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# Phylogenetic re-evaluation of previously identified *Chlamydomonas* (Chlorophyta, Chlamydomonadaceae) strains from *The Mosonmagyaróvár Algal Culture Collection*, Hungary, using molecular data



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# ABSTRACT

Systematic studies on 70 MACC isolates previously identified as '*Chlamydomonas*', a unicellular flagellate, were carried out based on partial 18S rRNA. The aim of this study was to determine the phylogenetic affiliations of *Chlamydomonas* strains in the MACC collection. The study found that most of the strains were not *Chlamydomonas*. Nine clusters of phylogenetically similar taxa were identified. The previous determinations were completed with their new phylogenetic affiliations (partly due to changes in green algae classification). Molecular data revealed that 3 of the 70 strains are from Arenicolinia, 14 are members of the phylogroup Stephanosphaerinia, 11 are Oogamochlamydinia, 1 is Chloromonadinia, 19 are Reinhardtinia, 2 are Polytominia, 9 are Scenedesmaceae, 5 are Moewusinia, and 6 are Chlorella. Clades were established by 18S rRNA similarity and p-distances. This study reveals the need to revise established culture collections whose isolates are solely identified with morphology.

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# 1. Introduction

The genus *Chlamydomonas*Ehrenberg (1833) is one of the largest green algal genera consisting of 400–600 species, most of which are solely morphologically described (Nakada and Tomita, 2011). However, as Ettl (1976, 1981) indicated before, the genus is not a natural assemblage. Phylogenetic studies have clearly showed that the genus is very narrow in the phylogenetic sense (Pröschold et al., 2001; Demchenko et al., 2012). Pröschold et al. (2018), after complex comparative studies, found out that currently only three strains, namely *C. incerta, C. reinhardtii* and *C. schloesseri*, are considered to be the member of the genus. *Chlamydomonas* is a model organism to study fundamental processes such as photosynthesis, cell motility, assembly and disassembly

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of cilia, cell cycle, fertilization, and stress responses of green microalgae (Pan, 2008; Harris, 2009; Ettl and Gärtner, 2014). Since *Chlamydomonas* is an essential biological tool, proper species designations and descriptions are needed.

Algal classifications are currently being revised due to expanding molecular data. Pröschold and Leliaert (2007) advocate using a polyphasic approach when revising algae, which combines molecular information, morphology, electron microscopy, life cycle analyses and ecology. The 18S rRNA gene (Němcová et al. 2011; Barsanti et al., 2013) is the preferred phylogenetic marker for the Volvocales, with hundreds of sequences deposited in public databases, such as GenBank (Nakada et al., 2008b). 18S rRNA gene also has variable regions, so this marker can also be used at lower taxonomic levels, including microevolutionary investigations (Gerloff-Elias et al., 2005; Skaloud, 2008). Nakada et al. (2008b) performed comprehensive molecular analyses of Volvocales, including Chlamydomonas species, based on 18S rRNA gene sequences and adopted the PhyloCode (International Code of Phylogenetic Nomenclature, Cantino and de Queiroz, 2010) to explicitly define individual clades (Yumoto et al., 2013, 2014). Apart from this, a few plastid genes (e.g. psaB, rbcL), as well as combined 18S and 28S rRNA

*Abbreviations*: MACC, the Mosonmagyaróvár Algal Culture Collection; TBE buffer, Tris/ Borate/EDTA; BLAST, Basic Local Alignment Search Tool; MUSCLE, MUltiple Sequence Comparison by Log-Expectation, MEGA, Molecular Evolutionary Genetics Analysis; TIM2 + G + I model, Transition model; RAxML, Randomized Axelerated Maximum Likelihood; OTU, Operational Taxonomic Unit.

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#### Table 1

Summary of the taxonomic reassessment of 70 Chlamydomonas strains from the MACC collection with NCBI GenBank accession number.

athina     exignmenty     segmenty     year     minit       Differing     475     Self-hand     Classifications 19, and Classification 19, and Classification 19, and Classification 19, and Classifi	Phylogenetic	MACC strain	Origin/Source	Previous morphological	Closest BLAST hit	Similarity	NCBI accession	
Chiorella     969     Tarnite bous: Euro     Vietos (Moleg. 2015)       Chiorella     972     Sul, Buzal     Chiorella musc spin-spin     972.81     No580.218       1913     Sul, Buzal     Chiorella musc spin-spin     Chiorella musc spin-spin     972.81     No580.218       1913     Sul, Buzal     Chiorella musc spin-spin     Chiorella musc spin-spin     972.81     NO580.221       186     Sul, Buzal     Chiorella musc spin-spin     Chiorella musc spin musc spin     972.81     NO580.221       186     Sul, Buzal     Chiorella musc spin	affiliation	code		assignment by		score	number	
IndexoffFundResultOldersOldersSetNetsNetsNoSetSetSetNetsNetsNetsNetsNetsNetsNetsNoSetNets <td< td=""><td></td><td></td><td></td><td>Vörös (Ördög, 2015)</td><td></td><td></td><td></td></td<>				Vörös (Ördög, 2015)				
771     Soil Read     Chienydonnona sp.     Clienyd scotbishna     9.2.5.     K 1986-223       773     Soil Read     Chienydonnona sp.     Clienyd scotbishna     9.7.5.     K 1986-223       774     Soil Read     Chienydonnona sp.     Clienydonnona     9.7.6.     K 1986-223       774     Soil Read     Chienydonnona sp.     Clienydonnona sp.     7.7.6.     K 1986-17       775     Soil Read     Chienydonnona sp.     Clienydonnona sp.     7.7.6.     K 1986-17       772     Soil Read     Chienydonnona sp.     Clienydonnona sp.     Clienydonnona sp.     7.7.6.     K 1986-17       772     Soil Read     Chienydonnona sp.     Clienydonnona sp.     K 1986-17     1.7.6.       772     Soil Read     Chienydonnona sp.     Clienydonnona sp.     K 1986-17     1.7.6.       773     Soil Read     Chienydonnona sp.     Clienydonnona sp.     1.7.6.     K 1986-17       773     K 1986-17     Chienydonnona sp.     Clienydonnona sp.     1.7.6.     K 1986-17       774     K 1986-17     Chienydonnona sp.     Clienydonnona sp.	Chlorella	406	Termite house, Brazil	Chlamydomonas sp.	Chlorella sp.	97.2%	KY864188	
		771	Soil. Brazil	Chlamydomonas sp.	Chlorella sorokiniana	96.2%	KY864216	
791 Sali Roil Olarigiones related Roberti 97.2 N98622   Morenzia 97.2 N98623 N98623   Morenzia Diarigiones net general 97.2 N98623   Morenzia Diarigiones net general 97.2 N98623   Morenzia Diarigiones net general 97.3 N98623   Morenzia CALA 28 Network Obergiones net general 97.8 N98617   Morenzia Diarigiones net general 97.8 N98617 N98617   Morenzia Diarigiones net general 97.8 N98617   Morenzia Diarigiones net genes 9		787	Soil. Brazil	Chlamydomonas sp.	Chlorella sorokiniana	99.5%	KY864223	
816     Sola, Rozi     Chlargidomans vi, Dibragidomans vi, CAM 25 (Further)     Columpidomans vi, Dibragidomans vicingina     87.2     V766211       817     Print Brain CAM 25 (Further)     Columpidomans vicingina     89.3     V766217       77     CCAM 25 (Further)     Chlargidomans vicingina     89.2     V766175       78     CCAM 25 (Further)     Chlargidomans vicingina     89.2     V766173       78     Columpidomans vicingina     79.2     V766173     V766173		793	Soil. Brazil	Chlamydomonas reinhardtii	Chlorella sorokiniana	97.9%	KY864225	
R51     Poed, Road     Charmaly-mouse op.     Charmaly-mouse op.     Charmaly-mouse op.     Resolution     Second and and and and and and and and and a		816	Soil. Brazil	Chlamydomonas sp.	Chlorella sorokiniana	96.7%	KY864231	
Meterusinia     7.1     CCLAL 253 (Tribon)     Chamyelenness andergan     97.7.     KY864170       7.0     CCALA 256 (Tribon)     Colonaryelenness andergan     Editaryelenness andergan     100.8     KY864173       7.0     CCALA 256 (Tribon)     Colonaryelenness andergan     Colonaryelenness andergan     100.8     KY864173       7.0     CCALA 256 (Tribon)     Colonaryelenness andergan     Colonaryelenness andergan     93.8     KY864178       7.0     CCALA 255 (Tribon)     Colonaryelenness ap.     Colonaryelenness ap.     Colonaryelenness ap.     KY864178       7.0     Colonaryelenness ap.     Colonaryelenness ap.     Colonaryelenness ap.     KY864178       7.0     KY864181     Colonaryelenness ap.     Colonaryelenness ap.     KY864178       7.0     KY864178     Colonaryelenness ap.     Colonaryelenness ap.     KY86417		823	Pond. Brazil	Chlamydomonas sp.	Chlorella sp.	99.5%	KY864236	
Image: Proceedings of the section of the s	Moewusinia	27	CCALA 238 (Trebon)	Chlamydomonas	Chlamydomonas noctigama	97.7%	KY864170	
77 CXLA 254 (Trebur) Columy:domess gib: Columy		20	CCALA 220 (Trobon)	aorsoventralis Chlamudomonas gaitlari	Chlamydomonas postigama	100%	KV96/171	
10     Tar. Soveral of the processing of the process of the proces of the process of the process of the proces of the p		30 77	CCALA 239 (Trebon)	Chlamydomonas oblonga	Chlamydomonas noctigama	08.0%	K1804171 KV86/175	
72 Sul, Rotal Cilenzydomoza Sp. Charnydomoza Sp. Charnydomoza Sp. Nisoler 12   216 Sevage platt. Hungo Cilenzydomoza Sp. Charnydomoza sp. Sp. Nisoler 12   225 Seval, Rotal Cilenzydomoza Sp. Charnydomoza sp. Sp. Nisoler 12   226 Seval, Rotal Cilenzydomoza Sp. Charnydomoza Sp. Nisoler 12   226 Seval, Rotal Cilenzydomoza Sp. Charnydomoza Sp. Nisoler 12   237 Sul, Rotal Cilenzydomoza Sp. Charnydomoza Sp. Nisoler 12   238 Sul, Rotal Cilenzydomoza Sp. Charnydomoza Sp. Nisoler 12   239 Sul, Brazil Cilenzydomoza Sp. Charnydomoza Sp. Nisoler 12   230 Sul, Brazil Cilenzydomoza Sp. Charnydomoza Sp. Nisoler 12   231 Sul, Brazil Cilenzydomoza Sp. Charnydomoza Sp. Nisoler 12   234 Sul, Brazil Cilenzydomoza Sp. Charnydomoza Sp. Nisoler 12   234 Sul, Brazil Cilenzydomoza Sp. Charnydomoza Sp. Nisoler 12   234 Sul, Brazil Cilenzydomoza Sp. Charnydomoza Sp. Nisoler 12   234 Sul, Brazil Cilenzydomoza Sp. Charnydomoza Sp. Nisoler 1		120	Tarn Slovenia	Chlamydomonas sp	Chlamydomonas nitschmannii	99.2%	KY864176	
Benkandrinue     51     CCAA 227 (Prebon)     Columydomonas chaparano     Usergitanoma debaryano     953.5     VY864172       256     Sowage Jasht Husging     Chamydomonas spinhardin     97.53     VY864173       257     Soil, Brazil     Chamydomonas spin     Chamydomonas spinhardin     97.93     VY864173       328     Soil, Brazil     Chamydomonas spin     Chamydomonas debaryano     95.78     VY864173       339     Soil, Brazil     Chamydomonas spin     Chamydomonas spin     95.78     VY864102       340     Soil, Brazil     Chamydomonas spin     Chamydomonas spin     97.78     VY864201       541     Soil, Brazil     Chamydomonas spin     Chamydomonas spin     97.78     VY864201       543     Soil, Brazil     Chamydomonas spin     Chamydomonas spin     97.78     VY864201       544     Koru Variano     Chamydomonas spin     Chamydomonas spin     97.88     VY864201       543     Soil, Brazil     Chamydomonas spin     Chamydomonas spin     97.88     VY864212       544     Koru Variano     Chamydomonas spin     Chamydomonas		782	Soil. Brazil	Chlamydomonas sp.	Chlamydomonas pitschmannii	98.9%	KY864218	
216 Severe plant. Hungen Colong-domonas reinhabitali 97.5 NY864 187   285 Keine, Ukraine Colong-domonas spon Colong-domonas advangence 98.55 NY864 181   371 Soil, Brazil Colong-domonas spon Colong-domonas advangence 97.5 NY864 181   372 Soil, Brazil Colong-domonas advangence 97.5 NY864 181   373 Soil, Brazil Colong-domonas advangence 97.6 NY864 101   374 Soil, Brazil Colong-domonas advangence 97.7 NY864 201   374 Soil, Brazil Colong-domonas pheromas advangence 97.7 NY864 201   374 Soil, Brazil Colong-domonas pheromas advangence 97.7 NY864 201   374 Soil, Hungary Colong-domonas advangence 97.7 NY864 214   375 Soil, Hungary Colong-domonas advangence 97.7 NY864 181	Reinhardtinia	53	CCALA 236 (Trebon)	Chlamydomonas debaryana	Chlamydomonas debaryana	98.5%	KY864172	
285 Kieu Ukoine Chanydomonas 9,0 Chanydomonas 9,0 Chanydomonas 9,0 Kieu Kiel 183   336 Soil Brail Chanydomonas 9,0 Chanydomonas 9,0 Kieu Kiel 183   336 Soil Brail Chanydomonas 9,0 Chanydomonas 4,000 Soil Kiel 183   437 Soil Brail Chanydomonas 9,0 Chanydomonas 4,000 Soil Kiel 183   438 Soil Brail Chanydomonas 9,0 Chanydomonas 9,00 Kiel 183   544 Soil Brail Chanydomonas 9,0 Chanydomonas 9,00 Kiel 183   544 Soil Brail Chanydomonas 9,00 Chanydomonas 4,000 Soil 18,000   544 Soil Brail Chanydomonas 9,00 Chanydomonas 9,00 Kiel 183   646 Got A,127 Chanydomonas 9,00 Chanydomonas 7,000 Kiel 183   772 Soil Brail Chanydomonas 9,00 Chanydomonas 7,000 Kiel 183   773 Soil Brail Chanydomonas 9,000 Chanydomonas 7,000 Kiel 183   774 Soil Brail Chanydomonas 9,000 Chanydomonas 7,000 Kiel 183   775 Soil Brail Chanydomonas 9,000 Chanydomonas 7,000 Kiel 183   775 Soil Brail Chanydomonas 9,000 Chanydomonas 7,000 Kiel 183   775		216	Sewage plant. Hungary	Chlamydomonas reinhardtii	Chlamydomonas reinhardtii	97.5%	KY864179	
32Soil BrailChanydomas sp.Chanydomas 200797.9%(N264182)335Soil BrailChanydomas sp.Chanydomas 400788.7%(N264182)342Natha BrailChanydomas sp.Chanydomas 400788.7%(N264182)343Soil BrailChanydomas sp.Chanydomas 400788.7%(N264182)344Soil BrailChanydomas sp.Chanydomas 400797.7%N26402544Soil BrailChanydomas sp.Chanydomas 400797.7%(N26402)544Soil HungaryChanydomas pp.Chanydomas 400797.7%(N26402)544Soil HungaryChanydomas pp.Chanydomas 400797.7%(N26402)544Soil HungaryChanydomas pp.Chanydomas 400797.7%(N26412)544Soil HungaryChanydomas pp.Chanydomas 400797.7%(N26412)638Soil BrailChanydomas reinhaldChanydomas 700497.7%(N26412)638Soil BrailChanydomas reinhaldChanydomas 700497.8%(N2642)772Soil BrailChanydomas pp.Chanydomas 700497.7%(N2642)784Soil BrailChanydomas pp.Chanydomas 700497.8%(N2642)785Soil BrailChanydomas pp.Chanydomas 700497.8%(N264181786Soil BrailChanydomas pp.Chanydomas 700497.8%(N264181787Soil BrailChanydomas pp.Chanydomas 70,8%(N264181786Soil Br		285	Kiev. Ukraine	Chlamydomonas sp.	Chlamydomonas zebra	98.9%	KY864180	
353     Soli, Brazil     Chlamydomnas sp.     Chlamydomnas thebuynan     98.72     KY854182       342     Soli, Brazil     Chlamydomnas sp.     Terraspon sp.     98.52     KY854183       351     Soli, Brazil     Chlamydomnas sp.     Terraspon sp.     98.52     KY854191       354     Soli, Brazil     Chlamydomnas sp.     Chlamydomnas shor     97.72     KY854201       354     Soli, Huagary     Chlamydomnas sp.     Chlamydomnas shor     97.72     KY854201       544     Soli, Huagary     Chlamydomnas sp.     Chlamydomnas shor     97.75     KY854201       548     Soli, Huagary     Chlamydomnas shor     Glamydomnas shor     97.75     KY854201       648     Key, Ukrain     Chlamydomnas shor     Chlamydomnas shor     97.75     KY854201       773     Soli, Huagary     Chlamydomnas shor     Chlamydomnas shor     97.75     KY854201       783     Soli, Huagary     Chlamydomnas shor     Chlamydomnas shor     97.75     KY85421       787     Soli, Huagary     Chlamydomnas shor     Chlamydomnas shor     Nor     <		327	Soil. Brazil	Chlamydomonas sp.	Chlamydomonas zebra	97.9%	KY864181	
382     Soli. Brazil     Chlamydomonas sp.     Chlamydomonas debaryana     98.25.     KY884189       415     Puddle. Brazil     Chlamydomonas debaryana     97.27.     KY884201       530     Soli. Brazil     Chlamydomonas debaryana     97.27.     KY884201       541     Soli. Hungary     Chlamydomonas debaryana     95.27.     KY884201       542     Soli. Hungary     Chlamydomonas debaryana     95.27.     KY884201       543     Soli. Hungary     Chlamydomonas sp.     Chlamydomonas debaryana     95.7.     KY884201       544     Soli. Hungary     Chlamydomonas sp.     Chlamydomonas merikana     97.3.     KY884201       658     Soli. Brazil     Chlamydomonas sp.     Chlamydomonas merikana     97.2.     KY884214       728     Soli. Brazil     Chlamydomonas rinharditi     95.05.     KY884214       728     Soli. Brazil     Chlamydomonas rinharditi     95.25.     KY884214       728     Soli. Brazil     Chlamydomonas rinharditi     95.25.     KY884214       728     Soli. Brazil     Chlamydomonas rinharditi     95.25.		335	Soil. Brazil	Chlamydomonas sp.	Chlamydomonas debaryana	98.7%	KY864182	
415     Pudde Brail     Chlamydomonas sp.     Teresgona sp.     98.5.5     KY864101       530     Soill Krazil     Chlamydomonas sp.     Chlamydomonas debarynan     92.7.5     KY864201       541     Soill Krazil     Chlamydomonas sp.     Chlamydomonas debarynan     92.7.7     KY864201       549     Soill Hungary     Chlamydomonas sp.     Chlamydomonas debarynan     92.7.7     KY864201       549     Soill Hungary     Chlamydomonas sp.     Chlamydomonas debarynan     92.7.7     KY864201       544     Soill Hungary     Chlamydomonas sp.     Chlamydomonas debarynan     92.7.7     KY864201       648     Soill Hungary     Chlamydomonas sp.     Chlamydomonas reichan 40     92.5.8     KY864213       722     Soill Hazil     Chlamydomonas sp.     Chlamydomonas reichan 40     92.7.8     KY864201       723     Soill Hungary     Chlamydomonas sp.     Chlamydomonas reichan 40     92.7.8     KY864201       724     Soill Hungary     Chlamydomonas sp.     Chlamydomonas reichan 40     92.7.8     KY864201       727     Soill Hungary     Chlamydomonas		382	Soil. Brazil	Chlamydomonas sp.	Chlamydomonas debaryana	98.2%	KY864183	
330     Soli, Brazil     Chlamydomnas sp.     Chlamydomnas debaynun     92.22     FY84202       541     Soli, Brazil     Chlamydomnas sp.     Chlamydomnas sp.     Chlamydomnas sp.     FY84202       549     Soli, Brazil     Chlamydomnas sp.     Chlamydomnas sp.     Chlamydomnas sp.     FY84202       549     Soli, Hungary     Chlamydomnas sp.     Chlamydomnas scharyana     93.73     FY864203       549     Soli, Brazil     Chlamydomnas sp.     Chlamydomnas renkrama     97.23     FY864212       644     Kav, Ukraine     Chlamydomnas renkrama     97.23     FY864213       728     Soli, Brazil     Chlamydomnas renkrama     97.23     FY864214       728     Soli, Brazil     Chlamydomnas renkrama     95.25     FY864214       728     Soli, Brazil     Chlamydomnas sp.     Chlamydomnas fy8.74     FY864214       728     Soli, Brazil     Chlamydomnas sp.     Chlamydomnas fy8.74     FY864214       739     Soli, Brazil     Chlamydomnas sp.     Chlamydomnas fy8.74     FY864214       747     Soli, Brazil     Chlamydomnas sp.		415	Puddle. Brazil	Chlamydomonas sp.	Tetraspora sp.	98.5%	KY864189	
531     Soil Brazil     Chlamydonomis sp.     Chlamydonomis sp.     Chlamydonomis sp.     FXR     KY84202       544     Soil Brazil     Chlamydonomis sp.     Collamydonomis sp.     Six KY84204       545     Soil Hungary     Chlamydonomis sp.     Chlamydonomis sp.     KY84205       534     Soil Hungary     Chlamydonomis sp.     Chlamydonomis sb.     Six KY84206       638     CALA 247 (Treban)     Chlamydonomis scharpana     97.8     KY84213       674     Kev. Ukcau     Chlamydonomis scharpana     97.8     KY84213       772     Soil Brazil     Chlamydonomis reinhardtii     95.8     KY86217       773     Soil Brazil     Chlamydonomis reinhardtii     95.8     KY86217       773     Soil Brazil     Chlamydonomis rainhardtii     95.8     KY86217       774     Soil Brazil     Chlamydonomis rainhardtii     95.8     KY86217       774     Soil Brazil     Chlamydonomis rainhardtii     95.7     KY86218       775     Soil Brazil     Chlamydonomis sp.     Chlamydonomis rainhardtii     95.7     KY864208		530	Soil. Brazil	Chlamydomonas sp.	Chlamydomonas debaryana	96.2%	KY864201	
SH4Sh1 Bra21Chamydonionis Sp.ColoryColis Maximis95-30KY894247SH9Soil HungaryChamydonionis schar92.51KY894247SH9Soil HungaryChamydonionis sp.Chamydonionis schar92.52KY894217SH9Soil Bra21Chamydonionis sp.Chamydonionis schar92.82KY894217SH9Soil Bra21Chamydonionis sinchia95.82KY894217SH9Soil Bra21Chamydonionis sinchia95.82KY894214T88Soil Bra21Chamydonionis sinchia95.83KY894214T88Soil Bra21Chamydonionis sinchia95.83KY894214T88Soil Bra21Chamydonionis sinchia95.83KY894244T88Soil Bra21Chamydonionis sinchia95.83KY894204T89Soil Bra21Chamydonionis sinchia95.83KY894204T89Soil Bra21Chamydonionis sinchia95.83KY894214T89Soil Bra21Chamydonionis sinchia95.83KY894214T89Soil Bra21Chamydonionis sinchia95.83KY894214T89Soil Bra21Chamydonionis sinchia95.83KY894214T89Soil Bra21Chamydonionis sinchia95.83KY894214T89Soil Bra21Chamydonionis sinchia95.83KY89418T89Soil Bra21Chamydonionis sinchia95.83KY89418T89Soil Bra21Chamydonionis sinchia95.84KY89418T89Soil Bra21Chamydonio		531	Soil. Brazil	Chlamydomonas sp.	Chlamydomonas debaryana	97.7%	KY864202	
3-19     Soli, Hungary     Chamydomonis score     97.7.8     K 188-4.0.5       578     Soli, Hungary     Chamydomonis Schuroma     55.7.     K 188-4.0.5       578     Soli, Hungary     Chamydomonis Schuroma     55.7.     K 188-4.0.5       568     Soli, Hungary     Chamydomonis Schuroma     55.7.     K 188-4.0.5       568     Koli, Hangary     Chamydomonis Schuroma     57.8.     K 188-4.0.5       578     Soli, Bozal     Chamydomonis rehandull     59.8.     K 188-4.0.5       772     Soli, Bozal     Chamydomonis rehandull     59.8.     K 188-4.0.5       773     Soli, Bozal     Chamydomonis rehandull     59.8.     K 188-4.0.5       773     Soli, Bozal     Chamydomonis rehandull     59.8.     K 188-4.0.5       774     Soli, Bozal     Chamydomonis rehandull     59.8.     K 188-4.0.5       775     Soli, Bozal     Chamydomonis rehandull     59.8.     K 188-4.0.5       775     Soli, Bozal     Chamydomonis rehandull     59.8.     K 188-4.0.5       775     Soli, Bozal     Chamydomonis rehandull     <		544	Soil, Brazil	Chlamydomonas sp.	Gloeococcus maximus	98.5%	KY864204	
1     2     2     2     1		549	Soil Hungary	Chiamydomonas intermedia	Chiamydomonas zebra	97.7%	KY864205	
Arenical in Angle 20     Change 20     Change 20     Probability       654     Cold Aren 20     Cold Aren 20     Probability     Probability       654     Soil, Brazil     Change 20     Change 20     Probability       772     Soil, Brazil     Change 20     Change 20     Probability     Probability       772     Soil, Brazil     Change 20     Change 20     Probability     Probability       772     Soil, Brazil     Change 20     Change 20     Probability     P		579	Soil Hungary	Chlamydomonas sp.	Chlamydomonas debaryana	98.3%	K1804207	
and constrainedCharangedomases and under Charangedomases and under Charangedomases and under Charangedomases and under Section 2014Constrained Charangedomases and under Section 2014Constrained Section 2014Constrained 		564 658	CCALA 247 (Trebon)	Chlamydomonas peterfii	Change Ch	96.7%	K1004209 KV864212	
Besk     Soli, Brazil     Chlomydomonas sp.     Chlomydomonas reinhardii     97.21     NY98-11       772     Soli, Brazil     Chlomydomonas reinhardii     Dolarydomonas reinhardii     95.35     NY98-121       878     Soli, Brazil     Chlomydomonas reinhardii     Dolarydomonas reinhardii     95.35     NY98-123       878     Soli, Brazil     Chlomydomonas sp.     Chloragnium elongatum     95.35     NY98-124       870     Soli, Hungay     Chlomydomonas sp.     Chloragnium elongatum     95.35     NY86-103       871     Soli, Brazil     Chlomydomonas sp.     Chloragnium elongatum     95.35     NY86-113       872     NY86-113     Chlomydomonas sp.     Chloragocuru elipoideuru     95.35     NY86-113       874     Soli, Brazil     Chlomydomonas sp.     Chloragocuru elipoideuru     95.35     NY86-113       974     Soli, Brazil     Chlomydomonas sp.     Chloragocuru elipoideuru     95.35     NY86-113       974     Soli, Brazil     Chlomydomonas sp.     Chloragocuru elipoideuru     95.35     NY86-123       974     Soli, Brazil     Chlomydomon		674	Kiev Ukraine	Chlamydomonas callunae	Chlamydomonas mexicana	99.2%	KV864212	
722     Soil. Bozall     Chlamydonnoars rinharditi     Sist. NYS64217       788     Soil. Bozall     Chlamydonnoars prinharditi     Sist. NYS64228       788     Soil. Bozall     Chlamydonnoars pp.     Chlamydonnoars pp.     Sist. NYS64228       788     Soil. Hungary     Chlamydonnoars pp.     Chlamydonnoars pp.     Sist. NYS64208       607     Soil. Hungary     Chlamydonnoars pp.     Chlarogonium olongarum     95.2%     NYS64208       607     Soil. Hungary     Chlamydonnoars pp.     Chlarococcum ellipsoideum     95.5%     NYS64184       395     Puddle. Razill     Chlamydonnoars pp.     Chlarococcum ellipsoideum     95.7%     NYS64195       476     Soil. Brazil     Chlamydonnoars pp.     Chlarococcum ellipsoideum     95.3%     NYS64195       478     Soil. Brazil     Chlamydonnoars pp.     Chlarococcum ellipsoideum     95.3%     NYS64217       478     Soil. Brazil     Chlamydonnoars pp.     Chlarococcum ellipsoideum     95.3%     NYS64213       478     Soil. Brazil     Chlamydonnoars pp.     Chlarococcum ellipsoideum     95.3%     NYS64223		688	Soil Brazil	Chlamydomonas sp	Chlamydomonas debarvana	97.7%	KY864214	
788Soil, BazilCilanydonnas reinharditi90.5%NYS64224ArencoliniaSoil, BazilCilanydonnas sp.Cilanydonnas rapa92.2%NYS64206581Soil, BuzilCilanydonnas sp.Cilorgoniun elongatum93.2%NYS64206581Soil, BuzilCilanydonnas sp.Cilorgoniun elongatum95.3%NYS64206581Soil, BuzilCilanydonnas sp.Cilorocccum ellipsoideum95.3%NYS64118582Soil, BazilCilanydonnas sp.Cilorocccum ellipsoideum95.3%NYS64193467Soil, BazilCilanydonnas sp.Cilorocccum ellipsoideum95.3%NYS64195467Soil, BazilCilanydonnas sp.Cilorocccum ellipsoideum95.3%NYS64196478Soil, BazilCilanydonnas sp.Cilorocccum ellipsoideum95.3%NYS64196479Soil, BazilCilanydonnas sp.Cilorocccum ellipsoideum95.3%NYS64196478Soil, BazilCilanydonnas sp.Ciloroccum ellipsoideum95.3%NYS64196478Soil, BazilCilanydonnas sp.Ciloroccum ellipsoideum95.3%NYS64203478Soil, BazilCilanydonnas sp.Ciloroccum ellipsoideum95.3%NYS64216478Soil, BazilCilanydonnas sp.Ciloroccum ellipsoideum95.3%NYS64236478Soil, BazilCilanydonnas sp.Ciloroccum ellipsoideum95.3%NYS64236479Soil, BazilCilanydonnas sp.Ciloroccum ellipsoideum95.3%NYS64236 <td></td> <td>772</td> <td>Soil, Brazil</td> <td>Chlamydomonas reinhardtii</td> <td>Chlamydomonas reinhardtii</td> <td>95.9%</td> <td>KY864217</td>		772	Soil, Brazil	Chlamydomonas reinhardtii	Chlamydomonas reinhardtii	95.9%	KY864217	
835Soil, BazilChlamydomans sp.Chlamydomans np.92.%NY864238Arenciolinia526Soil, BazilChlamydomans sp.Chlorogonium clorgarum95.%NY864206607Soil, HungaryChlamydomans sp.Chlorogonium clorgarum96.7%NY8642085rephanosphaerinia188Soil, BazilChlamydomans sp.Chlorocccum clipsoidem95.5%NY864134780Soil, BazilChlamydomans sp.Chlorocccum clipsoidem95.5%NY864136781Soil, BazilChlamydomans sp.Chlorocccum clipsoidem95.7%NY864136786Soil, BazilChlamydomans sp.Chlorocccum clipsoidem95.7%NY864136787Soil, BazilChlamydomans sp.Chlorocccum clipsoidem95.7%NY864136788Soil, BazilChlamydomans sp.Chlorocccum clipsoidem95.7%NY864136787Soil, BazilChlamydomans sp.Chlorocccum clipsoidem95.7%NY864236788Soil, BazilChlamydomans sp.Chlorocccum clipsoidem95.7%NY864236788Soil, BazilChlamydomans sp.Chlorocccum clipsoidem95.7%NY864236789Soil, BazilChlamydomans sp.Chlorocccum clipsoidem95.7%NY864236781Soil, BazilChlamydomans sp.Chloroccum clipsoidem95.7%NY864236781Soil, BazilChlamydomans sp.Chloroccum clipsoidem95.7%NY864236781Soil, BazilChlamydomans sp.Chloroccum cl		788	Soil. Brazil	Chlamydomonas reinhardtii	Chlamydomonas reinhardtii	99.5%	KY864224	
Arenciolinia526Soli. BrazilChlargdomonas sp.Chlorgonium elongatum95.9%KY864200607Soli. HungaryChlarydomonas sp.Chlorogonium elongatum95.7%KY864211538Soli. BrazilChlarydomonas sp.Chlorococum ellipsoideum95.7%KY864184647Soli. BrazilChlarydomonas sp.Chlorococum ellipsoideum98.5%KY864193476Soli. BrazilChlarydomonas sp.Chlorococum dipobionitcum98.5%KY864193476Soli. BrazilChlarydomonas sp.Chlorococum ellipsoideum98.5%KY864193477Soli. BrazilChlarydomonas sp.Chlorococum ellipsoideum98.5%KY864193478Soli. BrazilChlarydomonas sp.Chlorococum ellipsoideum98.5%KY864193679Soli. BrazilChlarydomonas sp.Chlorococum ellipsoideum98.5%KY864203671Mad. HungaryChlarydomonas sp.Chlorococum ellipsoideum98.5%KY864213673Soli. BrazilChlarydomonas sp.Chlorococum ellipsoideum98.5%KY864234786Soli. BrazilChlarydomonas sp.Chlorococum ellipsoideum98.5%KY864234787Soli. BrazilChlarydomonas sp.Chlorococum ellipsoideum98.5%KY864234788Soli. BrazilChlarydomonas sp.Chlorococum ellipsoideum98.5%KY864234789Soli. BrazilChlarydomonas sp.Chlorococum ellipsoideum98.5%KY864234781Soli. Brazil <td< td=""><td></td><td>835</td><td>Soil. Brazil</td><td>Chlamydomonas sp.</td><td>Chlamydomonas rapa</td><td>99.2%</td><td>KY864238</td></td<>		835	Soil. Brazil	Chlamydomonas sp.	Chlamydomonas rapa	99.2%	KY864238	
581Soli. HungaryChlamydannara sp.Chlorogonium elongatum98.2%KY856208Stephanosphaerini838Soli. BrazilChlamydannara sp.Chlorococcum ellipsoideum99.3%KY864185395Puddle. BrazilChlamydannara sp.Chlorococcum ellipsoideum99.3%KY864185467Soli. BrazilChlamydannara sp.Chlorococcum diplobiniticum98.7%KY864195476Soli. BrazilChlamydannara sp.Chlorococcum diplobinitum98.7%KY864195477Soli. BrazilChlamydannara sp.Chlorococcum diplobinitum98.7%KY8641964787Soli. BrazilChlamydannara sp.Chlorococum diplobinitum98.7%KY8642036710Mud. HungaryChlamydannara sp.Chlorococum diplobinitum98.7%KY8642156731Soli. BrazilChlamydannara sp.Chlorococum diplobinitum98.7%KY8642166731Soli. BrazilChlamydannara sp.Chlorococum diplobinitum98.7%KY864215786Soli. BrazilChlamydannara sp.Chlorococum diplobinitum98.7%KY8642167874Soli. BrazilChlamydannara sp.Chlorococum diplobinitum98.7%KY8642167876Soli. BrazilChlamydannara sp.Chlorococum diplobinitum98.7%KY8642167876Soli. BrazilChlamydannara sp.Chlorococum diplobinitum98.7%KY8642167876Soli. BrazilChlamydannara sp.Chlorococum diplobinitum98.7%KY8642177877 <td>Arenicolinia</td> <td>526</td> <td>Soil. Brazil</td> <td>Chlamydomonas sp.</td> <td>Chlorogonium elongatum</td> <td>95.9%</td> <td>KY864200</td>	Arenicolinia	526	Soil. Brazil	Chlamydomonas sp.	Chlorogonium elongatum	95.9%	KY864200	
607Soil, HungaryChlanydomonas sp. Chlanydomonas sp.Chlorococcum ellipsoideum95.7%KY86413395Pudlle, BrazilChlanydomonas sp. Chlorococcum ellipsoideum95.3%KY864193476Soil, BrazilChlanydomonas sp. Chlorococcum diplobionticum95.3%KY864193476Soil, BrazilChlanydomonas sp. Chlorococcum ellipsoideum95.3%KY864196478Soil, BrazilChlanydomonas sp. Chlorococcum ellipsoideum95.3%KY864196478Soil, BrazilChlanydomonas sp. Chlorococcum ellipsoideum95.3%KY864203601Mud, HungaryChlanydomonas sp. 		581	Soil. Hungary	Chlamydomonas sp.	Chlorogonium elongatum	98.2%	KY864208	
Stephanosphaerinia     388     Soil, Brazil     Chlanydomonas sp.     Chlorococcum ellipsoideum     95.%     KY864185       467     Soil, Brazil     Chlanydomonas sp.     Chlorococcum ellipsoideum     98.7%     KY864195       466     Soil, Brazil     Chlanydomonas sp.     Chlorococcum ellipsoideum     98.7%     KY864195       476     Soil, Brazil     Chlanydomonas sp.     Chlorococcum ellipsoideum     98.5%     KY864195       487     Soil, Brazil     Chlanydomonas sp.     Chlorococcum ellipsoideum     98.7%     KY864203       601     Mud. Hungary     Chlanydomonas sp.     Chlorococcum ellipsoideum     98.7%     KY864203       693     Soil, Brazil     Chlanydomonas sp.     Chlorococcum ellipsoideum     98.7%     KY864203       786     Soil, Brazil     Chlanydomonas sp.     Chlorococcum ellipsoideum     98.3%     KY864203       814     Soil, Brazil     Chlanydomonas sp.     Chlorococcum ellipsoideum     98.3%     KY864203       60gamochlanydinia     57.2     Pieforest. Hungary     Chlarydomonas sp.     Chlorococcum ellipsoideum     98.3%     KY864173 <td></td> <td>607</td> <td>Soil. Hungary</td> <td>Chlamydomonas sp.</td> <td>Chlorogonium elongatum</td> <td>96.7%</td> <td>KY864211</td>		607	Soil. Hungary	Chlamydomonas sp.	Chlorogonium elongatum	96.7%	KY864211	
935Puddle, BrazilChlanydomonas sp.Chlarococcum diplobionticum98.5%KY861193476Soil, BrazilChlanydomonas sp.Chlorococcum diplobionticum98.9%KY861195482Soil, BrazilChlanydomonas sp.Chlorococcum diplobionticum98.9%KY861195483Sevage water, BrazilChlanydomonas sp.Chlorococcum diplobionticum98.3%KY864193543Sevage water, BrazilChlanydomonas sp.Chlorococcum diplobionticum98.2%KY864203601Mud. HungaryChlanydomonas sp.Chlorococcum diplobionticum98.2%KY864215786Soil, BrazilChlanydomonas sp.Chlorococcum diplobionticum95.3%KY864216786Soil, BrazilChlanydomonas sp.Chlorococcum diplobionticum98.3%KY864230814Soil, BrazilChlanydomonas sp.Chlorococcum diplobionticum98.3%KY864234814Soil, BrazilChlanydomonas sp.Chlorococcum diplobionticum98.3%KY864234814Soil, BrazilChlanydomonas sp.Chlorococcum diplobionticum98.3%KY864234819Soil, BrazilChlanydomonas sp.Chlorococcum diplobionticum98.3%KY864234810Calarydomonas sp.Chlorococcum diplobionticum98.3%KY864234811Soil, BrazilChlanydomonas sp.Chlorococcum diplobionticum98.3%KY864234812Soil, BrazilChlanydomonas sp.Chlorococcum diplobionticum98.3%KY864234813	Stephanosphaerinia	388	Soil, Brazil	Chlamydomonas sp.	Chlorococcum ellipsoideum	99.5%	KY864184	
467Soil. BrazilChlamydomonas sp. Chlorococcum dipbolonicum98.7% 98.7% NT861195482Soil. BrazilChlamydomonas sp. Chlorococcum ellipsoideum98.5% 98.7% NT861196487Soil. BrazilChlamydomonas sp. Chlorococcum ellipsoideum98.9% 98.7% NT861198601Mud. Hungary Chlamydomonas sp.Chlorococcum ellipsoideum98.2% NT864198603Soil. BrazilChlamydomonas sp. Chlorococcum ellipsoideum98.2% NT864215786Soil. BrazilChlamydomonas sp. Chlorococcum ellipsoideum98.2% NT864215786Soil. BrazilChlamydomonas sp. Chlorococcum ellipsoideum98.2% NT864223807Soil. BrazilChlamydomonas sp. Chlorococcum ellipsoideum98.2% NT864233814Soil. BrazilChlamydomonas sp. Chlorococcum ellipsoideum98.2% NT864233819Soil. BrazilChlamydomonas sp. Chlorococcum ellipsoideum98.2% NT8642340gamochlamydinia10CCALA 246 (Trebon)Chlamydomonas sp. Chloromonas chlartat95.5% NT8642340gamochlamydinia10CCALA 246 (Trebon)Chlamydomonas sp. Chlorococcum ellipsoideum98.5% NT864173194Sunflower soil.Chlamydomonas sp. Chlorococcum ellipsoideum98.5% NT864173194Sunflower soil.Chlamydomonas sp. Chlorococcum ellipsoideum98.5% NT864173194Sunflower soil.Chlamydomonas sp. Chlamydomonas sp. Chlorococcum ellipsoideum98.5% NT864187194Sunflower soil. <td></td> <td>395</td> <td>Puddle. Brazil</td> <td>Chlamydomonas sp.</td> <td>Chlorococcum ellipsoideum</td> <td>98.5%</td> <td>KY864185</td>		395	Puddle. Brazil	Chlamydomonas sp.	Chlorococcum ellipsoideum	98.5%	KY864185	
476Soil. BrazilChlamydomnas sp.Chlorocccum dipbolonicum98.95%KY864195487Soil. BrazilChlamydomnas sp.Chlorocccum ellipsoideum98.5%KY864196543Sewage water. BrazilChlamydomnas sp.Chlorocccum ellipsoideum98.9%KY864203601Mud. HungaryChlamydomnas sp.Chlorocccum ellipsoideum98.9%KY864203603Soil. BrazilChlamydomnas sp.Chlorocccum ellipsoideum98.7%KY864221786Soil. BrazilChlamydomnas sp.Chlorocccum ellipsoideum98.2%KY864223787Soil. BrazilChlamydomnas sp.Chlorocccum ellipsoideum98.2%KY864230814Soil. BrazilChlamydomnas sp.Chlorocccum ellipsoideum98.2%KY864234819Soil. BrazilChlamydomnas sp.Chlorocccum ellipsoideum98.5%KY864234Chloronoccum ellipsoideum95.5%KY864234Chlamydomnas sp.Chloroccum ellipsoideum98.5%KY864234OgamochlamydiniaCCALA 249 (Trebon)Chlamydomnas sp.Chloronoccum ellipsoideum98.5%KY86417300gamochlamydiniaCCALA 249 (Trebon)Chlamydomnas sp.Chlorononas clathrata96.5%KY864174194Sunflower soil.Chlamydomnas sp.Lobochlamys culleus96.2%KY864187195Sunflower soil.Chlamydomnas sp.Lobochlamys culleus98.7%KY864187196Soil. BrazilChlamydomnas sp.Lobochlamys culleus97.3%KY864187<		467	Soil. Brazil	Chlamydomonas sp.	Chlorococcum diplobionticum	98.7%	KY864193	
482Soil, BrazilChlamydomonas sp.Chlamydomonas sp.Chlamydomonas sp.KY864198487Seivage water. BrazilChlamydomonas sp.Chlorococcum ellipsoideum98.9.%KY864203601Mud. HungaryChlamydomonas sp.Chlorococcum ellipsoideum98.2.%KY864210693Soil. BrazilChlamydomonas sp.Chlorococcum ellipsoideum98.2.%KY864212786Soil. BrazilChlamydomonas sp.Chlorococcum ellipsoideum98.2.%KY86422807Soil. BrazilChlamydomonas sp.Chlorococcum ellipsoideum98.3.%KY86423819Soil. BrazilChlamydomonas sp.Chlorococcum ellipsoideum98.3.%KY86423819Soil. BrazilChlamydomonas sp.Chlorococcum ellipsoideum98.3.%KY86423811Soil. BrazilChlamydomonas sp.Chlorococcum ellipsoideum98.3.%KY86423821Soil. BrazilChlamydomonas sp.Chloromocacu ellarhata96.9.%KY8642600ogamochlamydnini10CCALA 248 (Trebon)Chlamydomonas segnisLobochlamy culleus96.3.%KY86417375CCALA 249 (Trebon)Chlamydomonas sp.Lobochlamy culleus96.2.%KY86418684402Sunflower soil.Chlamydomonas sp.Lobochlamy culleus96.2.%KY86418784402Sunflower soil.Chlamydomonas sp.Lobochlamy culleus96.2.%KY86418684402Sunflower soil.Chlamydomonas sp.Lobochlamy culleus97.9.%		476	Soil. Brazil	Chlamydomonas sp.	Chlorococcum diplobionticum	98.9%	KY864195	
487Soil, BrazilChlanydomons sp.Chlarocccum ellipsoideum98.3%KY864198601Mud, HungaryChlanydomonas sp.Chlorocccum ellipsoideum98.3%KY864203693Soil, BrazilChlanydomonas sp.Chlorocccum ellipsoideum98.7%KY864215786Soil, BrazilChlanydomonas sp.Chlorocccum ellipsoideum98.3%KY864225807Soil, BrazilChlanydomonas sp.Chlorocccum ellipsoideum98.3%KY86423814Soil, BrazilChlanydomonas sp.Chlorocccum ellipsoideum98.3%KY864231817Soil, BrazilChlanydomonas sp.Chlorocccum ellipsoideum98.3%KY864231818Soil, BrazilChlanydomonas sp.Chlorocccum ellipsoideum98.3%KY864234Chlorochamydinia10CCALA 248 (Trebon)Chlanydomonas sp.Chlorononas clathrata98.3%KY86424Cogamochlanydinia557Pine forest, HungaryChlanydomonas sp.Chlorononas clathrata98.3%KY864173CogamochlanydiniaCCALA 248 (Trebon)Chlanydomonas sp.Lobochlamys culleus95.3%KY864174194Sunflower soil.Chlanydomonas sp.Lobochlamys culleus95.3%KY864187194Sunflower soil.Chlanydomonas sp.Lobochlamys culleus95.3%KY864187194Sunflower soil.Chlanydomonas sp.Lobochlamys culleus95.3%KY864192195Soil. BrazilChlanydomonas sp.Lobochlamys culleus95.3%KY864187 <td></td> <td>482</td> <td>Soil, Brazil</td> <td>Chlamydomonas sp.</td> <td>Chlorococcum ellipsoideum</td> <td>98.5%</td> <td>KY864196</td>		482	Soil, Brazil	Chlamydomonas sp.	Chlorococcum ellipsoideum	98.5%	KY864196	
543Sewage water, biz2iiChlamydomonas Sp.Chlorotoccum ellipsioleum95.3%KY864210601Mud, HungaryChlamydomonas Sp.Chlorococcum ellipsiodeum95.3%KY8642157866Soii, BrazilChlamydomonas Sp.Chlorococcum ellipsiodeum95.3%KY864227814Soii, BrazilChlamydomonas Sp.Chlorococcum ellipsiodeum95.3%KY864233819Soii, BrazilChlamydomonas Sp.Chlorococcum ellipsiodeum95.3%KY864231819Soii, BrazilChlamydomonas Sp.Chlorococcum oleglaciens99.2%KY864234ChloromonadiniaD0CCALA 248 (Trebon)Chlamydomonas Sp.Chloromonas clathrata96.5%KY864236Ogaanochlamydinia10CCALA 248 (Trebon)Chlamydomonas sp.Chloromonas clathrata95.5%KY864174194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus96.2%KY864177194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus96.2%KY864177194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus96.2%KY864187194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus96.2%KY864174194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus96.2%KY864174194MolecessiChlamydomonas sp.Lobochlamys culleus96.2%KY864174194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus97.5%KY86417419		487	Soil, Brazil	Chlamydomonas sp.	Chlorococcum ellipsoideum	98.9%	KY864198	
601Mult. HuigaryChlamydomonas Sp.Chlorotoctim appondutictim98.7%KY864215786Soii. BrazilChlamydomonas sp.Chlorococtum ellipsoideum99.5%KY86422807Soii. BrazilChlamydomonas sp.Chlorococtum ellipsoideum98.2%KY86423814Soii. BrazilChlamydomonas sp.Chlorococtum ellipsoideum98.3%KY86423819Soii. BrazilChlamydomonas sp.Chlorococtum oleg/actiens98.5%KY86423ChloroSoii. BrazilChlamydomonas sp.Chlorococtum oleg/actiens98.5%KY864234Ogamochlamydinia100CCALA 248 (Trebon)Chlamydomonas sp.Chloromonas clarhrata96.5%KY864234Ogamochlamydinia74CCALA 249 (Trebon)Chlamydomonas sgnisLobochlamys culleus96.2%KY864174194Sunflower soil.Chlamydomonas gleoganaLobochlamys culleus96.2%KY864174194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus96.2%KY864187194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus96.2%KY864187194Soii. BrazilChlamydomonas sp.Lobochlamys culleus96.2%KY864187195Soii. BrazilChlamydomonas sp.Lobochlamys culleus96.2%KY864181196Soii. BrazilChlamydomonas sp.Lobochlamys culleus95.5%KY864191196Soii. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864192196<		543	Sewage Water, Brazil	Chiamydomonas sp.	Chlorococcum ellipsoideum	98.9%	KY864203	
0.520.510.		603	Soil Brazil	Chlamydomonas sp.	Chlorococcum allipsoidaum	90.2%	K1004210 KV864215	
No.No.LowChlorydomona sp.Chlorococcum ellipsoideum98.2%KY864227814Soil. BrazilChlamydomonas sp.Chlorococcum ellipsoideum98.3%KY864233819Soil. BrazilChlamydomonas sp.Chlorococcum ellipsoideum98.3%KY864234821Soil. BrazilChlamydomonas sp.Chlorococcum ellipsoitens99.5%KY864234Chlorococcum idegiaciens99.5%KY864234KY864234Sill StazilSill StazilSill StazilSill StazilOgamochlamydinia10CCALA 248 (Trebon)Chlamydomonas segnisLobochlamys culleus99.5%KY86417374CCALA 249 (Trebon)Chlamydomonas segnisLobochlamys culleus96.2%KY864174194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus95.7%KY864177194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus95.7%KY864187194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus95.7%KY864187194YengaryChlamydomonas sp.Lobochlamys culleus96.2%KY864191195Soil. BrazilChlamydomonas sp.Lobochlamys culleus95.7%KY864193195Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864193195Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864194196Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864194196 <td< td=""><td></td><td>786</td><td>Soil Brazil</td><td>Chlamydomonas sp.</td><td>Chlorococcum ellipsoideum</td><td>99.5%</td><td>KY864222</td></td<>		786	Soil Brazil	Chlamydomonas sp.	Chlorococcum ellipsoideum	99.5%	KY864222	
814Soil, BrazilChlanydomona sp.Chlorococcum diplobionticum98.3%KY864230819Soil, BrazilChlamydomonas sp.Chlorococcum olegiciens99.2%KY864234Chloromonadinia557Pine forest. HungaryChlamydomonas sp.Chloromonac clathrata96.9%KY864206Oogamochlanydnina557Pine forest. HungaryChlamydomonas cpinsLobochlamys culleus95.5%KY8065374CCALA 248 (Trebon)ChlamydomonasChloromonas clathrata96.2%KY864173CCALA 249 (Trebon)Chlamydomonas gloeogamaLobochlamys culleus96.2%KY864174194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus98.7%KY864174194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus98.7%KY864174194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus98.7%KY864186Hungary <td colspa<="" td=""><td></td><td>807</td><td>Soil, Brazil</td><td>Chlamydomonas sp.</td><td>Chlorococcum ellipsoideum</td><td>98.2%</td><td>KY864227</td></td>	<td></td> <td>807</td> <td>Soil, Brazil</td> <td>Chlamydomonas sp.</td> <td>Chlorococcum ellipsoideum</td> <td>98.2%</td> <td>KY864227</td>		807	Soil, Brazil	Chlamydomonas sp.	Chlorococcum ellipsoideum	98.2%	KY864227
819Soil. BrazilChlamydomonas sp.Chlorococcum oleofaciens99.2%KY864234ChloromonadiniaSoil. BrazilChlamydomonas sp.Chlorococcum oleofaciens98.5%KY864234OogamochlamydiniaSoil. BrazilChlamydomonas sp.Chloromonas clathrata96.5%KY864206OogamochlamydiniaCCALA 248 (Trebon)Chlamydomonas sp.Lobochlamys culleus99.5%KY864206OogamochlamydiniaCCALA 248 (Trebon)Chlamydomonas subilisLobochlamys culleus96.2%KY86417374CCALA 249 (Trebon)Chlamydomonas subilisLobochlamys culleus96.2%KY864174194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus98.7%KY864177194AL/G-23. CzechChlamydomonas sp.Lobochlamys culleus99.5%KY864186194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus99.5%KY864187194YungaryHungary100KY864187100100402Sunflower soil.Chlamydomonas sp.Lobochlamys culleus96.2%KY864191460Puddle. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864192460Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864192460Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864194460Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864194461Puddle. BrazilChlamyd		814	Soil. Brazil	Chlamvdomonas sp.	Chlorococcum diplobionticum	98.3%	KY864230	
821Soil, BrazilChlamydomonas sp.Chlorococcum oleofaciens98.5%KY864236Chloromonadinina557Pine forest. HungaryChlamydomonas sp.Chloromonas clathrata96.5%KY806553Oogamochlamydini74CCALA 243 (Trebon)Chlamydomonas segnisLobochlamys culleus95.5%KY80655374CCALA 249 (Trebon)Chlamydomonas segnisLobochlamys culleus96.2%KY864174194Sunflower soil.Chlamydomonas gleogamaLobochlamys culleus96.2%KY864174194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus99.5%KY864187194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus99.5%KY864187194Sunflower soil.Chlamydomonas sp.Lobochlamys culleus96.2%KY864192402Sunflower soil.Chlamydomonas sp.Lobochlamys culleus96.2%KY864192405Soil. BrazilChlamydomonas sp.Lobochlamys culleus96.2%KY864192406Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.5%KY86419290gominiaAf5Soil. BrazilChlamydomonas sp.Lobochlamys culleus99.5%KY86419290gominiaAf5Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.5%KY86419290gominiaAf5Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.5%KY86419390gominiaAf5Soil. BrazilChlamydomonas sp.Lobochlamys culleus<		819	Soil. Brazil	Chlamydomonas sp.	Chlorococcum oleofaciens	99.2%	KY864233	
Chloromonadinia Oogamochlamydinia557Pine forest. Hungary CCALA 248 (Trebon)Chlamydomonas sp. Chlamydomonas segnis 		821	Soil. Brazil	Chlamydomonas sp.	Chlorococcum oleofaciens	98.5%	KY864234	
Oogamochlamydinia10CCALA 248 (Trebon) CCALA 234 (Trebon)Chlamydomonas segnis Chlamydomonas chlorococcidesLobochlamys culleus99.5% SK KY86417375CCALA 249 (Trebon)Chlamydomonas subitiis Inamydomonas subitiisLobochlamys culleus96.2%KY864174194Sunflower soil RepublicChlamydomonas subitiis HungaryLobochlamys culleus98.7%KY864177402Sunflower soil. RepublicChlamydomonas sp.Lobochlamys culleus99.5%KY864186402Sunflower soil. HungaryChlamydomonas sp.Lobochlamys culleus98.7%KY864187403Soil. BrazilChlamydomonas sp.Lobochlamys culleus98.5%KY864191404Soil. BrazilChlamydomonas sp.Lobochlamys culleus96.2%KY864192405Soil. HungaryChlamydomonas sp.Lobochlamys culleus90.5%KY864192460Puddle. BrazilChlamydomonas sp.Lobochlamys culleus90.5%KY864192460Soil. BrazilChlamydomonas sp.Lobochlamys culleus100%KY864192460Soil. BrazilChlamydomonas sp.Lobochlamys culleus90.5%KY86419276Soil. BrazilChlamydomonas sp.Lobochlamys culleus90.5%KY864226822Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864193764Soil. BrazilChlamydomonas sp.Polytoma uvella98.2%KY864197784Soil. BrazilChlamydomonas sp.Secn	Chloromonadinia	557	Pine forest. Hungary	Chlamydomonas sp.	Chloromonas clathrata	96.9%	KY864206	
74CCALA 234 (Trebon) chlorococcidesChlomydomonas chlorococcidesChlomydomonas clathrata98.5%KY86417375CCALA 249 (Trebon) HungaryChlamydomonas subtilis Lobochlamys culleusLobochlamys culleus96.2%KY864177194Sunflower soil. HungaryChlamydomonas sp. ExpublicLobochlamys culleus98.7%KY864186402Sunflower soil. HungaryChlamydomonas sp. HungaryLobochlamys culleus96.2%KY864187425Soil. BrazilChlamydomonas sp. Lobochlamys culleus96.2%KY864191460Puddle. BrazilChlamydomonas sp. Lobochlamys culleus96.2%KY864192406Soil. HungaryJobochlamys culleus9.5%KY86419296Soil. ImagrayLobochlamys culleus9.5%KY86419296Soil. BrazilChlamydomonas sp. Lobochlamys culleus9.5%KY864192806Soil. BrazilChlamydomonas sp. Lobochlamys culleus9.5%KY86419290lytominia75Soil. BrazilChlamydomonas sp. Lobochlamys culleus9.5%KY8641945cenedesmaceae215IPAS D-292. RussiaChlamydomonas sp. Chlamydomonas sp.Lobochlamys culleus9.7%KY8641785cenedesmaceae215IPAS D-292. RussiaChlamydomonas sp.Scenedesmus communis9.8%KY864190485Pond. BrazilChlamydomonas sp.Desmodesmus communis9.8%KY864191783Soil. BrazilChlamydomonas sp.Desmodesmus communis<	Oogamochlamydinia	10	CCALA 248 (Trebon)	Chlamydomonas segnis	Lobochlamys culleus	99.5%	KY806553	
chlorococides75CCALA 249 (Trebon)Chlamydomonas subilisLobochlamys culleus96.2%KY864174194Sunflower soil.Chlamydomonas gleeogamaLobochlamys culleus98.7%KY864177Hungary298AL/G-23. CzechChlamydomonas sp.Lobochlamys culleus98.5%KY864186Republic402Sunflower soil.Chlamydomonas sp.Lobochlamys culleus96.2%KY864197460Puddle. BrazilChlamydomonas sp.Lobochlamys culleus96.2%KY864192460Puddle. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864192496Soil. HungaryChlamydomonas sp.Lobochlamys culleus99.5%KY864192496Soil. BrazilChlamydomonas sp.Lobochlamys culleus99.5%KY864235Polytominia475Soil. BrazilChlamydomonas sp.Lobochlamys culleus99.5%KY864236Scenedesmaceae215IPAS D-292. RussiaChlamydomonas sp.Lobochlamys culleus97.2%KY86420Scenedesmaceae215IPAS D-292. RussiaChlamydomonas sp.Scenedesmus coundurus97.3%KY864190485Pond. BrazilChlamydomonas sp.Scenedesmus coundurus97.3%KY864191783Soil. BrazilChlamydomonas sp.Scenedesmus coundurus97.3%KY864191784Soil. BrazilChlamydomonas sp.Scene		74	CCALA 234 (Trebon)	Chlamydomonas	Chloromonas clathrata	98.5%	KY864173	
75CCALA 249 (Trebon)Chlamydomonas subtilisLobochlamys culleus96.2%KY864174194Sunflower soil. HungaryChlamydomonas gloeogamaLobochlamys culleus98.7%KY864177398AL/G-23. Czech RepublicChlamydomonas sp.Lobochlamys culleus99.5%KY864186402Sunflower soil. HungaryChlamydomonas sp.Lobochlamys culleus98.9%KY864191402Sunflower soil. HungaryChlamydomonas sp.Lobochlamys culleus96.2%KY864192403Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864192404Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864192405Soil. HungaryChlamydomonas sp.Lobochlamys culleus97.9%KY864192406Soil. HungaryChlamydomonas sp.Lobochlamys culleus97.9%KY864192406Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864235806Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864194784Soil. BrazilChlamydomonas reinhardtiiPolytoma uvella98.2%KY8641785cenedesmaceae215IPPAS D-292. RussiaChlamydomonas sp.Scenedesmus vacuolatus97.7%KY864190485Pond. BrazilChlamydomonas sp.Scenedesmus vacuolatus97.7%KY864191783Soil. BrazilChlamydomonas sp.Scenedesmus vacuolatus97.7%KY864191784<				chlorococcoides				
194Suthiower soli. HungaryChiamydomonas gioeoganaLobochlamys culieus98.7%KY864177398AL/G-23. Czech RepublicChiamydomonas sp.Lobochlamys culieus99.5%KY864186402Sunflower soil. HungaryChiamydomonas sp.Lobochlamys culieus98.9%KY864187425Soil. BrazilChiamydomonas sp.Lobochlamys culieus96.2%KY864191460Puddle. BrazilChiamydomonas sp.Lobochlamys culieus97.9%KY864192496Soil. BrazilChiamydomonas sp.Lobochlamys culieus97.9%KY864192496Soil. BrazilChiamydomonas sp.Lobochlamys culieus99.5%KY864226806Soil. BrazilChiamydomonas sp.Lobochlamys culieus99.5%KY864226822Soil. BrazilChiamydomonas sp.Lobochlamys culieus97.9%KY864226821Soil. BrazilChiamydomonas sp.Lobochlamys culieus97.9%KY864226822Soil. BrazilChiamydomonas sp.Polytoma uvella98.2%KY8642065cenedesmaceae215IPAS D-292. RussiaChiamydomonas sp.Scenedesmus vacuolatus97.4%KY864178485Pond. BrazilChiamydomonas sp.Desmodesmus communis98.5%KY86421485Soil. BrazilChiamydomonas sp.Scenedesmus vacuolatus97.7%KY86421485Soil. BrazilChiamydomonas sp.Scenedesmus vacuolatus98.7%KY864221486Soil. BrazilChiamy		/5	CCALA 249 (Trebon)	Chlamydomonas subtilis	Lobochlamys culleus	96.2%	KY864174	
Inligaty398AL/G-23. Czech RepublicChlamydomonas sp.Lobochlamys culleus99.5%KY864186402Sunflower soil. HungaryChlamydomonas sp.Lobochlamys culleus98.9%KY864187425Soil. BrazilChlamydomonas sp.Lobochlamys culleus96.2%KY864191460Puddle. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864192466Soil. HungaryChlamydomonas sp.Lobochlamys culleus97.9%KY864192466Soil. BrazilChlamydomonas sp.Lobochlamys culleus99.5%KY864226496Soil. BrazilChlamydomonas sp.Lobochlamys culleus99.5%KY864226822Soil. BrazilChlamydomonas sp.Lobochlamys culleus99.5%KY86423670lytominia475Soil. BrazilChlamydomonas sp.Polytoma uvella97.2%KY864194784Soil. BrazilChlamydomonas sp.Polytoma uvella97.2%KY8641965cenedesmaceae215IPPAS D-292. RussiaChlamydomonas sp.Scenedesmus vacuolatus97.4%KY864197783Soil. BrazilChlamydomonas sp.Desmodesmus communis100%KY864219784Soil. BrazilChlamydomonas sp.Desmodesmus communis100%KY864219783Soil. BrazilChlamydomonas sp.Desmodesmus communis100%KY864219783Soil. BrazilChlamydomonas sp.Ceelastrella rubescens98.9%KY864228810 <td></td> <td>194</td> <td>Sunflower soll.</td> <td>Chiamyaomonas gioeogama</td> <td>Lobochiamys culleus</td> <td>98.7%</td> <td>КҮ864177</td>		194	Sunflower soll.	Chiamyaomonas gioeogama	Lobochiamys culleus	98.7%	КҮ864177	
402KypenneChlamydomonas sp.Lobochlamys culleus98.9%KY864187405Soil. BrazilChlamydomonas sp.Lobochlamys culleus96.2%KY864191460Puddle. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864192496Soil. HrugaryChlamydomonas sp.Lobochlamys culleus97.9%KY864199806Soil. BrazilChlamydomonas sp.Lobochlamys culleus99.5%KY864226806Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864235807Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864235808Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864235809Soil. BrazilChlamydomonas sp.Polytoma uvella98.2%KY864194784Soil. BrazilChlamydomonas reinhardtiiPolytoma uvella97.2%KY8641785cenedesmaceae215IPPAS D-292. RussiaChlamydomonas sp.Scenedesmus vacuolatus97.4%KY864190485Pond. BrazilChlamydomonas sp.Desmodesmus communis90.7%KY864197783Soil. BrazilChlamydomonas sp.Desmodesmus vacuolatus98.7%KY864221785Soil. BrazilChlamydomonas sp.Coelastrella rubescens98.9%KY864221781Soil. BrazilChlamydomonas sp.Coelastrella rubescens98.9%KY864229785Soil. BrazilChlamydomonas sp.Coelastrella striolata <td></td> <td>398</td> <td>AL/G-23. Czech</td> <td>Chlamydomonas sp.</td> <td>Lobochlamys culleus</td> <td>99.5%</td> <td>KY864186</td>		398	AL/G-23. Czech	Chlamydomonas sp.	Lobochlamys culleus	99.5%	KY864186	
425Soil, BrazilChlamydomonas sp.Lobochlamys culleus96.2%KY864191460Puddle. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864192496Soil. HungaryChlamydomonas sp.Lobochlamys culleus100%KY864199806Soil. BrazilChlamydomonas sp.Lobochlamys culleus99.5%KY864226822Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864235Polytominia475Soil. BrazilChlamydomonas sp.Polytoma uvella98.2%KY864194784Soil. BrazilChlamydomonas reinhardtiiPolytoma uvella97.2%KY864178Scenedesmaceae215IPPAS D-292. RussiaChlamydomonas sp.Polytoma uvella97.4%KY864178424Soil. BrazilChlamydomonas sp.Scenedesmus vacuolatus97.4%KY864190485Pond. BrazilChlamydomonas sp.Desmodesmus communis98.5%KY864219783Soil. BrazilChlamydomonas sp.Desmodesmus cullatus98.7%KY864221785Soil. BrazilChlamydomonas sp.Coelastrella rubescens98.7%KY864228810Soil. BrazilChlamydomonas sp.Coelastrella rubescens98.5%KY864228811Soil. BrazilChlamydomonas sp.Coelastrella striolata99.5%KY864228813Soil. BrazilChlamydomonas sp.Coelastrella striolata99.5%KY864228814Soil. BrazilChlamydomonas sp.C		402	Sunflower soil.	Chlamydomonas sp.	Lobochlamys culleus	98.9%	KY864187	
460Puddle, BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864192496Soil, HungaryChlamydomonas sp.Lobochlamys culleus100%KY864199806Soil, BrazilChlamydomonas sp.Lobochlamys culleus99.5%KY864226822Soil, BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864235Polytominia475Soil, BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864236Scenedesmaceae215IPAS D-292. RussiaChlamydomonas sp.Polytoma uvella98.2%KY864178Scenedesmaceae215IPAS D-292. RussiaChlamydomonas sp.Scenedesmus vacuolatus97.4%KY864178485Pond. BrazilChlamydomonas sp.Scenedesmus vacuolatus97.4%KY864190485Pond. BrazilChlamydomonas sp.Desmodesmus communis98.5%KY864197783Soil. BrazilChlamydomonas sp.Desmodesmus communis98.7%KY864219785Soil. BrazilChlamydomonas sp.Coelastrella rubescens98.7%KY864228810Soil. BrazilChlamydomonas sp.Coelastrella rubescens98.7%KY864228811Soil. BrazilChlamydomonas sp.Coelastrella rubescens98.5%KY864229818Soil. BrazilChlamydomonas sp.Coelastrella striolata99.5%KY8642232825Soil. BrazilChlamydomonas sp.Coelastrella striolata99.5%KY864233825Soil. Brazil<		425	Soil. Brazil	Chlamydomonas sp.	Lobochlamys culleus	96.2%	KY864191	
496Soil. HungaryChlamydomonas sp.Lobochlamys culleus100%KY864199806Soil. BrazilChlamydomonas sp.Lobochlamys culleus99.5%KY864226822Soil. BrazilChlamydomonas sp.Lobochlamys culleus97.9%KY864235Polytominia475Soil. BrazilChlamydomonas sp.Polytoma uvella98.2%KY864194784Soil. BrazilChlamydomonas reinhardtiiPolytoma uvella97.2%KY864178Scenedesmaceae215IPPAS D-292. RussiaChlamydomonas sp.Scenedesmus vacuolatus97.4%KY864178424Soil. BrazilChlamydomonas sp.Desmodesmus communis98.5%KY864190485Pond. BrazilChlamydomonas sp.Desmodesmus communis98.5%KY864219783Soil. BrazilChlamydomonas sp.Desmodesmus communis98.7%KY864219785Soil. BrazilChlamydomonas sp.Scenedesmus vacuolatus98.7%KY864228810Soil. BrazilChlamydomonas sp.Coelastrella rubescens98.5%KY864229811Soil. BrazilChlamydomonas sp.Coelastrella striolata99.5%KY864229818Soil. BrazilChlamydomonas sp.Coelastrella striolata99.5%KY864232825Soil. BrazilChlamydomonas sp.Coelastrella striolata99.5%KY864232825Soil. BrazilChlamydomonas sp.Coelastrella striolata99.5%KY864232825Soil. BrazilChlamydomonas sp.<		460	Puddle. Brazil	Chlamydomonas sp.	Lobochlamys culleus	97.9%	KY864192	
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genes, have highlighted numerous chlamydomonadalean lineages (Buchheim et al., 1990, Buchheim et al., 1996, Buchheim et al., 1997a, Buchheim et al., 2013; Nozaki et al., 2000, 2003; Matsuzaki et al., 2010; Lemieux et al., 2015). To confirm the genetic results, Pröschold et al. (2018) used cross experiments of sporangium wall autolysins (VLE), because this enzyme is a good biochemical marker for classification of *Chlamydomonas* species.

Our study focuses on the systematic relationships of 70 MACC strains previously identified as *Chlamydomonas*. No molecular phylogenetic data were available for the *Chlamydomonas* strains in the MACC collection, therefore, we sequenced partial 18S rRNA gene to determine their phylogenetic position and assign them to subgroups defined by Nakada et al. (2008b). The aim of this research is to provide a proper platform for future systematic and biodiversity research.

#### 2. Materials and methods

#### 2.1. Strains and cultivation

Seventy *Chlamydomonas* (Table 1) strains were selected from MACC, Széchenyi István University (Mosonmagyaróvár, Hungary). The former nomenclature is based on the morphological identification, which was carried out in the 1990's by the Balaton Limnological Institute team (Ördög, 2015). Forty-six of the 70 MACC *Chlamydomonas* strains were from Brazil, 11 from Hungary, nine from the Czech Republic, 2 from Ukraine, 1 from Slovenia, and another from Russia. Cultures of *Chlamydomonas* were maintained and cultivated in modified Z8 medium (Kótai, 1972; NIVA, 1976) at 24–26 °C under a light intensity of 20 µmol m<sup>-2</sup> s<sup>-1</sup> provided by Lumoflor and cool white light (16:8 h light:dark cycle).

#### 2.2. Genomic DNA extraction and PCR

One mL of cell culture was centrifuged at 14,000 rpm for 2 min to spin down the culture. From the spin down, a 10 mg pellet was resuspended in 500  $\mu$ L of 10% Chelex-100 diluted in ddH<sub>2</sub>O. The suspension was vortexed for 30 s and incubated at 95 °C for 10 min. The sample was cooled to 20 °C and vortexed again for 10 s and centrifuged at 14000 rpm for 2 min. One  $\mu$ L of the supernatant was used in each 20  $\mu$ L PCR reaction. The 18S rRNA gene was amplified with primers EUK528F (5-'CCGCGGTAATTCCAGCTC-3') (Elwood et al., 1985; Keresztes et al., 2012) and Chlo02R (5'-CTTCGAGCCCCCAACTTTC-3') (Zhu et al., 2005). The PCR mixture contained 10  $\mu$ L Phusion Flash High-Fidelity PCR Master Mix, 7  $\mu$ L dH<sub>2</sub>O, 1  $\mu$ L of each primer (0.5 um final concentration per primer) and 1  $\mu$ L purified DNA (50–100 ng) to give 20  $\mu$ L final volume of PCR reaction.

For PCR amplification, an initial denaturation was carried out at 98 °C for 30 s, followed by denaturation at 98 °C for 10 s, annealing at 58 °C for 20 s, extension at 72 °C for 30 s, and the final extension at 72 °C for 1 min over 40 cycles. After DNA amplification, the products were run for 45 min at 120 V in 0.5% TBE buffer and visualized on a 1.5% agarose gel. The PCR products were purified using GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA). PCR products were sequenced using a LifeTech 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) capillary sequencer at the Biological Research Centre of the Hungarian Academy of Sciences (BRC) (Szeged, Hungary).

## 2.3. Sequence alignment and phylogenetic analyses

Reference sequences of Chlamydomonadaceae strains (Lemieux et al., 2015; Nakada et al., 2016; Possmayer et al., 2016; Watanabe and Lewis, 2017) were retrieved from GenBank (NCBI) (Altschul et al., 1997), in addition to closest BLAST hits. The sequences were aligned using MUSCLE (Edgar, 2004) on MEGA 7 (Kumar et al., 2016). The jModelTest 2 confirmed a TIM2 + G + I model of substitution (Darriba et al., 2012). The maximum likelihood (ML) analysis was run

on a dataset of 279 sequences using RAxML (Stamatakis, 2014) with 1000 bootstraps and *Ulothrix zonata* UTEX 745 was used to root the tree. The final phylogenetic tree (Fig. 1) was edited using Adobe Illustrator CC version 2014.01. The similarity matrix (percentages) for MACC strains comparing partial sequences of the 18S rRNA gene was calculated by Geneious 10.2.3 (Table S2). Due to lack of space, only a few representatives of each cluster were shown in the table

# 3. Results

The sequences formed c.a. 400 bp alignments, including our strains, was composed of 279 OTUs with nine phylogroups within three different orders (following Nakada 2008b) (Fig. 1). One phylogroup belonged to the order Chlorellales (*Chlorella* phylogroup with 6 MACC strains), one in the order Sphaeropleales (Scenedesmaceae phylogroup with 9 MACC strains) and seven phylogroups in the order Chlamydomonadales (*Moewusinia* with 5, *Reinhardtinia* with 19 strains, *Arenicolinia* with 3, *Stephanosphaerinia* with 14, *Chloromonadinia* with 1, *Oogamochlamydinia* with 11 and *Polytominia* phylogroup with 2 MACC strains). Results of present phylogenetic analysis are shown in Table 1.

# 3.1. Arenicolinia phylogroup

The Arenicolinia phylogroup (Fig. 1) formed a well-supported cluster (BS 99%) with two reference sequences (Chlorophycean sp. SEV3VF14 AF513371 and *Chlorosarcinopsis arenicola* UTEX 1697 AB218701) and three MACC strains (MACC 526, 581, 607). The sequence similarity between *Chlorosarcinopsis arenicola* UTEX 1697 AB218701 and MACC 607 was 97.5% (Table S2).

#### 3.2. Stephanosphaerinia phylogroup

The Stephanosphaerinia phylogroup (Fig. 1) included 14 MACC strains (MACC 395, 388, 467, 476, 482, 487, 543, 601, 693, 786, 807, 814, 819, 821) and forms a well-supported clade (B.S. 99%).

Five strains of the first subclade (MACC 482, 487, 543, 819, 821) had 100% B.S. support with *Chlorosarcinopsis aggregata* UTEX 779 AB218695, but their sequence similarity ranged between 90.4 and 93.7%. MACC 543 originated from sewage water (Brazil), while the remaining four strains were terrestrial (Brazil). MACC 819 and 821 are likely to belong to the same *Chlorosarcinopsis* taxon, since they were 100% homologous, and isolated from the same area.

The sequence similarity of the second subcluster groups MACC 388, 395, 693, 786 and 807 together with *Chlorococcum ellipsoideum* UTEX 972U70586 ranged from 97.2 to 98.2%. MACC 388, 395, 693, 786, and 807 (Table 1) are strains with 96% bootstrap support.

MACC 601 was 97.7% similar (Table S2) to *Nautococcus solutes* SAG 76 80 AB360749. This is the only Hungarian strain in this phylogroup and it originated from mud, whereas the other 13 strains were from Brazil (11 from soil, MACC 395 from a puddle and MACC 543 from sewage water).

MACC 467, 476 and 814 fell close to *Deasonia multinucleata* UTEX 2013 U63106. Their 18S rRNA similarity ranged from 95.3% to 95.7%. They were all isolated from soil (Brazil).

# 3.3. Oogamochlamydinia phylogroup

The Oogamochlamydinia phylogroup (Fig. 1) is a mixture of genera containing *Chlamydomonas, Lobochlamys, Oogamochlamys, Sarcinochlamys* and 11 MACC taxa (MACC 10, 74, 75, 194, 398, 402, 425, 460, 496, 806, 822) (Table 1). The Oogamochlamydinia group was composed of two subclusters: the first subcluster included MACC 74, 75, and 425. MACC 74 and 75 are from CCALA. As for their 18S rRNA similarity, both showed low values (<50%) with *Chlamydomonad sp.* BogD8 18T2w AY220581. MACC 74 and 75 did not group with the reference sequences, thus further analysis is needed to classify these



Fig. 1. 18S rRNA gene phylogenetic analysis based on 279 OTUs demonstrating the position of 70 MACC strains. MACC strain names are in bold. The bootstrap support (>50%) for maximum likelihood (ML) is shown next to the nodes. Ulothrix zonata was used as outgroup.



Fig. 1 (continued).

strains. MACC 425 originated from soil (Brazil) and grouped with the Chlamydomonad sp. BogD8 18T2w AY220581.

The second subcluster of the Oogamochlamydinia group included MACC 10, 194, 398, 402, 460, 496, 806, 822 and *Lobochlamys*. Their sequence similarity ranged from 96% to 98.7%. MACC 398 and 460 grouped with *Lobochlamys segnis* SAG 1.79 AJ410457, MACC 822 and 806 with Lobochlamys culleus SAG 64.72 AJ410463, and MACC 194, 402, and 496 with Lobochlamys culleus SAG 17.73 AJ410461. MACC 10 was also part of this subcluster but distant from the other strains. Interestingly, MACC 10 was designated as Chlamydomonas segnis and originated from CCALA. MACC 10 neighbored Lobochlamys segnis, which was previously designated as *Chlamydomonas segnis* SAG 1 79 AJ410457. The strains of this subcluster had various places of origin.

#### 3.4. Chloromonadinia phylogroup

The Chloromonadinia phylogroup (Fig. 1) contains mostly *Chloromonas* sequences and the only MACC member of this phylogroup is MACC 557, which is from a pine forest (Hungary) (Table S2). According to the phylogenetic analysis, its closest neighbor is *Chloromonas actinochloris* UTEX 578U57695, but had higher sequence similarity to *Chloromonas asteroidea* SAG 11-47U70783.

#### 3.5. Reinhardtinia phylogroup

The Reinhardtinia phylogroup (Fig. 1) contained 19 MACC strains (MACC 53, 216, 285, 327, 335, 382, 415, 530, 531, 544, 549, 579, 584, 658, 674, 688, 772, 788, 835) and three subclades were identified (Table 1).

The first subclade (100% bootstrap) contained two strains, MACC 674 and 835, along with *Heterochlamydomonas* reference sequences. MACC 674 was 74% similar to *Heterochlamydomonas lobata* UTEX 728 AF367858 (Table S2), whereas MACC 835 was 66% similar to *Heterochlamydomonas inaequalis* UTEX 1705 AF367857. MACC 674 is from Kiev and MACC 835 is from Brazilian soil samples.

The second subclade contained two strains, MACC 544 and 658. MACC 658 originated from Trebon, Czech Republic (*Chlamydomonas peterfii* CCALA 247) and grouped with *Chlamydomonas asymmetrica* SAG 70 72U70788 (97.3% sequence similarity). MACC 544 originated from soil (Brazil), and was placed next to *Heterotetracystis akinetes* UTEX 1675 AB244242. Despite this, MACC 544 showed the greatest sequence similarity (98.3%) to *Hormotila blennista* UTEX 1239U83123, thus further analysis is required to accurately delineate this strain.

The third subclade included 15 MACC strains (MACC 53, 216, 285, 327, 335, 382, 415, 530, 531, 549, 579, 584, 688, 772, 788) from which MACC 415 fell outside and grouped with Vitreochlamys nekrassovii SAG 1110 LC086372. MACC 415 was the only strain in the Reinhardtinia phylogroup that originated from a puddle (Brazil), whereas the remaining strains were terrestrial (Brazil and Hungary). The remaining 14 MACC strains (MACC 53, 216, 285, 327, 335, 382, 530, 531, 549, 579, 584, 688, 772, 788) grouped together with such strains as Edaphochlamys debaryana SAG 26.72 AF008240, Chlamydomonas reinhardtii UTEX 1061M32703 02949, Chlamydomonas zebra SAG 10.83U70792 and two other sequences (Chlamydomonad sp. WTwin 8 18 P5d AY220084 and Volvox carteri f. nagariensis UTEX 1885 X53904). MACC 285, 327 and 549 were most similar to Chlamydomonas zebra SAG 10.83 (97-97.5%) whereas MACC 53, 216, 335, 382, 530, 531, 579, 584, 688, 772, and 788 were most similar to Chlamydomonas reinhardtii UTEX 1061M32703J02949 and ranged from 96.7% to 98.2% similarity. In a phylogenetic sense, this latter together with Chlamydomonas incerta and Chlamydomonas schloesseri are considered to be 'real' Chlamydomonas genera. They are in a green shaded area on Fig. 1.

## 3.6. Polytominia phylogroup

The Polytominia cluster (Fig. 1) has two *Chlamydomonas applanata*, two *Chlamydomonas pulsatilla* and four *Polytoma* OTUs as reference sequences. MACC 475 and 784 (Table 1) are 98.5% (Table S2) similar to *Chlamydomonas applanata* UTEX 225U13984 and *C. applanata* UTEX 2399U13985 strains, though their subclade is not well supported (B.S. 84%). Both MACC 475 and 784 were isolated from Brazilian soil samples.

#### 3.7. Scenedesmaceae phylogroup

The Scenedesmaceae phylogroup (Fig. 1) had nine MACC strains (MACC 215, 424, 485, 783, 785, 810, 811, 818, 825) (Table 1) that were isolated from Brazilian soils [except MACC 215 which was from IPPAS D-292 (Russia) and MACC 485 originating from a pond (Brazil)]. MACC 811 fell into the same clade as *Scenedesmus costatus* Hegewald 19862 AB037090 with 98.7% sequence similarity (Table S2). MACC 485 and MACC 783 had the highest bootstrap support of this cluster (100%) and grouped together with *Scenedesmus costato-granulatus* SAG 18 81 X91265, *Scenedesmus communis* UTEX 76 X73994 and *Scenedesmus abundans* UTEX 343 X73995. They showed the highest sequence similarity to *Scenedesmus communis* UTEX 76 X73994 (97.5%). Four MACC strains (785, 810, 818 and 825) were in the same group as *Scenedesmus vacuolatus* SAG 211-8b and their sequence similarity ranged from 97.2% to 98.5%. The remaining two strains (MACC 215 and 424) grouped together with *Ettlia texensis* SAG 79.80 GU292343.

#### 3.8. Moewusinia phylogroup

The Moewusinia phylogroup (Fig. 1) was well-supported [Bootstrap (B.S.) 99%] and included five MACC strains (MACC 27, 30, 77, 120, 782). MACC 120 grouped with *Chlamydomonas moewusii* CCAP 11/16F FR865565 and their 18S rRNA similarity was 98.2%. MACC 782 was only 94.9% similar to *Chlamydomonas raudensis* SAG 49.72 JN903981. Both MACC 27 and 30 were close to *Chlamydomonas noctigama* (also known as *Chlamydomonas pinicola*) UTEX 1339 AF008241, the former with 97.5% similarity, and the latter 99.1% (Table S2). MACC 77 was related to *Chlamydomonas sordida* SAG 18.73 AB290341 and their 18S rRNA similarity was 97%. MACC 27, 30 and 77 were from CCALA (Trebon, Czech Republic) (Table 1). MACC 120 was isolated from a tarn (Slovenia), whereas MACC 782 was terrestrial (Brazil).

#### 3.9. Chlorella phylogroup

The *Chlorella* phylogroup (Fig. 1) contained six MACC strains (MACC 406, 771, 787, 793, 816, 823). MACC 787 and 816 strains were tightly clustered with the reference sequence *Chlorella sorokiniana* SAG 211-8k X62441. Their 18S rRNA similarity was 99% with *Chlorella sorokiniana* SAG 211-8k (Table S2). The subcluster itself was supported by 66% (ML). MACC 406, 771, 793 and 823 were directly grouped with *Chlorella vulgaris* SAG 21111b X13688.

As for the place of origin, MACC 771, 787, 793, 816 are from soil samples (Brazil), whereas MACC 823 was isolated from a pond (Brazil) (Table 1).

#### 4. Discussion

This is the first study using molecular data to determine phylogenetic relationships of MACC green algae strains. The phylogenetic tree, resulting from the analysis of 70 partial 18S rRNA sequences, revealed nine different phylogroups. There is a need for proper identification of culture collections that do not have molecular data. Correcting these descriptions contributes to refining taxonomic models and methods and provides a proper platform for future work.

Phenotypic plasticity combined with constant nomenclatural updates makes it difficult to identify and classify isolates based solely on morphology. Methods of molecular genotyping and subsequent phylogenetic analyses enable more precise determination of the taxonomic position of new isolates (Kravtsova et al., 2013). The circumscription of species in *Chlamydomonas* is problematic because many original descriptions were based on light microscopy of a few specimens from natural samples without considering the plasticity of morphological characters within a population or life history (Pröschold et al., 2001). In addition, several molecular phylogenetic analyses have shown that *Chlamydomonas* is highly polyphyletic (e.g. Buchheim et al., 1997b; Pröschold et al., 2001; Nakada et al., 2008a; Nakada and Tomita, 2011; Demchenko et al., 2012, Watanabe and Lewis, 2017, Pröschold et al., 2018). Nakada et al. (2008b) adopted the phylogenetic classification system of the order Volvocales based on PhyloCode (Cantino and de Queiroz, 2010), and recognized 21 'primary clades' and we found this code to be the best way to determine the appropriate taxonomic positions of MACC green algae strains.

The MACC strains were distributed among nine clades. Performing molecular analyses (based on partial 18S rRNA gene sequences) allowed the reclassification of 70 strains previously assigned to the Chlamydomonas genus into various phylogenetic affiliations. The results showed that most strains previously assigned to Chlamydomonas, were not Chlamydomonas in the phylogenetic sense. This indicates how molecular approaches to systematics are fundamental for classifying algae. The first explanation for the results could be that the morphological features of Chlamydomonas are very variable and overlapping. Therefore, the initial light microscope identification carried out in the 1990's lead to misidentification, and then there is the constantly changing Chlamydomonas taxonomy. In addition, 15 of the strains belonged to non-Chlamydomonas-like phylogroups, such as Scenedesmacea and Chlorella. Probably the Chlorella or Scenedesmus species were contaminations in the original Chlamydomonad isolates and later predominated in the culture

Molecular biologists are currently screening the contents of public strain collections, however, these collections are not representative of the large diversity of taxa in the field and the designations of many taxa in culture collections are doubtful. Many algae are difficult to maintain in cultures and are not available for molecular analysis (Day et al., 2004; Hegewald, 1989; Krienitz and Bock, 2012). Future goals for advanced characterization of the strains within the MACC include fluorescent microscopy and ultrastructural analyses. Watanabe and Lewis (2017) showed that certain ultrastructural traits can be used to diagnose monophyletic lineages and that the investigated traits are informative at different phylogenetic depths, from genera to phylogroups. Morphological characters used for taxonomy (Nakazawa et al., 2001) often disagree with specific lineages or are not diagnostic as originally proposed. Apart from this, using more and longer genes (SSU, ITS rDNA, plastid-coding rbcL) and cross experiments of sporangium wall autolysins (Pröschold et al., 2018), could result more firm phylogenetic separation.

#### 5. Conclusion

This study examined and resolved 70 MACC strains based on molecular methods. This collection offers an unexploited potential as a repository of taxonomic data for algal diversity in relation to unexamined public algal collections. By placing 70 MACC strains on the algal tree of life, we provide references for future systematic and biodiversity research. The re-examination of these strains may contribute to a better understanding of *Chlamydomonas* classification. Based on our findings, we recommend that other culture collections regularly update the status of their strains, thus establishing a more accurate algal taxonomic framework.

#### **Declarations of competing interest**

All authors declare that there is no conflict of interest regarding the publication of this paper.

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# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.sajb.2019.06.028.

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