape and the position of some cell organelles. Unfortunately, no cultures are available of these species. Whether *C. monadina* var. *ovalis* Playfair (1915) and *C. braunii* f. *elliptica* Moewus (1940) belong to *Microglena* is doubtful: it is impossible to decide because the descriptions and illustrations are too poor (see Ettl, 1976). As shown in Fig. 1, several psychrophilic strains isolated from snow and ice fields of Svalbard (Norway: Leya, 2004) and Antarctica (Liu *et al*., 2006; Eddie *et al*., 2008) belong to *Microglena*. Broady (1979) described an isolate called *Chlamydomonas* sp. from South Georgia (Antarctica), which probably belongs to *Microglena* and represents a new species.

As we note above, according to their sexual reproduction, *C. heterogama* (Gerloff, 1940), and *C. praecox* (Pascher, 1943) probably belong to *Microglena*, and for similar reasons also *C. upsaliensis* Skuja (Skuja, 1949) and *C. goroschankinii* Chmiliewski (Pascher, 1927), but the cell morphology of these species differs from the species we describe above and no cultures are available for further studies.

Whereas the freshwater and psychrophilic species probably have a monophyletic origin (Fig. 1), the marine species *Microglena reginae*, *M. uva-maris* and two strains designated as *C. kuwadae* Gerloff (1940), and an unidentified species of *Chlamydomonas* represent three different lineages within *Microglena*, indicating the origin of *Microglena* species in marine habitats. *Chlamydomonas kuwadae* was originally described by Kuwada (1916) as an unidentified *Chlamydomonas* species from a marine habitat. The strain NIES 968 identified as *C. kuwadae* by Nozaki *et al*. (2002) belongs to *Microglena* (see Fig. 1), but needs further investigation. All strains studied so far have in common that they are adapted to low temperature. Our study and the studies of Eddie *et al*. (2008), Liu *et al*. (2006) and Leya (2004) have shown that *Microglena* strains have a maximal growth temperature at or below 20°C, and most grow optimally below 15°C. Some psychrophilic strains are also halotolerant (Eddie *et al*., 2008).

Systematics and taxonomic revision of the genus *Microglena*

As shown in Fig. 1, the genus *Chlamydomonas* (in the traditional sense) is polyphyletic (see also Pröschold *et al*., 2001, and references therein). Pröschold & Silva (2007) proposed *C. reinhardtii* as the conserved type of *Chlamydomonas*, with the consequence that all species not closely related to *C. reinhardtii* have to be transferred to other or new genera. The taxonomic revision was initiated by Pröschold *et al*. (2001), who established two new genera, *Oogamochlamys* and *Lobochlamys*. In this study, we have demonstrated the monophyly of the *Monadina*-clade *sensu* Pröschold *et al*. (2001) and suggest that this group should be recognized as an independent genus. As discussed earlier (see Introduction), the name *Microglena* is available for any genus containing *M. monadina*, which is the green alga we describe here. As a consequence of our study, and according to the ICBN, we emend the diagnosis of the genus *Microglena* and propose 11 new combinations and two new species as below. The later-described species of *Microglena* summarized in Ettl (1978) are chrysophytes and need to be transferred to another genus.

Microglena Ehrenberg *emend.* Demchenko, Mikhailyuk & Pröschold.

Original description: Ehrenberg (1832). *Abh. Königl. Akad. Wiss. Berlin* (*Phys. Kl.*), **1831**: 64, pl. 1, fig. 1.

Emended description**:** Unicellular green alga with two equal flagella and a clockwise basal body orientation. Cells ellipsoidal to widely ellipsoid or spherical; cell wall thick; papilla broad, trapezoidal or crown-like. Chloroplast cup-shaped, with or without a thick basal part; pyrenoid variable, from one wide ellipsoidal to elongated quarter-half-ring- or ring-shaped, or sausage-like body, to

fragmented into several round pyrenoids; pyrenoid surrounded by many small or several big starch grains oriented in parallel, located in the thick basal part or in lateral thickenings of the chloroplast in basal, medial or upper positions. Stigma bright, ellipsoidal, rod-like or fusiform (sometimes drop- or patch-like) and in an anterior to medial position. Two apical contractile vacuoles. Nucleus central or basal.

Asexual reproduction by sporulation into two or four zoospores, cell division with or without rotation of protoplast by 90°. Akinetes spherical, with layered smooth cell wall, yellowish.

Sexual reproduction by homothallic advanced anisogamy. Macro- and microgametes covered by cell wall. During copulation microgametes attach to macrogametes by the papilla or laterally near the anterior part; both gametes lose their flagella during copulation. Mature zygotes with layered smooth or corrugated cell wall, yellowish.

TYPE SPECIES: *Microglena monadina* Ehrenberg *emend.* Demchenko, Mikhailyuk & Pröschold.

COMMENTS: *Microglena* differs from other *Chlamydomonas*-like genera by its characteristic pyrenoid, which is surrounded by many or several starch grains oriented in parallel rows, and which varies from ellipsoidal to characteristic horseshoe- half-ring- to almost ring-shapes; its variably perforated cup-shaped chloroplast; its trapezoidal or crown-like papilla; and sexual reproduction by advanced anisogamy. Unique ultrastructural characters of *Microglena* are the structure of pyrenoid (single thylakoids or thylakoid pairs penetrate the pyrenoid body), stigma (a single thylakoid or thylakoid band lies between two layers of pigmented globules), and mitochondrion (located around the nucleus and not between cell wall and chloroplast: Rosowski & Hoshaw, 1988). According to the ICBN, the name of *Microglena* is the oldest of any applicable to monadoid green flagellates and will have priority if further studies show that other genera are closely related to *Microglena*.

Microglena monadina Ehrenberg *emend.* Demchenko, Mikhailyuk & Pröschold

(Fig. 5a–d)

ORIGINAL DESCRIPTION: Ehrenberg (1832). *Abh. Königl. Akad. Wiss. Berlin* (*Phys. Kl.*), **1831**: 64, pl. 1, fig. 1.

SYNONYMS: *Chlamydomonas monadina* (Ehrenberg) F. Stein (1878): legend to pl. 15, figs 38, 39. *Chlamydomonas sphaerica* Troitskaya (1923): p. 82 *non Chlamydomonas sphaerica* Migula. *Chlamydomonas cingulata* Pascher (1927): p. 271, figs 230, 230a.

EMENDED DESCRIPTION: Cells 19–23 × 12–18 μm, ellipsoidal to widely ellipsoidal, with two flagella about as long as the cell. Papilla trapezoidal. Chloroplast cup-shaped, without a thick basal part. One half-ring-shaped pyrenoid in a lateral medial thickening of the chloroplast. Stigma bright, short rod-like, in an anterior to medial position. Nucleus central.

Asexual reproduction by sporulation into two or four zoospores, cell division with or without rotation of protoplast by 90°. Akinetes spherical, with layered smooth cell wall, yellowish.

Sexual reproduction by advanced anisogamy: macrogametangium with a single macrogamete, morphologically similar like the vegetative cell; 8–16 microgametes formed in the microgametangium; microgametes 8.5–15.7 × 6.0–11.2 μm, drop-shaped, with cell wall and two flagella that are twice as long as the cell; during copulation microgametes attach to the macrogamete by the papilla or any part of the anterior; both gametes lose their flagella during copulation; young zygotes immobile, formed inside macrogametangium or sometimes released; mature zygotes with layered smooth cell wall, yellowish.

ITS-2 DNA BARCODE: Barcode A in Fig. 3.

LECTOTYPE: Ehrenberg (1832), pl. I: fig. I, designated in Kusber *et al.* (2004).

EPITYPE (DESIGNATED HERE TO SUPPORT THE LECTOTYPE SPECIFIED ABOVE): The strain SAG 55.72 (proposed here as the authentic strain of *M. monadina*) permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

COMMENTS: Kusber *et al.* (2004) designate an epitype (a sample of dried specimen II Polygastrica No. CXVIII: 1 in BHUPM, shown as fig. 6 in Kusber *et al.* [2004]). Their figure shows high similarity to our micrographs (Fig. 5a–d). Therefore we designate the cryopreserved strain SAG 55.72 as epitype, because this strain provides essential additional information to typify the species. The epitype strain generally corresponds to the descriptions of *C. monadina* in Ehrenberg (1832), Stein (1878) and Ettl (1983). According to these publications the cells are widely ellipsoidal to almost spherical, and 18–35 μm in diameter. The cells of SAG 55.72 are slightly smaller and more elongated.

The proposal to transfer *Chlamydomonas sphaerica* and *C. cingulata* to this species is based on the strong morphological similarity to *M. monadina*

and was previously proposed by Pascher (1927) and Ettl (1976, 1983). The pyrenoid shape and size of *C. sphaerica* and *C. cingulata* is variable (Troitskaya, 1923; Pascher, 1927), but we think that they are within the limits of morphological variability of *M. monadina*.

***Microglena braunii* (Goroschankin) Demchenko, Mikhailyuk & Pröschold, *comb. nov*.**

(Fig. 5e–h)

Basionym: *Chlamydomonas braunii* Goroschankin (1890). *Bull. Soc. Imp. Nat. Moscou*, N.S. **4**: 502, pl. 14, 15.

Synonym: *Chlamydomonas monadina* Stein *sensu* Korshikov (1938): p. 75, fig. 28.

Emended description: Cells 17–25 × 11–23 μm, ellipsoidal to widely ellipsoidal and almost spherical, with two flagella about as long as the cell. Papilla trapezoidal. Chloroplast of young cells cup-shaped with a thick basal part; in mature cells cup-shaped without a basal thickening. One horseshoe-like, half-ring-shaped or wide-ellipsoid pyrenoid, in a near-basal lateral thickening of the chloroplast. Stigma bright, short rod-like or ellipsoidal, anterior to medial. Nucleus central.

Asexual reproduction by sporulation into two or four zoospores, cell division with or without rotation of protoplast by 90°.

Sexual reproduction by advanced anisogamy; macrogametangium with a single macrogamete, morphologically similar to vegetative cells; eight microgametes formed in the microgametangium; microgametes (7.6–) 8.3–14.4 (−15.7) × 5.0–12.2 μm, elongated oviform or drop-shaped, with a cell wall and two flagella twice as long as the cell; during copulation microgametes attach to the macrogamete at the papilla or near the anterior; both gametes lose their flagella during copulation; young zygotes immobile, formed inside macrogametangium or sometimes released; mature zygotes with a layered smooth cell wall, yellowish.

ITS-2 DNA Barcode: Barcode C in Fig. 3.

Lectotype (designated here): Goroschankin (1890), pl. 14, fig. 1.

Epitype (designated here in support of the lectotype designated here): The strain SAG 50.86 (proposed here as the authentic strain of *M. braunii*) permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

Comments: The epitype strain generally corresponds to the diagnosis of *C. braunii* (Goroschankin, 1890), with minor differences in cell shape and details of chloroplast structure. According to the original diagnosis, cells are widely ellipsoidal to almost spherical and the chloroplast is cup-shaped with a thick bottom in which the horseshoe-like pyrenoid is situated (Goroschankin, 1890). The cells of the epitype strain are slightly more elongated. The chloroplast of young cells is as indicated by Goroschankin, but with age its basal part becomes thinner and the elongated pyrenoid occupies a lateral thickening of the chloroplast in a near-basal position.

Korshikov used the diagnosis and figure of *C. braunii* (Goroschankin, 1890) for the description of *C. monadina* in his monograph (1938, p. 75, fig. 28). Therefore we consider *C. monadina sensu* Korshikov to be a synonym of *C. braunii*.

***Microglena coccifera* (Goroschankin) Demchenko, Mikhailyuk & Pröschold, *comb. nov*.**

(Fig. 5i–l)

Basionym: *Chlamydomonas coccifera* Goroschankin (1905). *Flora* **94**: 420, pl. 3, figs 1–9.

Synonym: *Chlamydomonas coccifera* var. *mesopyrenigera* Skuja (1949): p. 601, fig. 3.

Emended description: Cells (16–) 22–24 (−27) × (13–) 19–23 (−27) μm, widely ellipsoidal, with two flagella about as long as the cell. Papilla trapezoidal. Chloroplast cup-shaped, without a thick basal part. Pyrenoids 1 to 4 or 5 (rarely 8) separated, wide-ellipsoidal to round, in lateral thickenings of the chloroplast, irregularly distributed. Stigma large, bright, elongated rod- or drop-like or fusiform, in an anterior to medial position. Nucleus central.

Asexual reproduction by sporulation into two or four zoospores, cell division with or without rotation of protoplast by 90°.

Sexual reproduction by advanced anisogamy; macrogametangium with a single macrogamete, morphologically similar to vegetative cells but larger (to 28–35 μm in diameter), without flagella; 16 microgametes formed in the microgametangium; microgametes 7.6–9.0 × 5.0–8.0 μm, spherical to oviform, with a cell wall and two flagella twice as long as the cell; during copulation microgametes attach to the macrogamete by the papilla or near the anterior; microgametes lose their flagella during copulation; young zygotes immobile, formed inside macrogamete cell wall or sometimes released; mature zygotes with a layered smooth cell wall, yellowish.

ITS-2 DNA Barcode: Barcode E in Fig. 3.

Lectotype (designated here): Goroschankin (1905), pl. 3, fig. 1.

Epitype (designated here in support of the lectotype designated here): The strain SAG 55.91 (proposed here as the authentic strain of *M. coccifera*) permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

Comments: The epitype strain generally corresponds to the diagnosis of Goroschankin (1905), with minor differences in the number and shape of pyrenoids. Cells have 5–8 separated pyrenoids according to the original diagnosis. The epitype strain often has a half-ring-shaped pyrenoid, which fragments into 4 or 5 separate pyrenoids depending on the stage of cell ontogenesis.

We propose that *C. coccifera* var. *mesopyrenigera* is a synonym of *M. coccifera* because there are few morphological differences between them. *Chlamydomonas coccifera* var. *mesopyrenigera* differs from the type variety of *C. coccifera* mainly by the location of pyrenoids in the equatorial part of chloroplast surrounding the nucleus (Skuja, 1949). However, these differences fall within the morphological variability of the epitype strain.

Microglena charkoviensis (Korshikov) Demchenko, Mikhailyuk & Pröschold, *comb. nov.*

(Fig. 5m–p)

Basionym: *Chlamydomonas monadina* var. *charkoviensis* Korshikov (1938). Volvocinae. In *Vyznacnyk prisnovodnych vodorostej Ukrainskoj RSR* (Roll, Y.V., editor), p. 76, fig. 30b.

Synonyms: *Chlamydomonas proboscigera* var. *charkowiensis* (Korshikov) L.S. Peterfi (1968): p. 222.

Emended description: Cells 10–16.5 × 8–14.5 μm, ellipsoidal to widely ellipsoidal, with two flagella about as long as the cell. Papilla trapezoidal. Chloroplast cup-shaped, with a thick basal part. A single widely ellipsoidal pyrenoid in the basal part of the chloroplast. Stigma bright, rod- or fusiform-like, in an anterior to medial position. Nucleus central.

Asexual reproduction by sporulation, producing two or four zoospores; cell division with or without rotation of protoplast by 90°.

Sexual reproduction not observed.

ITS-2 DNA Barcode: Barcode D in Fig. 3.

Lectotype (designated here): Korshikov (1938), fig. 30b.

Epitype (designated here in support of the lectotype designated here): The strain ACKU 274-03 (proposed here as the authentic strain of *M. charkoviensis*) permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

Comments: The epitype strain corresponds in every way to the original diagnosis of *C. monadina* var. *charkoviensis* (Korshikov, 1938): no morphological differences could be observed. This variety of *C. monadina* was previously published by Pascher (1927) as a variety of his newly described species *C. cingulata*. However, no description was provided and therefore, according to the ICBN, his variety is invalid. Korshikov (1938) described the variety as *C. monadina* var. *charkoviensis*, including the type figure (fig. 30b), without mentioning that this figure had previously been published by Pascher (1927) as his fig. 230b, a. Therefore we designate Korshikov (1938)'s illustration as the lectotype and not Pascher (1927), even though it is obvious that their figures are identical.

Peterfi (1968) transferred the variety *Chlamydomonas monadina* var. *charkoviensis* to *C. proboscigera* var. *charkowiensis*. However, the type variety of *C. proboscigera* Korshikov *ex* Pascher differs from all species of *Microglena* by having another type of sexual reproduction (isogamy with formation of a protoplasmic bridge; Pascher, 1927).

Microglena globulifera (Korshikov) Demchenko, Mikhailyuk & Pröschold, *comb. nov.*

(Fig. 6a–d)

Basionym: *Chlamydomonas monadina* var. *globulifera* Korshikov (1938). Volvocinae. In *Vyznacnyk prisnovodnych vodorostej Ukrainskoj RSR* (Roll, Y.V., editor), p. 76, fig. 31.

Emended description: Cells 16–22 × 10–19 μm, widely ellipsoidal to spherical, with two flagella about as long as the cell. Papilla trapezoidal. Chloroplast cup-shaped without a thick basal part, perforated by small fissures. Pyrenoid single, horseshoe-like or half-ring-shaped, in a near-basal lateral thickening of the chloroplast in young cells; in mature cells fragmented into 3–5 separate widely ellipsoid to round pyrenoids in lateral medial or near-basal thickenings of the chloroplast. Stigma large, bright, elongated rod-like or fusiform, in an anterior to medial position. Nucleus central.

Asexual reproduction by sporulation, with formation of two or four zoospores; cell division with or without rotation of protoplast by 90°.

Sexual reproduction not observed.

ITS-2 DNA Barcode: Barcode F in Fig. 3.

Lectotype (designated here): Korshikov (1938), fig. 31.

Epitype (designated here in support of the lectotype designated here): The strain CCAC 0017 (proposed here as the authentic strain of *M. globulifera*) permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

Comments: The epitype strain generally corresponds to the original diagnosis of *C. monadina* var. *globulifera* (Korshikov, 1938) and differs by only small differences in the number and shape of the pyrenoids, as well as details of chloroplast structure. Cells have up to four separated pyrenoids according to Korshikov. However, the epitype strain often has one half-ring-shaped pyrenoid, which is fragmented into 3 or 4 separate pyrenoids, depending on the stage of cell ontogenesis. The chloroplast of the epitype strain has small irregular perforations, whereas this characteristic was not mentioned, but illustrated by Korshikov (1938). This variety of *C. monadina* was previously published by Pascher (1927) as a variety of his newly described species *C. cingulata*. However, no description was provided in Pascher (1927) and this variety is therefore invalid. Korshikov (1938) described the variety as *C. monadina* var. *globulifera* without mentioning that his figure (fig. 31) had previously been published by Pascher (1927, fig. 230b, b).

Microglena indica (Iyengar *in* Iyengar & Desikachary) Demchenko, Mikhailyuk & Pröschold, *comb. nov.*

(Fig. 6e–h)

Basionym: *Chlamydomonas monadina* var. *indica* Iyengar *in* Iyengar & Desikachary (1981). *Volvocales*, pp. 290, 485, fig 164: 1–7.

Synonyms: *Chlamydomonas monadina* var. *cingulata* (Pascher) Korshikov (1938): p. 76, fig. 29; *non Chlamydomonas cingulata* Pascher 1927.

Emended description: Cells (9)14–18 × (7)13–17 μm, spherical to widely ellipsoidal, with two flagella about as long as the cell. Papilla trapezoidal. Chloroplast cup-shaped, without a thicker basal part. Pyrenoid a single, half-ring-shaped to ring-shaped, in a lateral medial thickening of chloroplast surrounding the nucleus. Stigma bright, rod-like, in an anterior to medial position. Nucleus central.

Asexual reproduction by sporulation, with formation of two or four zoospores; cell division without rotation of protoplast by 90°.

Sexual reproduction not observed.

ITS-2 DNA Barcode: Barcode G in Fig. 3.

Lectotype (designated here): Iyengar & Desikachary (1981), fig. 164: 1.

Epitype (designated here in support of the lectotype designated here): The strain SAG 46.96 (proposed here as the authentic strain of *M. indica*) permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

Comments: The epitype strain generally corresponds to the original diagnosis of *C. monadina* var. *indica* (Iyengar & Desikachary, 1981), with small differences in cell size and papilla shape. According to the diagnosis, cells are 15 × 13–14 μm and have a thin blunt papilla. Cells of the epitype strain are slightly bigger and the papilla is wider.

We propose that *C. monadina* var. *cingulata* is synonymous because of the essential morphological similarity to *M. indica* (Korshikov, 1938); *C. cingulata* Pascher (Pascher, 1927; Korshikov, 1938), however, is a synonym of *M. monadina*.

Microglena skujae Demchenko, Mikhailyuk & Pröschold, *nom. nov.*

(Fig. 6i–l)

Synonym: *Chlamydomonas nova* Skuja (1956). *Nova Acta R. Soc. Sc. Upsal.*, ser. 4. **16**(3): 128, pl. 18, figs 19–21; *non Chlamydomonas nova* Sörensen (1948).

Emended description: Cells (12–) 18–23 (−25) × (8–)14–20 μm, ellipsoidal to widely ellipsoidal, with two flagella about as long as the cell. Papilla trapezoidal. Chloroplast cup-shaped without a thicker basal part, dissected into several lobes. Pyrenoid single, wide-ellipsoidal or half horseshoe-like, lying in a lateral medial thickening of the chloroplast. Stigma bright, rod-like, in an anterior to medial position. Nucleus central.

Asexual reproduction by sporulation, producing two or four zoospores; cell division with or without rotation of protoplast by 90°.

Sexual reproduction not observed.

ITS-2 DNA Barcode: Barcode L in Fig. 3.

Lectotype (designated here): Skuja (1956), pl. 18, fig. 19.

Epitype (designated here in support of the lectotype designated here): The strain SAG 16.90 (proposed here as the authentic strain of *M. skujae*) permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

COMMENTS: The epitype strain generally corresponds to the diagnosis of *C. nova* (Skuja, 1956) and exhibits only small differences in cell size, pyrenoid shape and details of chloroplast structure. According to the original diagnosis, cells are 20–30 × 12–20 μm and have a spherical pyrenoid. Cells of the epitype strain are shorter; the pyrenoid varies from spherical to ellipsoidal and half horseshoe-like; and the chloroplast is dissected into lobes.

We have given a new name for this taxon because Skuja's (1956) name '*Chlamydomonas nova*' is invalid, the name having been used previously by Sörensen (1948). We do not agree with Ettl's (1983) suggestion that *C. nova* is a synonym of *C. anulata* (Nygaard, 1949). The latter species is characterized by a completely different position of nucleus (basal) and, as discussed above, we think that *C. anulata* represents another species of *Microglena*.

Microglena opisthopyren* (Skuja) Demchenko, Mikhailyuk & Pröschold, *comb. nov.

(Fig. 6m–p)

BASIONYM: *Chlamydomonas opisthopyren* Skuja (1956). *Nova Acta R. Soc. Sc. Upsal.*, ser. 4, **16**(3): 130, pl. 18, figs 26–29.

EMENDED DESCRIPTION: Cells 10–20 × (8)11–17 μm, ellipsoidal to widely ellipsoidal, with two flagella about as long as the cell. Papilla trapezoidal. Chloroplast of young cells cup-shaped with a thick basal part, in mature cells cup-shaped but without a basal thickening. Pyrenoid single, widely ellipsoidal, horseshoe-like, or half-ring-shaped, in a near-basal lateral thickening of the chloroplast. Stigma bright, elongated rod-like, in an anterior to medial position. Nucleus central.

Asexual reproduction by sporulation, producing two or four zoospores; cell division with or without rotation of protoplast by 90°.

Sexual reproduction not observed.

ITS-2 DNA BARCODE: Barcode J in Fig. 3.

LECTOTYPE (DESIGNATED HERE): Skuja (1956), pl. 18, fig. 26.

EPITYPE (DESIGNATED HERE IN SUPPORT OF THE LECTOTYPE DESIGNATED HERE): The strain SAG 8.87 (proposed here as the authentic strain of *M. opisthopyren*) permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

COMMENTS: The epitype strain generally corresponds to the original diagnosis of *C. opisthopyren* (Skuja, 1956) and exhibits only small differences in cell size and the shapes of the papilla and pyrenoid. According to Skuja, the cells are 12–26 × 9–21 μm and have a wide semicircular papilla and one or two spherical pyrenoids in a lateral near-basal thickening of the chloroplast. However, one of Skuja's figures shows an elongated dumb-bell-shaped pyrenoid (Skuja, 1956, fig. 28). The epitype strain has smaller cells, a trapezoidal papilla, and an ellipsoidal to horseshoe-like pyrenoid.

Microglena longirubra* (Ettl) Demchenko, Mikhailyuk & Pröschold, *comb. nov.

(Fig. 7e–i)

BASIONYM: *Chlamydomonas monadina* var. *longirubra* Ettl (1976). *Nova Hedwigia, Beih.* **49**: 442, pl. 77, fig. 1.

SYNONYM: *Chlamydomonas monadina* var. *longistigma* Ettl (1976): p. 928 (invalid name).

EMENDED DESCRIPTION: Cells (16–) 20–26 × (10–) 17–20 μm, ellipsoidal to widely ellipsoidal, with two flagella about as long as the cell. Papilla trapezoidal. Chloroplast cup-shaped without a thickened basal part. Pyrenoid single, half-ring-shaped, in a lateral medial thickening of the chloroplast. Stigma bright, large, elongated rod-like or fusiform, sometimes curved, in an anterior to medial position, a third as long as the cell. Nucleus central.

Asexual reproduction by sporulation, producing two or four zoospores; cell division with or without rotation of protoplast by 90°.

Sexual reproduction by advanced anisogamy; macrogametangium with a single macrogamete morphologically similar to vegetative cells, with flagella; 16 microgametes formed in the microgametangium; microgametes small, 15.0 × 10.0 μm, ellipsoidal to oviform, with two flagella as long as the cell; during copulation, microgametes attach to the macrogamete near the anterior; both gametes lose their flagella during copulation; young zygotes immobile, formed inside the macrogamete cell wall or sometimes released; mature zygotes with a layered smooth cell wall, yellowish.

ITS-2 DNA BARCODE: Barcode I in Fig. 3.

LECTOTYPE (DESIGNATED HERE): Ettl (1976), pl. 77, fig. 1.

EPITYPE (DESIGNATED HERE IN SUPPORT OF THE LECTOTYPE DESIGNATED HERE): The strain SAG 5.92 (proposed here as the authentic strain of *M. longirubra*) permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

COMMENTS: The epitype strain agrees completely with the original diagnosis of *C. monadina var. longirubra* (Ettl, 1976).

Microglena uva-maris* (Butcher) Demchenko, Mikhailyuk & Pröschold, *comb. nov.

(Fig. 7a–d).

BASIONYM: *Chlamydomonas uva-maris* Butcher (1959). *Smaller Algae of British Coastal Waters. Part I: Introduction and Chlorophyceae*. Fisheries Invest, ser. 4, p. 53, pl. 3: 2, pl. 9: 2, pl. 13: 9.

EMENDED DESCRIPTION: Cells 8–17 × 6–13 µm, ellipsoidal to widely ellipsoidal, with two flagella about as long as the cell. Papilla trapezoidal. Chloroplast cup-shaped with a thick basal part. Pyrenoid single, widely ellipsoidal, located in the basal part of the chloroplast. Stigma bright, ellipsoidal to elongate-ellipsoidal and drop-like, in an anterior to medial position. Two apical contractile vacuoles, which do not pulse in seawater. Nucleus central.

Asexual reproduction by sporulation, producing two or four zoospores; cell division with or without rotation of protoplast by 90°.

Sexual reproduction not observed.

ITS-2 DNA BARCODE: Barcode M in Fig. 3.

LECTOTYPE (DESIGNATED HERE): Butcher (1959), pl. 9, fig. 2.

EPITYPE (DESIGNATED HERE IN SUPPORT OF THE LECTOTYPE DESIGNATED HERE): The strain SAG 19.89 (proposed here as the authentic strain of *M. uva-maris*) permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

COMMENTS: The epitype generally corresponds with the original diagnosis of *C. uva-maris* by Butcher (1959), with small differences in cell size, the shape of the stigma, and the presence of contractile vacuoles. According to Butcher, the cells are 8–15 × 6–8 µm, have a large, elongated, patch-like stigma, and are characterized by the absence of contractile vacuoles. The epitype strain has slightly bigger cells and a smaller stigma. Contractile vacuoles are present in the epitype strain; these do not function in salt water but do pulse in fresh water.

Microglena reginae* (Ettl & J.C. Green) Demchenko, Mikhailyuk & Pröschold, *comb. nov.

BASIONYM: *Chlamydomonas reginae* Ettl and J.C. Green (1973). *J. Mar. Biol. Assoc. UK*, **53**: 975, text-fig. 1 A–G.

ITS-2 DNA BARCODE: Barcode K in Fig. 3.

LECTOTYPE (DESIGNATED HERE): Ettl and Green (1973), text-fig. 1A.

EPITYPE (DESIGNATED HERE IN SUPPORT OF THE LECTOTYPE DESIGNATED HERE): The strain SAG 17.89 (proposed here as the authentic strain of *M. reginae*) permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

COMMENT: The SAG 17.89 strain agrees in morphology and reproduction with the original description of Ettl and Green (1973) and no emendations are necessary.

Microglena lobata* Demchenko, Mikhailyuk & Pröschold, *sp. nov.

(Fig. 7j–m)

DESCRIPTION: Cells (12–) 17–23 × (9–) 13–20 µm, ellipsoidal to widely ellipsoidal, with two flagella about as long as the cell. Papilla trapezoidal. Chloroplast cup-shaped without a thick basal part, dissected into several (4–6) lobes. Pyrenoid single, half-ring-shaped, located in a lateral medial thickening of the chloroplast. Stigma small, rod-like, in an anterior to medial position. Nucleus central.

Asexual reproduction by sporulation producing two or four zoospores; cell division with or without rotation of protoplast by 90°.

Sexual reproduction not observed.

ITS-2 DNA BARCODE: Barcode H in Fig. 3.

HOLOTYPE: The strain SAG 31.72 permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

ICONOTYPE: Fig. 7j–m.

TYPE LOCALITY: collected by R.C. Starr, 1953, from Yellowwood fish ponds, Bloomington, IN, USA.

ETYMOLOGY: This taxon is named after the characteristically lobed chloroplast.

AUTHENTIC CULTURE: SAG 31.72.

COMMENTS: This species is the most similar to *M. monadina*, but characterized by its thinner long pyrenoid and deeply dissected chloroplast, with several distinct lobes.

Microglena basinucleata* Demchenko, Mikhailyuk & Pröschold, *sp. nov.

(Fig. 7n–q)

DESCRIPTION: Cells 15–22 × 12–20 µm, ellipsoidal to widely ellipsoidal, with two flagella about as long

as the cell. Chloroplast cup-shaped without a thick basal part, dissected into several (4 or 5) lobes. Pyrenoid single, half-ring-shaped, naked and almost invisible, located in a lateral upper thickening of the chloroplast. Stigma bright, elongate-ellipsoidal, in an anterior to medial position. Nucleus posterior.

Asexual reproduction by sporulation, producing two or four zoospores; cell division with or without rotation of protoplast by 90°.

Sexual reproduction not observed.

ITS-2 DNA Barcode: Barcode B in Fig. 3.

Holotype: The strain SAG 67.72 permanently preserved in a metabolically inactive state (cryopreserved in liquid nitrogen) in the Sammlung von Algenkulturen, University of Göttingen, Germany.

Iconotype: Fig. 7n–q.

Type locality: collected by W. Koch, 1958, from a pond in the Old Botanical Garden of the University, Göttingen, Germany.

Etymology: This taxon is named after its characteristically posterior nucleus.

Authentic culture: SAG 67.72.

Acknowledgements

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (PR 682/1-1 and PR 682/1-2). We thank Tatyana Darienko for helping us with the pictures, Michael Melkonian for his support during the grant project, and Maria Pavlovska for technical support. We are also very grateful to Olga Ettova, who provided us with unpublished material (drawings and comments) by her husband Hanus Ettl. This contribution is dedicated to him; his morphological observations in his unpublished material were of great help for our investigations.

Supplementary material

The following supplementary material is available for this article, accessible via the Supplementary Content tab on the article's online page at http://dx.doi.org/10.1080/09670262.2012.678388.

Supplementary Table S1. Strains used in this study and their origins.

Supplementary Table S2. Comprehensive CBC-analysis among the species of *Microglena*.

Supplementary Figure S1. ITS-1 and ITS-2 secondary structures of the *Microglena* species.

Supplementary Figure S2. Molecular phylogeny of *Microglena* based on SSU and ITS rDNA sequence comparisons.

References

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, **19**: 716–723.

Bock, C., Krienitz, L. & Pröschold, T. (2011). Taxonomic reassessment of the genus *Chlorella* (Trebouxiophyceae) using molecular signatures (barcodes), including description of seven new species. *Fottea*, **11**: 293–312.

Broady, P.A. (1979). A preliminary survey of the terrestrial algae of the Antarctic Peninsula and South Georgia. *British Antarctic Survey Bulletin*, **48**: 47–70.

Buchheim, M.A., Limieux, C., Otis, C., Gutell, R.R., Chapman, R.L. & Turmel, M. (1996). Phylogeny of the *Chlamydomonas* (Chlorophyceae): a comparison of ribosomal RNA sequences from the nucleus and chloroplast. *Molecular Phylogeny and Evolution*, **5**: 391–402.

Buchheim, M.A., Turmel, M., Zimmer, E.A. & Chapman, R.L. (1990). Phylogeny of *Chlamydomonas* (Chlorophyta) based on cladistic analysis of nuclear 18S ribosomal-RNA sequence data. *Journal of Phycology*, **26**: 689–699.

Butcher, R.W. (1959). *An Introductory Account of the Smaller Algae of the British Coastal Waters. Part I: Introduction and Chlorophyceae*. Ministry of Agriculture, Fisheries and Food, Fishery Investigations (series 4). HMSO, London.

Coleman, A.W. (2000). The significance of a coincidence between evolutionary landmarks found in mating affinity and a DNA sequence. *Protist*, **151**: 1–9.

Coleman, A.W. (2009). Is there a molecular key to the level of 'biological species' in eukaryotes? A DNA guide. *Molecular Phylogeny and Evolution*, **50**: 197–203.

Eddie, B., Krembs, C. & Neuer, S. (2008). Characterization and growth response to temperature and salinity of psychrophilic, halotolerant *Chlamydomonas* sp. ARC isolated from Chukchi Sea ice. *Marine Ecology Progress Series*, **354**: 107–117.

Ehrenberg, C.G. (1832). Über die Entwickelung und Lebensdauer der Infusionsthiere; nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. *Abhandlungen der Königlichen Akademie der Wissenschaften, Berlin*, **1831**: 1–154.

Ehrenberg, C.G. (1834). Dritter Beitrag zur Erkenntnis groβer Organisation in der Richtung des kleinsten Raumes. *Abhandlungen der Königlichen Akademie der Wissenschaften, Berlin*, **1833**: 145–336.

Ettl, H. (1965). Beiträge zur Kenntnis der Morphologie der Gattung *Chlamydomonas* Ehrenberg. *Archiv für Protistenkunde*, **108**: 271–430.

Ettl, H. (1976). Die Gattung *Chlamydomonas* Ehrenberg (*Chlamydomonas* und die nächstverwandten Gattungen II). *Nova Hedwigia, Beiheft*, **49**: 1–1122.

Ettl, H. (1978). Xanthophyceae, 1.Teil. In *Süβwasserflora von Mitteleuropa*, vol. 3: 1 (Ettl, H., Gerloff, J. & Heynig, H., editors). Gustav Fischer, Stuttgart.

Ettl, H. (1979). Die Protoplastenteilung und der Verlauf der Chloroplastenteilung während der asexuellen Fortpflanzung von *Chlamydomonas* (Volvocales, Chlorophyceae). *Algological Studies*, **22**: 40–113.

Ettl, H. (1983). Chlorophyta I. Phytomonadina. In *Süβwasserflora von Mitteleuropa*, vol. 9: 1 (Ettl, H., Gerloff, J. Heynig, H. & Mollenhauer, D., editors). Gustav Fischer, Stuttgart and New York.

Ettl, H. (1988). Unterschiedliche Teilungsverläufe bei den Phytomonaden (Chlorophyta). *Archiv für Protistenkunde*, **135**: 81–101.

Ettl, H. & Green, J.C. (1973). *Chlamydomonas reginae* sp. nov. (Chlorophyceae), a new marine flagellate with unusual chloroplast differentiation. *Journal of the Marine Biological Association of the United Kingdom*, **53**: 975–985.

Gerloff, J. (1940). Beiträge zur Kenntnis der Variabilität und Systematik der Gattung *Chlamydomonas*. *Archiv für Protistenkunde*, **94**: 31–502.

Gerloff-Elias, A., Spijkerman, E. & Pröschold, T. (2005). Effect of external pH on the growth, photosynthetic electron transport of *Chlamydomonas acidophila* Negoro, isolated from an

extremely acidic lake (pH 2.6). *Plant, Cell and Environment*, **28**: 1218–1229.

Gibson, A., Gowri-Shankar, V., Higgs, P.G. & Rattray, M. (2005). A comprehensive analysis of mammalian mitochondrial genome base composition and improved phylogenetic methods. *Molecular Biology and Evolution*, **22**: 251–264.

Gilbert, D.G. (1992). *loopDloop, a Macintosh program for visualizing RNA secondary structure*. ftp://iubio.bio.indiana.edu/molbio/loopdloop/

Goroschankin, J.N. (1890). Beiträge zur Kenntnis der Morphologie und Systematik der Chlamydomonaden. I. *Chlamydomonas braunii*. *Bulletin de la Société Imperiale des Naturalistes de Moscou, Nouvelle Serié*, **4**: 498–520.

Goroschankin, J.N. (1905). Beiträge zur Kenntnis der Morphologie und Systematik der Chlamydomonaden. III. *Chlamydomonas coccifera*. *Flora*, **94**: 420–423.

Hepperle, D., Nozaki, H., Hohenberger, S., Huss, V.A.R., Morita, E. & Krienitz, L. (1998). Phylogenetic position of the Phacotaceae within the Chlamydophyceae as revealed by analysis of 18S rDNA and rbcL sequences. *Journal of Molecular Evolution*, **47**: 420–430.

Higgs, P., Jameson, D., Jow, H. & Rattray, M. (2003). The evolution of tRNA-Leu genes in animal mitochondrial genomes. *Journal of Molecular Evolution*, **57**: 435–445.

Hu, H. & Wei, Y. (2006). *The Freshwater Algae of China. Systematics, Taxonomy and Ecology*. Science China Press, Beijing.

Huber-Pestalozzi, G. (1961). *Das Phytoplankton des Süßwassers, 5. Teil. Chlorophyceae. Ordnung: Volvocales*. Schweizerbart, Stuttgart.

Hudelot, C., Gowri-Shankar, V., Jow, H., Rattray, M. & Higgs, P. (2003). RNA-based phylogenetic methods: application to mammalian mitochondrial RNA sequences. *Molecular Phylogeny and Evolution*, **28**: 241–252.

Huelsenbeck, J.P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, **17**: 754–755.

Iyengar, M.O.P. & Desikachary, T.V. (1981). *Volvocales*. ICAR, New Delhi.

Jow, H., Hudelot, C., Rattray, M. & Higgs, P. (2002). Bayesian phylogenetics using an RNA substitution model applied to early mammalian evolution. *Molecular Biology and Evolution*, **19**: 1591–1601.

Korshikov, O.A. (1938). Volvocineae. In *Vyznacnyk prisnovodnych vodorostej Ukrainskoj RSR* (Roll, Y.V., editor). Akad. Nauk USSR, Kyiv (in Ukrainian).

Kostikov, I.Y., Demchenko, E.M. & Berezovskaya, M.A. (2009). Culture collection of algae of Taras Shevchenko Kyiv National University. Catalogue of strains (2008). *Chernomorskij Botanicheskij Journal*, **5**: 37–79 (in Russian).

Kusber, W.-H., Glück, K. & Jahn, R. (2004). AlgaTerra Informationssystem für Mikroalgen: Inhalte und Stand der Modellierung. *Deutsche Gesellschaft für Limnologie* (*DGL*) – *Tagungsbericht 2003* (Köln), Berlin: 394–399.

Kuwada, Y. (1916). Some peculiarities observed in the culture of *Chlamydomonas*. *Botanical Magazine Tokyo*, **30**: 347–358.

Leya, T. (2004). *Feldstudien und genetische Untersuchungen zur Kryophilie der Schneealgen Nordwestspitzbergens*. Shaker, Aachen.

Liu, C., Huang, X., Wang, X., Zhang, X. & Li, G. (2006). Phylogenetic studies on two strains of Antarctic ice algae based on morphological and molecular characteristics. *Phycologia*, **45**: 190–198.

Luo, W., Pflugmacher, S., Pröschold, T., Walz, N. & Krienitz, L. (2006). Genotype *versus* phenotype variability in *Chlorella* and *Micractinium* (Chlorophyta, Trebouxiophyceae). *Protist*, **157**: 315–333.

Marin, B., Palm, A., Klingberg, M. & Melkonian, M. (2003). Phylogeny and taxonomic revision of plastid-containing euglenophytes based on SSU rDNA sequence comparisons and synapomorphic signatures in the SSU rRNA secondary structure. *Protist*, **154**: 99–145.

Massjuk, N.P. & Demchenko, E.N. (2001). A new type of cell division in Chlamydomonadaceae (Chlorophyta). *International Journal on Algae*, **3**: 18–30.

Mathews, D.H., Sabina, J., Zuker, M. & Turner, D.H. (1999). Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *Journal of Molecular Biology*, **288**: 911–940.

Mayrhofer, H. & Poelt, J. (1985). Die Flechtengattung *Microglaena sensu* Zahlbruckner in Europa. *Herzogia*, **7**: 13–79.

Moewus, F. (1940). Die Analyse von 42 erblichen Eigenschaften der *Chlamydomonas eugametos* Gruppe. Teil I–III. *Zeitschrift für Induktive Abstammungslehre und Vererbungslehre*, **78**: 418–522.

Moniz, M.B.J. & Kaczmarska, I. (2009). Barcoding diatoms: is there a good marker? *Molecular Ecology Resources*, **9**: 65–74.

Müller, T., Philippi, N., Dandekar, T., Schultz, J. & Wolf, M. (2007). Distinguishing species. *RNA*, **13**: 1469–1472.

Nakada, T., Misawa, K. & Nozaki, H. (2008). Molecular systematics of Volvocales (Chlorophyceae, Chlorophyta) based on exhaustive 18S rRNA phylogenetic analyses. *Molecular Phylogeny and Evolution*, **48**: 281–291.

Nozaki, H., Onishi, K. & Morita, E. (2002). Differences in pyrenoid morphology are corrected with differences in the rbcL genes of members of the *Chloromonas* lineage (Volvocales, Chlorophyceae). *Journal of Molecular Evolution*, **55**: 414–430.

Nygaard, G. (1949). Hydrobiological studies on some Danish ponds and lakes. Part II. *Det Kongelige Danske Videnskabernes Selskabs Biologiske Skrifter*, **7**: 1–293.

Pascher, A. (1927). Volvocales. In *Die Süsswasser-Flora Deutschlands, Österreichs und der Schweiz*, Vol. 4 (Pascher, A., editor). Gustav Fischer, Jena.

Pascher, A. (1932). Zur Kenntnis der einzelligen Volvocalen. *Archiv für Protistenkunde*, **76**: 1–82.

Pascher, A. (1943). Beiträge zur Morphologie der ungeschlechtlichen und geschlechtlichen Vermehrung der Gattung *Chlamydomonas*. *Beihefte zum Botanischen Centralblatt*, **62/A**: 197–220.

Pascher, A. & Jahoda, R. (1928). Neue Polyblepharidinen and Chlamydomonadinen aus dem Almtümpeln um Lunz. *Archiv für Protistenkunde*, **61**: 239–281.

Peterfi, L. (1968). Studies on taxonomy and ecology of Roumanian Volvocales II. *Nova Hedwigia*, **16**: 215–249.

Playfair, G.I. (1915). Freshwater algae of the Lismore district. *Proceedings of the Linnean Society of New South Wales*, **40**: 310–362.

Posada, D. (2008). ModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**: 1253–1256.

Pröschold, T. & Leliaert, F. (2007). Systematics of the green algae: conflict of classic and modern approaches. In *Unravelling the Algae: The Past, Present, and Future of Algal Systematics* (Brodie, J. & Lewis, J., editors), 123–153. Taylor and Francis, London.

Pröschold, T. & Silva, P.C. (2007). Proposal to change the listed type of *Chlamydomonas* Ehrenb., nom. cons. (Chlorophyta). *Taxon*, **56**: 595–596.

Pröschold, T., Marin, B., Schlösser, U.G. & Melkonian, M. (2001). Molecular phylogeny and taxonomic revision of *Chlamydomonas* (Chlorophyta). I. Emendation of *Chloromonas* Gobi, and description of *Oogamochlamys* gen. nov. and *Lobochlamys* gen. nov. *Protist*, **152**: 265–300.

Pröschold, T., Harris, E.H. & Coleman, A.W. (2005). Portrait of a species: *Chlamydomonas reinhardtii*. *Genetics*, **170**: 1601–1610.

Ronquist, F. & Huelsenbeck, J.P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**: 1572–1574.

Rosowski, J.R. & Hoshaw, R.W. (1988). Advanced anisogamy in *Chlamydomonas monadina* (Chlorophyceae) with special reference to vacuolar activity during sexuality. *Phycologia*, **27**: 494–504.

Schlösser, U.G. (1994). SAG-Sammlung von Algenkulturen at the University of Göttingen. Catalogue of strains 1994. *Botanica Acta*, **107**: 113–186.

Schlösser, U.G. (1997). Additions to the culture collection of algae since 1994. *Botanica Acta*, **110**: 424–429.

Siebert, S. & Backofen, R. (2005). MARNA: multiple alignment and consensus structure prediction of RNAs based on sequence structure comparisons. *Bioinformatics*, **21**: 3352–3359.

Skuja, H. (1949). Drei Fälle von sexueller Reproduktion in der Gattung *Chlamydomonas* Ehrnb. *Svensk Botanisk Tidskrift*, **43**: 586–602.

Skuja, H. (1956). Taxonomische und biologische Studien über das Phytoplankton schwedischer Binnengewässer. *Nova Acta Regiae Societatis Scientiarum Upsaliensis, series 4*, **19/3**: 1–404.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**: 2688–2690.

Stein, F. von (1878). *Der Organismus der Infusionsthiere*. Engelmann, Leipzig.

Swofford, D.L. (2002). *PAUP*. Phylogenetic Analysis using parsimony* (* *and other methods*). Version 4.0b10. Sinauer Associates, Sunderland, MA, USA.

Tavare, S. (1986). Some probabilistic and statistical problems on the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences*, **17**: 262–272.

Telford, M.J., Wise, M.J. & Gowri-Shankar, V. (2005). Consideration of RNA secondary structure significantly improves likelihood-based estimates of phylogeny: examples from the bilateria. *Molecular Biology and Evolution*, **22**: 1129–1136.

Tillier, E. & Collins, R. (1998). Maximum likelihood with multiparameter models of substitution. *Journal of Molecular Evolution*, **39**: 409–417.

Troitskaya, O.V. (1923). About a new chlamydomonad: *Chlamydomonas sphaerica* Troitzk. nov. sp. *Botanicheskiye Materialy*, **2**: 81–82 (in Russian).

Vlk, W.H. (1939/40). Über die Morphologie und Entwicklung von *Chlamydomonas cingulata* Pascher im Vergleich mit anderen durch ihr Pyrenoid auffallenden Arten. *Lotos*, 87: 1–12.

Wolf, M., Friedrich, J., Dandekar, T. & Müller, T. (2005). CBCAnalyzer: inferring phylogenies based on compensatory base changes in RNA secondary structures. *In Silico Biology*, **5**: 291–294.

Wuyts, J., De Rijk, P., Van de Peer, Y., Pison, G., Rousseeuw, P. & De Wachter, R. (2000). Comparative analysis of more than 3000 sequences reveals the existence of two pseudoknots in area V4 of eukaryotic small subunit ribosomal RNA. *Nucleic Acids Research*, **28**: 4698–4708.

Zuker, M. (2003). Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research*, **31**: 3406–3416.