

Genotypic diversity of *Dictyosphaerium*–morphospecies (Chlorellaceae, Trebouxiophyceae) in African inland waters, including the description of four new genera

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Abstract: Trebouxiophytes of the *Dictyosphaerium*–morphotype from inland waters of Africa were studied using a polyphasic approach of SSU and ITS rDNA phylogeny, secondary structure of the ITS and observations made with a light microscope. Although the morphological criteria for differentiating species and genera are scarce, the genetic diversity of these algae was very high. Based on our genetic analysis findings, we described four new genera containing five new species: *Compactochlorella dohrmannii*, *Compactochlorella kochii*, *Kalenjinia gelatinosa*, *Marasphaerium gattermannii* and *Masaia oloidia*. Diversity and distribution of *Chlorella*–related colonial chlorophytes in the tropical and temperate zones were compared and discussed.

Key words: Inland waters of Africa, *Chlorella*, *Compactochlorella* nov. gen., *Dictyosphaerium*, diversity, geographic distribution, green algae, *Kalenjinia* nov. gen., *Marasphaerium* nov. gen., *Masaia* nov. gen.

Introduction

Cocoid green algae are among the most diverse microphytes. In their famous handbook, KOMÁREK & FOTT (1983) compiled more than 1200 taxa. However, these taxa are defined by morphology. To discover the real genotypic diversity of cocoid green algae only few reports with limited progress have been made. For example, POTTER et al. (1997) came up with a thesis of convergent evolution of morphology that masks the extensive biodiversity among cocoid picoplankton. FAWLEY et al. (2004) studied the molecular diversity of cocoid and monadoid green algae in 20 lakes of North America and found 93 distinct SSU rRNA genotypes, 89 of those were new to science. FAWLEY et al. (2005) evaluated the morphospecies concept in Selenastraceae and concluded that numerous cryptic species are hidden by one and the same morphotype. PRÖSCHOLD & LELIAERT (2007), COESEL & KRIENITZ (2008) and RINDI et al. (2010) discussed the state of the art in diversity and taxonomic conceptions in green algae

and recommended studies using a polyphasic approach combining morphological and molecular phylogenetic methods.

Studies about algal biodiversity in Africa have been hampered by several circumstances. For more than 100 years, algae in phytoplankton have been investigated. However, a general limitation of this classical research, especially in the early years is that only fixed samples were taken by scientific travellers and later studied in various laboratories. These findings can be brought into agreement only with difficulties with the today's views over the systematics of algae. The second half of the 20th century saw an increase in the documentation of detailed morphological characteristics leading to the generation of data useful for comparative studies. The most usable findings were published on lakes of the southern and eastern Africa (HUBER–PESTALOZZI 1929; TALLING 1987; COCQUYT et al. 1993). However, the majority of systematic considerations on material from tropical countries are based on identification keys established for temperate zone taxa. Furthermore, investigations

on living and cultured algal material including sequence analyses are largely missing. Until now, only few cultured strains of coccoid green algae from tropical Africa have been investigated by modern systematic approaches (LUO et al. 2006, 2010; BOCK et al. 2010, 2011a; KRIENITZ & BOCK 2011; KRIENITZ et al. 2010, 2011).

It is widely accepted that microbial diversity differs fundamentally from biodiversity of larger animals and plants (NORTON et al. 1996). Some workers, including FENCHEL & FINLAY (2004), have taken up the hypothesis of BAAS-BECKING (1934) based on a metaphor by BEIJERINCK, which suggests that free-living microbes have a cosmopolitan distribution and that most protistan organisms (microalgae and protozoa), smaller than one millimeter in size, have a worldwide distribution wherever their required habitats are realised (“everything is everywhere, but, the environment selects” – see DE WIT & BOUVIER 2006). This is a result of their ubiquitous dispersal driven by huge population sizes, and consequently a low probability of local extinction. However, this hypothesis is only based on the phenotypic (“morphospecies”) approach and depends on clear identification of the microorganisms. For microalgae, the hypothesis is controversial and remains the subject of ongoing discussions (COLEMAN 2002; FINLAY & FENCHEL 2002; FOISSNER 2006). Further studies, including more isolates from different regions of the world, are needed to prove or reject the hypothesis on the universal dispersal of microalgae.

One of the most common morphotypes of coccoid green algae in the phytoplankton of inland waters is represented by members of the genus *Dictyosphaerium*. The genus is characterized by green spherical cells interconnected by mucilaginous strands in colonies surrounded by thick gelatinous envelopes (Fig. 1). Although this morphotype has a world-wide distribution, it is difficult to compare findings from different climatic regions. On the base of their morphology, about 11 species are known (KOMÁREK & PERMAN 1978). The type species *Dictyosphaerium ehrenbergianum* NÄGELI was found in European waters, and closely related morphotypes were also identified as very common in tropical inland waters. Microscopic studies have revealed that in comparison to the phytoplankton from other continents, samples from Africa appear to be especially rich in specimens with thick mucilaginous envelopes. This unusual phenomenon may be a response to

water chemistry or the strong interaction with consumer and decomposer populations in the waters studied. The question that remains very contentious is the amount of morphological differences that are necessary to reflect the genotypic diversity of these *Dictyosphaerium*-like green algae. Molecular phylogenetic studies have shown that the *Dictyosphaerium*-morphotype evolved independently in different clades of the Chlorophyta (BOCK et al. 2010, 2011b; KRIENITZ et al. 2010, 2011). Out of a collection of 27 chlorellacean strains isolated from different inland waters of Africa, 24 strains belonged to the *Dictyosphaerium*-morphotype. Based on these strains this study will address the questions (i) how diverse are these coccoid green algae in terms of morphology and phylogeny, and (ii) are the tropical *Dictyosphaerium*-like algae genotypically identical with those from the temperate climatic region? The outcome of this study is the description of four new genera including five new species.

Material and Methods

In this study 67 strains belonging to the class Trebouxiophyceae were used (Table 1). Out of 28 African strains, 26 strains were isolated by one of the authors from eight inland waters of Kenya, and one or two waters of Angola, Tanzania, Tunisia, Uganda, and Zambia, respectively. These habitats were of different characteristics. Beside the large Lake Victoria, smaller lakes of the Rift Valley, the lakes Baringo, Naivasha and Oloiden were chosen as sampling area. Other strains were isolated from ephemeral pools in the Ngorongoro Crater in Tanzania, Nakuru National Park in Kenya and from a park pond in Nairobi. We also isolated strains from sewage oxidation ponds, such as the final sewage pond of the Nakuru town sewage plant, and from a sewage pond on the Djerba island in Tunisia. Additional strains were collected in rivers or water channels such as the Mara river in the Masai Mara National Reserve in Kenya, from the Kunene and Okavango in Angola, and from the Kazinga channel in Uganda, which is connected to lakes Albert and George.

We isolated single *Chlorella*-like cells or colonies of the *Dictyosphaerium*-morphotype by glass-capillaries from the field samples and transferred them to a liquid medium. All strains were maintained at the strain collection of the

IGB (Leibniz–Institute of Freshwater Ecology and Inland Fisheries). The strains were grown in a modified BOURRELLY medium (HEGEWALD et al. 1994; KRIENITZ & WIRTH 2006) on agar at 15 °C or in suspensions at room temperature under a 14 h : 10 h light–dark regime. New strains were deposited at the Culture Collection of Algae and Protozoa (CCAP, Oban, UK). The algae were investigated using a Nikon Eclipse E600 light microscope with differential interference contrast. Microphotographs were taken with a Nikon digital camera DS–Fi1, and Nikon software NIS–Elements D (Nikon Corporation, Tokyo, Japan).

In this study, we sequenced the SSU and ITS rRNA gene sequences of 13 strains to establish new sequence data, which were submitted to GenBank. Additionally, 57 sequences from GenBank were included in this data set (Table 1).

Genomic DNA was extracted using a lysozym/sodium phosphate method. The algal cells were mechanically disrupted with glass beads using the TissueLyserII (Qiagen GmbH, Hilden, Germany) in the presence of 600 µl sodium phosphate buffer (120 mM) and 100 µl SDS (25%). After centrifugation for 6 min, the liquid phase was transferred to a clean reaction tube and incubated with 200 µl Lysozym at 37 °C for 1 hour. Afterwards, the probes were incubated at 55 °C overnight after adding 150 µl SDS (25%) and 12.5 µl proteinase K. Protein precipitation was done by adding 7.5 M ammonium acetate (0.4 times of the existing volume) and incubating on ice for 5 min. The supernatant was transferred to a clean reaction tube after a centrifugation step and DNA was purified with 0.7% isopropanol and centrifugation for 1 hour. The liquid was discarded and DNA pellet was washed with ethanol (80%). The SSU and ITS rRNA genes were amplified and sequenced as described by BOCK et al. (2011a).

For the phylogenetic analyses, a data set of 68 taxa with 2693 aligned base positions and a dataset with 71 taxa and 2815 bases were used respectively. In order to obtain an adequate representation of chlorellacean algae, different sequences were selected according to BOCK et al. (2011a, b) with *Catena viridis* as outgroup in addition to the newly sequenced strains. The two phylogenetic trees presented in our results were inferred using two different ways of alignment: manual alignment according to the secondary structure; and the ClustalW algorithm integrated in SOAP v.1.2 alpha 4 (LOYTYNOJA & MILINKOVITCH 2001).

For the first phylogenetic tree, the SSU was manually aligned on the basis of the predicted secondary structure model for *Micractinium pusillum* (LUO et al. 2006). Stems and loop regions of the 18S of the strains were aligned to each other respectively. The ITS regions were more difficult to align due to a high degree of divergence between the sequences. Within these regions, we aligned them strictly according to their predicted secondary structure, e.g. stem regions were aligned separately from loops and unmatching regions. Dubiously aligned regions were excluded. The phylogenetic tree was inferred by maximum likelihood settings on a partitioned data set using Treefinder (JOBBERG 2008). Models for each partition, as proposed by Treefinder under AICc criterion, were as follows: 18S (J2:G:5 model, 1797 bases), ITS1 (J1:G:5 model, 408 bases), 5.8S (HKY model, 141 bases), ITS2 (GTR:G:5 mode, 347 bases). To test the confidence of the tree topology, bootstrap analyses were calculated by distance (neighbor–joining; NJ; 1000 replicates) and maximum parsimony (MP; 1000 replicates; with heuristic search options based on simple taxon addition, tree–bisection–reconnection (TBR) branch swapping algorithm and Multrees option enabled) using PAUP*, portable version 4.0b10 (SWOFFORD 2002) and maximum likelihood criteria using Treefinder (ML; 1000 replicates; settings as described above). The Bayesian inference (MB) was calculated using MrBayes version 3.1 (HUELSENBECK & RONQUIST 2001). Two runs with four chains of Markov chain Monte Carlo (MCMC) iterations were performed with tree sampling every 100 generations. The model GTR+I+G with gamma shape parameter and proportion of invariable sites was used for each partition. The parameters were unlinked and allowed to vary across the partitions. The stationary distribution was assumed after 2,000,000 generations when the average standard deviations of split frequencies between two runs were below 0.01. The first 25% of the calculated trees were discarded as burn–in. A 50% majority–rule consensus tree was calculated for posterior probabilities (PP).

For the second phylogenetic tree, the sequences were initially aligned using the ClustalW algorithm integrated in SOAP v.1.2 alpha 4 (LOYTYNOJA & MILINKOVITCH 2001). The stability of the alignment was assessed using SOAP by comparison of different ClustalW alignments using gap penalties from 7 to 20 by steps of 2.5 and extension penalties from 2 to 10 by steps

Table 1. Strains used in this study. The strains are grouped according to the topology in the phylogram (Fig. 8). Authentic strains in bold letters.

Strain designation	Strain-number in the IGB-Collection ^{b)}	Species	Origin	Accession number (SSU + ITS)	Reference
<i>Chlorella</i>-clade ^{a)}					
CCAP 211/81		<i>Chlorella vulgaris</i>	Germany, saline pond Salzteich, Trebbichau, Sachsen-Anhalt	FM205854	Luo et al. 2010
ACOI 856		<i>C. pituita</i>	Portugal, Serra da Estrela	FM205856	Luo et al. 2010
SAG 37.88		<i>C. lobophora</i>	Russia, soil from forest, Krasnyj Rog, Brianskaia district	FM205833	Luo et al. 2010
CCAP 211/84		<i>C. variabilis</i>	USA, endosymbiont of <i>Paramecium</i> , NC84A	AB206549	HOSHINA et al. 2005
SAG 211-6		<i>C. variabilis</i>	USA, endosymbiont of <i>Paramecium</i> , J.B. Loefer, 1934	FM205849	Luo et al. 2010
CCAP 260/11	KR 2007/5	<i>C. rotunda</i>	Angola, River Okavango near Mutango	HQ111433	Bock et al. 2011a
SAG 3.83		<i>C. heliozoae</i>	Canada, endosymbiont in <i>Acanthocystis</i> , Newfoundland, bog pool in Terra Nova National Park	FM205850	Luo et al. 2010
SAG 222-2a		<i>C. pulchelloides</i>	France, near Amiens, E.A. George	FM205857	Luo et al. 2010
CCAP 211/118	CB 2008/50	<i>C. pulchelloides</i>	Germany, Lake Feldberger Haussee, Mecklenburg-Western Pomerania	HQ111431	Bock et al. 2011a
CCAP 211/116	CB 2008/110	<i>C. chlorelloides</i>	Germany, Lake Pragsdorfer See, Mecklenburg-Western Pomerania	HQ111432	Bock et al. 2011a
UTEX 938		<i>C. colonialis</i>	USA, unknown	FM205862	Luo et al. 2010
CCAP 211/119	CB 2008/73	<i>C. singularis</i>	Kenya, Sewagepond Nakuru	HQ111435	Bock et al. 2011a
CCAP 222/18	Wolf 2000/1	<i>C. elongata</i>	Germany, Berlin, Steglitz, fountain-pool	FM205858	Luo et al. 2010
CCAP 211/120	CB 2008/69	<i>C. volutis</i>	Kenya, Rhinopool, Nakuru National Park	HQ111434	Bock et al. 2011a
CCALA 260		<i>C. sorokiniana</i>	Slovakia, Piestany, thermal spring	FM205860	Luo et al. 2010
CCAP 211/8k		<i>C. sorokiniana</i>	USA, University, Austin, Texas, Waller Creek	FM205859	Luo et al. 2010

Table 1. Cont.

CCAP 211/90	<i>C. lewini</i>		Chile, soil from the edge of a permanent freshwater pond in a crater, Easter Island, R.A. Lewin	FM205861	Luo et al. 2010
CCAP 222/71	<i>Hindakia tetrachotoma</i>	CB 2007/25	Germany, Lake Pragsdorfer See, Mecklenburg-Western Pomerania	GQ487228	Bock et al. 2010
CCAP 222/73	"	CB 2007/27	Germany, Waldstich Zehdenick, Brandenburg	GQ487231	Bock et al. 2010
CCAP 222/81	"	CB 2008/70	Kenya, Lake Baringo	GQ487230	Bock et al. 2010
CCAP 222/82	"	CB 2008/71	Kenya, Lake Baringo	GQ487232	Bock et al. 2010
CCAP 222/80	"	CB 2008/48	Germany, Lake Kleiner Tietzensee, Brandenburg	GQ487233	Bock et al. 2010
CCAP 222/29	<i>Hindakia fallax</i>	KR 2006/317	Kenya, Lake Victoria, Dunga	GQ487223	Bock et al. 2010
CCAP 222/30	"	KR 2006/320	Kenya, Lake Victoria, Dunga	GQ487224	Bock et al. 2010
CCAP 222/47	<i>Heynigia riparia</i>	KR 2007/12	Angola, River Kunene near Epupa Falls	GQ487225	Bock et al. 2010
CCAP 222/2D	<i>Heynigia dictyosphaerioides</i>		UK, Windermere, Cumbria	GQ487221	Bock et al. 2010
CCAP 248/5	<i>Micractinium pusillum</i>		Kenya, Sewagepond, Nakuru	FM205836	Luo et al. 2010
SAG 42.98	<i>M. belenophorum</i>		Germany, Berlin, Lake Lietzensee	FM205879	Luo et al. 2010
SAG 30.92	<i>Didymogenes palatina</i>		Germany, water tank, Jülich, E. Hegewald 1982/83	FM205840	Luo et al. 2010
SAG 18.91	<i>Didymogenes anomala</i>		Germany, River Rhein near Linz, E. Hegewald, 1990/11	FM205839	Luo et al. 2010
SAG 2015	<i>Actinastrum hantzschii</i>	KR 1996/4	Germany, River Elbe near Aken, Sachsen-Anhalt	FM205841	Luo et al. 2010
CCMP 2446	<i>Meyerella planctonica</i>		USA, Lake Itasca, Minnesota	AY543040 AY543045	FAWLEY et al. 2005
Parachlorella-clade					
CCAP 222/20	<i>Dictyosphaerium ehrenbergianum</i>	KR 2006/302	Kenya, Lake Naivasha	GQ487192	Bock et al. 2011b
CCAP 222/21	"	KR 2006/303	Kenya, Lake Naivasha	GQ487193	Bock et al. 2011b
CCAP 222/26	"	KR 2006/311	Kenya, Lake Naivasha	GQ487195	Bock et al. 2011b

Table 1. Cont.

CCAP 222/22	KR 2006/304	"	Kenya, Lake Naivasha	GQ487194	BOCK et al. 2011b
CCAP 222/27	KR 2006/312	"	Kenya, Lake Naivasha	GQ477062	BOCK et al. 2011b
UTEX 75		"	UK, pond near Cambridge, E.G. Pringsheim, 1940	GQ176856	KRIENITZ et al. 2010
CCAP 222/1A		"	UK, pond near Cambridge, E.G. Pringsheim, 1940	GQ176861	KRIENITZ et al. 2010
CCAP 222/10	LW 2003/183	"	Germany, Lake Geron, Gransee	GQ176857	KRIENITZ et al. 2010
CCAP 222/23	KR 2006/305	"	Kenya, Lake Victoria, Homa Bay	GQ176859	KRIENITZ et al. 2010
CCAP 222/14	KR 2006/31	"	Tunisia, oxidationpond, Jerba	GQ176858	KRIENITZ et al. 2010
CCAP 222/92	CB 2008/76	<i>D. libertatis</i>	Kenya, Uhuru-Pond Nairobi	GQ487211	BOCK et al. 2011b
CCAP 222/85	CB 2008/54	<i>Dictyosphaerium lacustre</i>	Germany, Oxidationpond Neuglobsow, Brandenburg,	GQ487206	BOCK et al. 2011b
SAG 2046	KR 1999/1	<i>Parachlorella beijerinckii</i>	Germany, Nonnenbach brook, a tributary of Lake Tollense, Mecklenburg-Western Pomerania	FM205845	LUO et al. 2010
SAG 211-11g		<i>Parachlorella kessleri</i>	USA, New York, M. Winokur, 1945	FM205885	LUO et al. 2010
CCAP 222/25	KR 2006/310	<i>Dictyosphaerium</i> morphotype	Uganda, Kazinga-Channel	GQ176862	KRIENITZ et al. 2010
CCAP 222/24	KR 2006/309	<i>Marasphaerium gattermannii</i>	Kenya, River Mara	GQ477057	this study
Heg. 1983/17		<i>Marasphaerium gattermannii</i>	Germany, Berlin, Lake Stölpchensee	HQ322127	this study
ACOI 1988		<i>Dictyosphaerium</i> morphotype	unknown	GQ176863	KRIENITZ et al. 2010
CCAP 222/1C		<i>Dictyosphaerium</i> morphotype	UK Windermere, Cumbria, G. Jaworski	GQ176864	KRIENITZ et al. 2010
CCAP 222/93	CB 2008/19	<i>Mucidosphaerium sphagnale</i>	Germany, Lake Wollingster See, Lower Saxony	GQ487218	BOCK et al. 2011b

Table 1. Cont.

CCAP 222/96	CB 2008/6	<i>Mucidosphaerium palustris</i>	Germany, pond Klipsmoorheide, Lower Saxony	GQ487216	Bock et al. 2011b
UTEX 731		<i>Mucidosphaerium pulchellum</i>	Canada, Nova Scotia, R.A. Lewin 1952	GQ176861	KRIENITZ et al. 2010
ACOI 1719		<i>Mucidosphaerium planctonicum</i>	Portugal, Abrantes, Campo Militar de Sta Margarida, Barragem do Monte Novo	GQ487201	Bock et al. 2011b
CCAP 222/61	CB 2007/8	<i>Compactochlorella kochii</i>	Germany, Lake Jabeler See, Mecklenburg-Western Pomerania	HQ322125	this study
	CB 2008/47	<i>Compactochlorella kochii</i>	Germany, Lake Grosser Tietzensee near Rheinsberg, Brandenburg	HQ322124	this study
	CB 2008/104	<i>Compactochlorella kochii</i>	Kenya, Rhinopool Nakuru National Park	HQ322126	this study
CCAP 222/7	LW 2003/37	<i>Compactochlorella kochii</i>	Tanzania, ephemeral Yellow-Bill-Pool, Ngorongoro Crater	GQ487244	this study
CCAP 222/5	KR 2002/24	<i>Compactochlorella dohrmannii</i>	Kenya, Sewagepond, Nakuru	GQ477058	this study
CCAP 211/85	KR 2003/30	<i>Masaia oloidia</i>	Kenya, Lake Oloidien, cruci	GQ477059	this study
CCAP 222/32	KR 2006/322	<i>Masaia oloidia</i>	Kenya, Lake Oloidien, cruci	GQ477060	this study
	CB 2008/72	<i>Masaia oloidia</i>	Kenya, Sewagepond, Nakuru	HQ322128	this study
SAG 11.86		<i>Closteriopsis acicularis</i>	Germany, Lake Grömitzer See, E. Hegewald 1977/126	FM205847	LUO et al. 2010
CCAP 222/43	KR 2007/3	<i>Dictyosphaerium</i> morphotype	Angola, River Okavango near Mutango	GQ477066	this study
	CB 2008/94	<i>Kalenjinia gelatinosa</i>	Kenya, Sewagepond, Nakuru	HQ322129	this study
CCAP 222/8	LW 2003/125	<i>Kalenjinia gelatinosa</i>	Kenya, Sewagepond, Nakuru	GQ477061	this study
SAG 41.98		<i>Diclostera acuatius</i>	Ukraine, pond at Krasne	FM205848	LUO et al. 2010

Table 1. Cont.

Outgroup	Accession	Species	Location	Reference
SAG 65.94	KR 1991/4	<i>Catena viridis</i>	Germany, pond Oxidationsteich Neuglobsow, Brandenburg	BOCK et al. 2010
CCAP 283/1		<i>Hegewaldia parvula</i>	USA, Lemoncove, California	PRÖSCHOLD et al. 2010
CCAP 283/2		<i>Hegewaldia parvula</i>	USA, Lemoncove, California	PRÖSCHOLD et al. 2010

^{a)} Abbreviations:

ACOI = Coimbra Collection of Algae, Portugal
 CAUP = Culture Collection of Algae of Charles University in Prague, Czech Republic
 CCALA = Culture Collection of Autotrophic Organisms, Trebon, Czech Republic
 CCAP = Culture Collection of Algae and Protozoa, Oban, UK
 CCMP = The Provasoli-Guillard National Center for Culture of Marine Phytoplankton, USA
 SAG = Sammlung von Algenkulturen der Universität Göttingen, Germany
 UTEX = The Culture Collection of Algae at The University of Texas at Austin, USA

^{b)} For own isolates the initials of the isolator were given: CB = Christina Bock, KR = Lothar Krienitz, LW = Luo Wei

of 1.5. Regions of instability were excluded by computing a 90% consensus alignment, resulting in 2815 aligned bases. The phylogenetic tree and the corresponding bootstrap values were interfered as described above with the ML models: 18S (GTR:G:5 model, 1807 bases), ITS1 (J1:G:5 model, 470 bases), 5.8S (HKY model, 139 bases), ITS2 (GTR:G:5 mode, 399 bases).

The ITS2 secondary structure was constructed with the help of mfold with the *D. ehrenbergianum* structure as template (BOCK et al. 2011b; KRIENITZ et al. 2010) to locate nonhomoplasious synapomorphies (NHS), hemi-compensatory base changes (h-CBCs) and compensatory base changes (CBCs) according to MARIN et al. (2003) and COLEMAN (2003, 2007). Structures were drawn by PseudoViewer (BYUN & HAN 2006).

Results

Under field conditions (Figs 1–5), colonies with *Dictyosphaerium*-morphotype covered by mucilaginous envelopes (Fig. 1) were clearly identifiable. The inner anatomy of the cells was identical to *Chlorella*: the parietal, cup-shaped chloroplast carried a starch-sheathed pyrenoid. Cell shape and size and several features of colony organization showed a certain amount of variability. The cells, which were 3–14 µm in diameter, varied from spherical to ovoid. The interconnecting strands between the cells, established by mother cell wall remnants after liberation of the autospores were simple, cross-shaped (Fig. 2) or more complex (Fig. 3) depending on the number of cells joined together within the colony. The stalks attached the cells at the apical (Fig. 1) or at the longitudinal side (Figs 4 and 5). Under culture conditions (Figs 6 and 7), some strains became solitary but most showed more or less the characteristic colonies of the *Dictyosphaerium*-morphotype. Generally, the colonial life form disappeared after longer maintenance under the conditions of the strain collection. Colonies were best visible on fresh agar-cultures or occasionally during the first days of freshly inoculated suspension cultures.

In strains belonging to the close relatives of *Chlorella*, such as the members of the genus *Hindakia* (Fig. 6), the mucilaginous stalks attached the cells at their apical end. In contrast, cells of the *Parachlorella/Dictyosphaerium*-relationship

Table 2. Comparison of CBCs and hemi-CBCs within the ITS2 between the newly erected species.

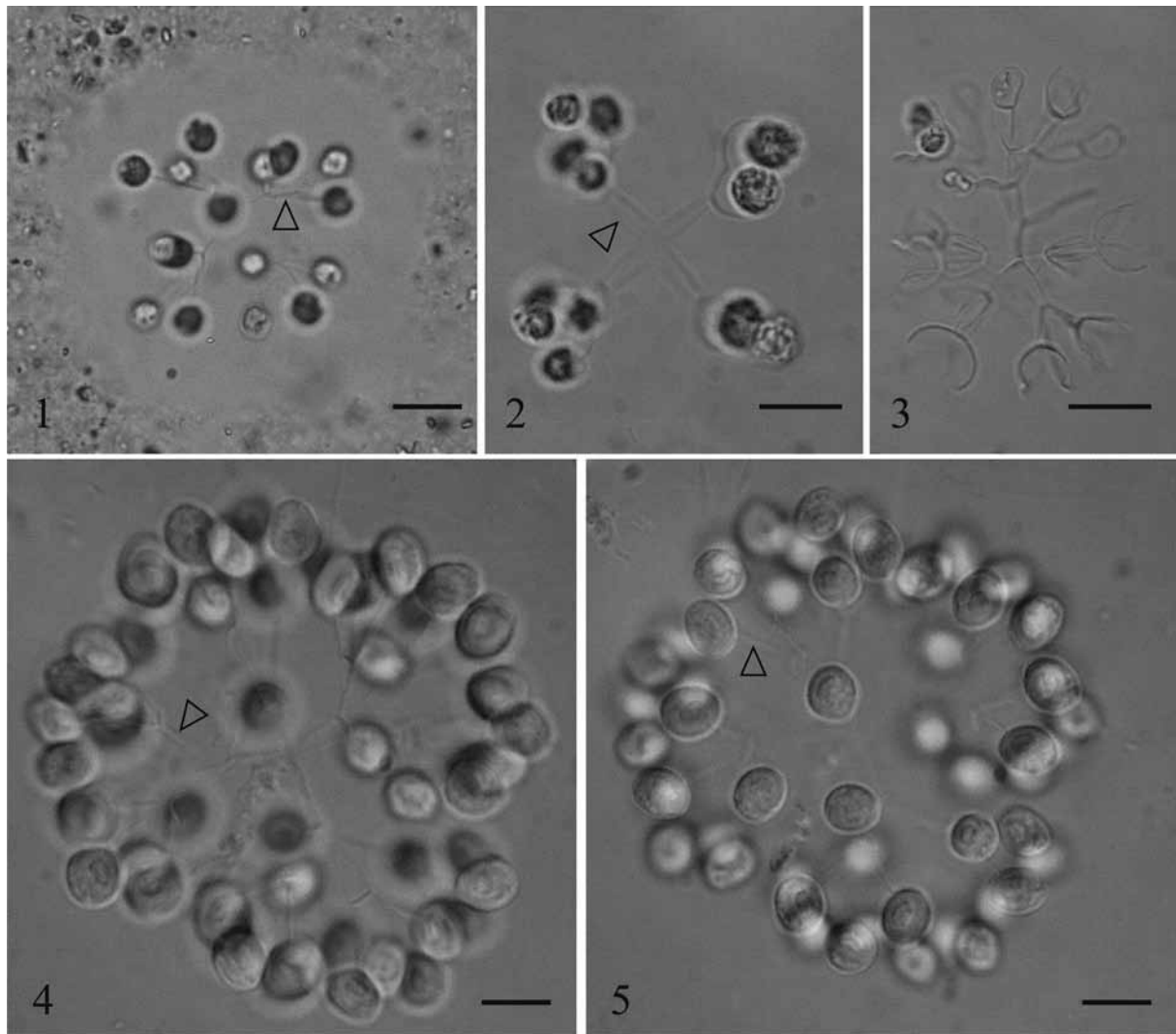
	Helix I CBC/ hemi- CBC	Helix II CBC/ hemi- CBC	Helix III CBC/ hemi-CBC	Helix IV CBC/ hemi-CBC
<i>Marasphaerium gattermannii</i> vs <i>Compactochlorella kochii</i>	– / –	5 / –	– / 2	6 / –
<i>Compactochlorella kochii</i> vs <i>Compactochlorella dohrmannii</i>	– / –	2 / 1	1 / 2	1 / 1
<i>Compactochlorella dohrmannii</i> vs <i>Masaia oloidia</i>	1 / 1	3 / –	– / 2	1 / 1
<i>Masaia oloidia</i> vs <i>Kalenjinia gelatinosa</i>	– / –	2 / 1	1 / 3	3 / –

were attached by the stalks at their longitudinal end (Fig. 7).

Three of the African strains of Chlorellaceae were solitary and belonged unambiguously to the genus *Chlorella*. The 24 colony-forming strains of *Dictyosphaerium*–morphotype from African waters evolved in seven different lineages of Chlorellaceae (Figs 8 and 9). Four strains, from the lakes Victoria and Baringo, belonged to the genus *Hindakia* within the *Chlorella*–clade. The other strains occurred in different clusters of the *Parachlorella*–clade. Whereas the sister-relationship of the *Chlorella*– and the *Parachlorella*–clade was supported sufficiently, the general topology of the lineages within these two clades was not supported by our analyses. However, the position of the genus *Hegewaldia* previously assigned to the *Chlorella*–clade is unclear (Fig. 9).

Eight African isolates belonged to the genus *Dictyosphaerium*, seven of them were designated to the type species *D. ehrenbergianum* NÄGELI, and the other species was determined as *D. libertatis* C. BOCK, PRÖSCHOLD et KRIENITZ. Closely related to these two clusters is the strain CCAP 222/25 from Uganda, which will be studied in more detail by Pavel Škaloud and his team. Two European strains (ACOI 1988 and CCAP 222/1C) were related to

a cluster containing the strain CCAP 222/24 from the Mara river in Kenya, which is described as *Marasphaerium gattermannii* gen. et sp. nov. in this paper. Next to these lineages evolved members of the genera *Mucidosphaerium* and a cluster containing three African strains described below as *Compactochlorella dohrmannii* and *C. kochii* gen. and sp. nov. From *C. kochii* four strains were analysed, two of them from Africa and two from Germany. Unfortunately, the DNA sequence from the African strain in the culture collection (CCAP 222/7) was imprecise at the end of the ITS2 and therefore we selected the cultured strain CCAP 222/61 from Germany as authentic strain those sequence was complete. Adjacent to these lineages, three strains from Kenya's standing waters, which are newly described here as *Masaia oloidia* gen. et sp. nov., established its own cluster. The strain CCAP 222/43 exhibited typical colonies of a *Dictyosphaerium*–morphotype and clustered together with the needle-shaped *Closteriopsis acicularis* (G.M. SMITH) BELCHER et SWALE. Furthermore, two new spherical and colonial strains from the Nakuru sewage oxidation pond, described here as *Kalenjinia gelatinosa* gen. et sp. nov., established a lineage placed between *Closteriopsis* and the colonial needle-shaped *Dicloster acuatus* JAO, WEI et HU.

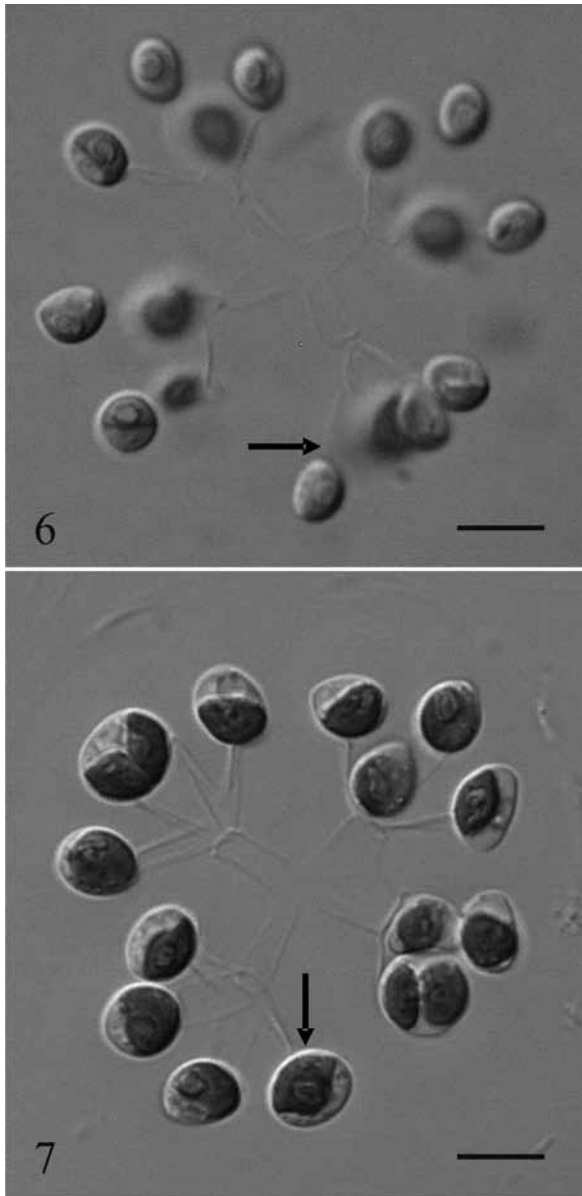


Figs 1 – 5. The *Dictyosphaerium*–morphotype in field samples from Lake Victoria (Figs 1–3) and a sewage pond at Nakuru (Figs 4, 5). The empty arrowhead indicates the interconnecting strands between the cells. (1) spherical colony covered by a thick mucilaginous envelope which is made visible by silt particles and picoplanktonic cyanobacteria. (2) colony with two-, or four-celled mother cells and cross-shaped interconnecting strands; (3) articulated strands of a colony of mother cells which already liberated the autospores (only two autospores remained in the upper left mother cell). Scale bars 10 μm .

The topology of the phylogenetic trees recovered from two different alignments (manual alignment, Fig. 8, and alignment computed with ClustelW, Fig. 9) was in general congruent. No major discrepancies occurred between the different alignment methods; differences were only observed in the placements of lineages that received no statistical support in either method. Within the *Chlorella*–clade, the cluster with sequences of *Micractinium* and *Didymogenes* exchanged position with the *Hindakia/Heynigia* cluster. Within the *Parachlorella*–clade, the cluster containing *Marasphaerium*, *Mucidosphaerium*, *Compactochlorella* and ACOI 1988/CCAP222/1C (Fig. 8) is separated in different lineages (Fig. 9). Small differences were also observed within the

bootstrap values of the lineages.

To evaluate the newly erected five species according to the CBC concept, we compared the number of CBCs and hemi-CBCs and found a remarkable number of differences among the taxa (for details see Fig. 10, and Table 2). *Marasphaerium gattermannii* and *Compactochlorella kochii* differ in 11 CBCs and 2 hemi-CBCs with additional base pairs in all helices within the ITS2. The two species belonging to the new genus *Compactochlorella* differ in 4 CBCs and 4 hemi-CBCs and additional base pairs in Helices I, II and IV. Considerable CBCs occurred between the genera *Compactochlorella* and *Masaia* (5 CBCs and 4 hemi-CBCs) and between *Masaia* and *Kalenjinia* (6 CBCs; 4 hemi-CBCs).



Figs 6, 7. The *Dictyosphaerium*–morphotype in culture. The black arrows indicate the place of attachment of the interconnecting strands to the cell wall. (6) colony of *Hindakia fallax* (CCAP 222/29), a member of the *Chlorella*–clade; the strands are attached to the apical cell side; (7) colony of *Dictyosphaerium ehrenbergianum* (CCAP 222/27), a member of the *Parachlorella*–clade; the strands are attached to the longitudinal cell side. Scale bars 10 μm .

To align the phylogenetic findings with the morphology features, the five authentic strains of the new taxa were subjected again to microscopic investigations. Micrographs of these authentic strains are given in Figs 11–15, whereas drawings including the iconotypes are shown in Figs 16–20. Results of the comparison of their morphological features are provided in Table 3.

Generic and species descriptions

Marasphaerium KRIENITZ, C. BOCK, KOTUT et PRÖSCHOLD gen. nov.

Latin diagnosis: Cellulae viridis, sphaericae, planctonicae. Chloroplastus unicus, parietalis, poculiformis, pyrenoide granis amyliis tecto. Reproductio asexualis autosporum ope, reproductio sexualis ignota. Cellulae solitariae vel in coloniis, 2–4 cellularis, interdum tegumento gelatinoso vestitae. A generibus ceteris familiae ordine nucleotidorum in 18S rDNA et ITS differt.

Cells green, spherical, planktonic. Single cup-shaped chloroplast with starch-covered pyrenoid. Asexual reproduction by autosporulation, sexual reproduction not observed. Cells solitary or in colonies of 2–4 cells, covered by a gelatinous envelope. Genus differs from other genera of the family by the order of the nucleotides in SSU and ITS rRNA gene sequences.

Typus generis: *Marasphaerium gattermannii* KRIENITZ, C. BOCK, KOTUT et PRÖSCHOLD sp. nov.

Etymology: the genus is named according to its locus classicus, the Mara river in the Masai Mara National Reserve, Kenya.

Marasphaerium gattermannii KRIENITZ, C. BOCK, KOTUT et PRÖSCHOLD sp. nov.

Latin diagnosis: Cellulae solitariae vel in coloniis, planctonicae, interdum tegumento gelatinoso vestitae. Coloniae parvae, 2–4 cellularis, cellulis funibus subtilibus hyalinis iunctis. Cellulae sphaericae, raro late ovalis, 4–13 μm in diametro. Chloroplastus unicus, parietalis, poculiformis, pyrenoide granis amyliis tecto. Reproductio asexualis autosporum ope. A speciebus ceteris generis ordine nucleotidorum in 18S rDNA et ITS differt.

Cells solitary or in colonies, planktonic, covered by a gelatinous envelope. Colonies small, 2–4 celled, cells connected by hyaline mother cell wall remnants. Cells spherical, seldom broad oval, 4–13 μm in diameter. Single cup-shaped chloroplast with starch-covered pyrenoid. Asexual reproduction by autosporulation. Differs from species of other genera by the order of the nucleotides in SSU and ITS rRNA gene sequences.

Holotype: material of the authentic strain CCAP 222/24 is cryopreserved in metabolic inactive state at the Culture Collection of Algae and Protozoa, Oban, Scotland.

Isotype: an air-dried as well as a formaldehyde-fixed sample of the authentic strain CCAP 222/24, deposited at the Botanical Museum at Berlin–

Dahlem under the designation B400040739.

Type locality: Mara river in the Masai Mara National Reserve, Kenya.

Etymology: The species is named in memory of the late Rolf Gattermann, who was a leading authority in Zoology and behavioural biology of mammals. He was impressed by the wildlife in East Africa and inspired the first author to study African nature.

Authentic strain: CCAP 222/24.

Iconotype: Figure 16*

***Compactochlorella* KRIENTZ, C. BOCK, KOTUT et PRÖSCHOLD gen. nov.**

Latin diagnosis: Cellulae viridis, sphaericae, planctonicae. Chloroplastus unicus, parietalis, poculiformis, pyrenoide granis amyliis tecto. Reproductio asexualis autosporum ope, reproductio sexualis ignota. Cellulae solitariae vel in coloniis, 2–4 cellularis, vel in aggregationibus compactis, interdum tegumento gelatinoso vestitae. A generibus ceteris familiae ordine nucleotidorum in 18S rDNA et ITS differt.

Cells green, spherical, planktonic. Single cup-shaped chloroplast with starch-covered pyrenoid. Asexual reproduction by autosporulation, sexual reproduction not observed. Cells solitary or in colonies of 2–4 cells, or in compact aggregations, covered by a gelatinous envelope. Genus differs from other genera of the family by the order of the nucleotides in SSU and ITS rRNA gene sequences.

Typus generis: *Compactochlorella kochii* KRIENTZ, C. BOCK, KOTUT et PRÖSCHOLD sp. nov.

Etymology: The genus is named according to the compact aggregations which were often developed by the colonies of the type species.

***Compactochlorella kochii* KRIENTZ, C. BOCK, KOTUT et PRÖSCHOLD sp. nov.**

Latin diagnosis: Cellulae solitariae vel in coloniis, 2–4 cellularis, vel in aggregationibus compactis, interdum tegumento gelatinoso vestitae, planctonicae. Cellulis funibus subtilis hyalinis iunctis. Cellulae ovoides, ovalis vel sphaericae, 3–12 × 2.5–12 µm. Chloroplastus unicus, parietalis, poculiformis, pyrenoide granis amyliis tecto. Reproductio asexualis autosporum ope. A speciebus ceteris generis ordine nucleotidorum in 18S rDNA et ITS differt.

Cells solitary or in 2–4 celled colonies, or in compact aggregations, covered by a gelatinous envelope, planktonic. Cells connected by hyaline stalks. Cells ovoid, oval or spherical, 3–12 ×

Table 3. Morphological characteristics of the species newly described in this study.

Species	Autospores	Vegetative Cells	Mother Cells	Colonies
<i>Marasphaerium gattermannii</i> CCAP 222/24	Spherical, rarely broad oval, 4–6 µm	Spherical, 5–9 µm	Spherical, 9–13 µm, with 2, 4 or 8 autospores	With 2 or 4 cells, very unstable
<i>Compactochlorella kochii</i> CCAP 222/61	Ovoid, 3–5 × 2.5–4 µm	Spherical or oval, 5.5–8.5 µm	Spherical, rarely oval, 8–12 µm, with 2, or 4 autospores	With 2 or 4 cells, developing lumpy complexes of colonies of different stage
<i>Compactochlorella dohrmannii</i> CCAP 222/5	Ovoid, 3–4.5 × 2.5–3 µm	Spherical or oval, 5–7.5 µm	Spherical, rarely oval, 7–12 µm, with 2 or 4 autospores	With 2 or 4 cells, sometimes cells are closely attached to tetrads
<i>Masaia oloidia</i> CCAP 222/32	Ovoid, 3.5–6 × 3–5 µm	Spherical or oval, 5.5–8 µm	Spherical, rarely oval, 8–12 µm, with 2, 4 or 8 autospores,	With 2, 4 or 8 cells
<i>Kalenjinia gelatinosa</i> CCAP 222/8	Ovoid or drop-shaped, 4–6 × 3.5–5.5 µm	Spherical or oval, 6–9 µm	Spherical, rarely oval, 9–14 µm, with 2 or 4 autospores	With 2 or 4 cells

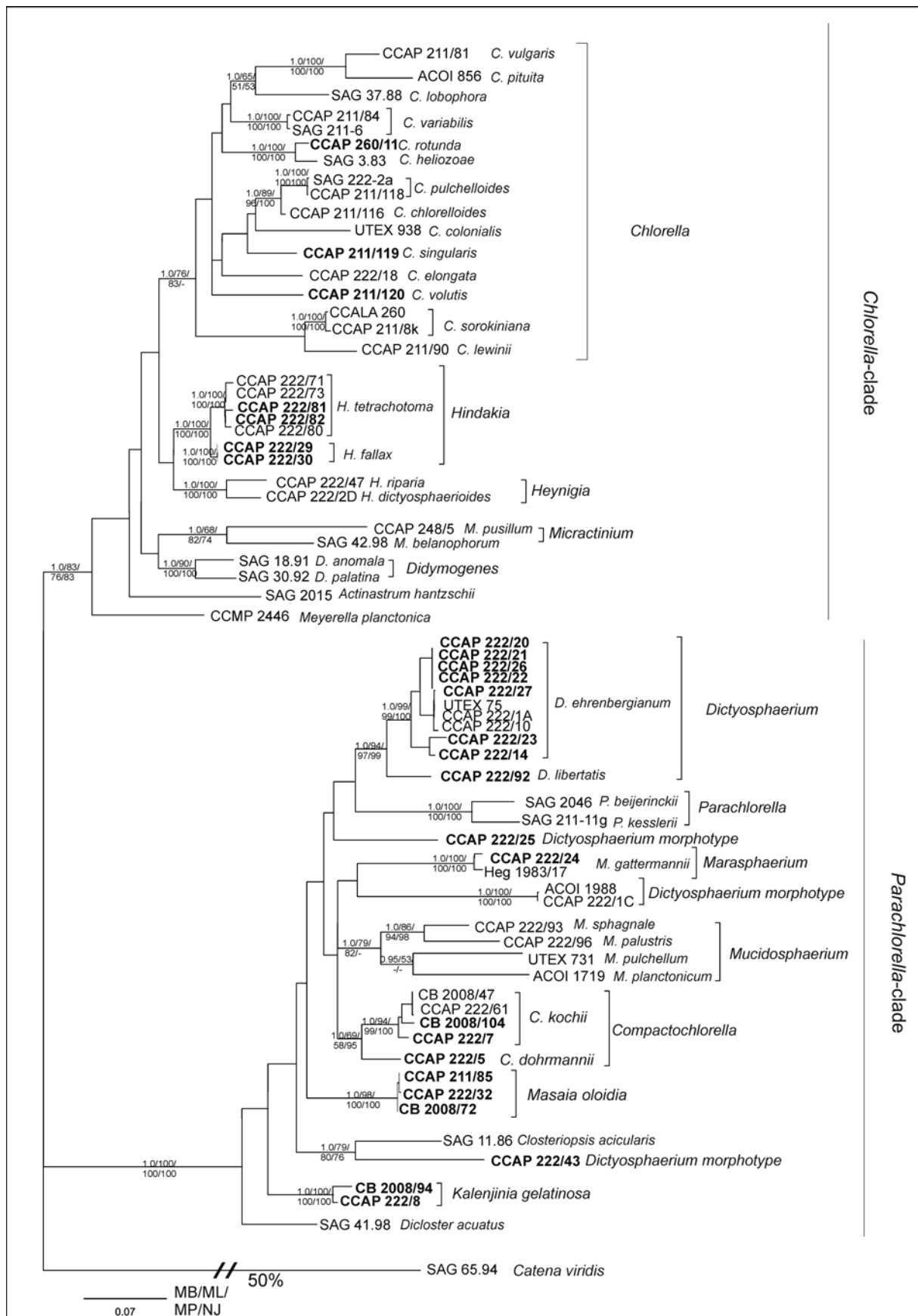


Fig. 8. Maximum likelihood phylogram of the Chlorellaceae with *Catena viridis* as outgroup inferred from a concatenated set of SSU and ITS sequences. The phylogram is based on a partitioned dataset with manually aligned sequences according to their secondary structure. Hyphens correspond to values below 50 for BP and below 0.95 for PP. African strains in bold. Scale bar indicates substitutions per site.

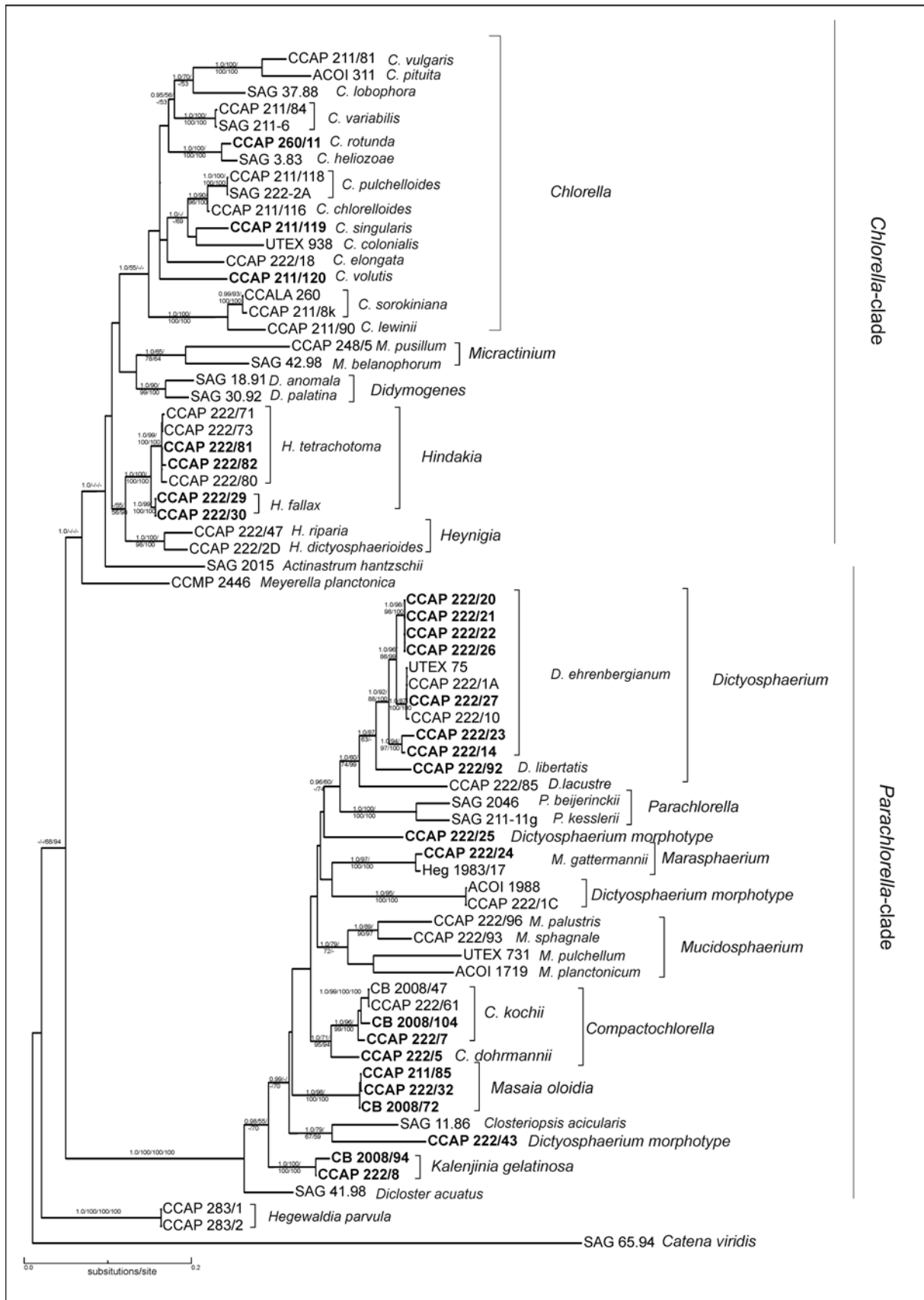


Fig. 9. Maximum likelihood phylogram of the Chlorellaceae with *Catena viridis* as outgroup inferred from a concatenated set of SSU and ITS sequences. The phylogram is based on a partitioned dataset of a 90% consensus alignment predicted by SOAP (LOYTYNOJA & MILINKOVITCH 2001) under different gap/extension penalties. Hyphens correspond to values below 50 for BP and below 0.95 for PP. African strains in bold. Scale bar indicates substitutions per site.

2.5–12 µm. Single cup-shaped chloroplast with starch-covered pyrenoid. Asexual reproduction by autospore. Differs from other species of the genus by the order of the nucleotides in SSU and ITS rRNA gene sequences.

Holotype: material of the authentic strain CCAP 222/61 is cryopreserved in metabolic inactive state at the Culture Collection of Algae and Protozoa, Oban, Scotland.

Isotype: an air-dried as well as a formaldehyde-fixed sample of the authentic strain CCAP 222/61, deposited at the Botanical Museum at Berlin-Dahlem under the designation B40004040.

Type locality: lake Jabeler See, Mecklenburg-Western Pomerania, Germany.

Etymology: the species is named in honour of Frank Koch, an authority in entomology. He makes frequent work visits to Africa, and inspired the first author to study African nature.

Authentic strain: CCAP 222/61.

Iconotype: Figure 17*

***Compactochlorella dohrmannii* KRIENITZ, C. BOCK, KOTUT et PRÖSCHOLD sp. nov.**

Latin diagnosis: Cellulae planctonicae, solitariae vel in coloniis, 2–4 cellularis, interdum tegumento gelatinoso vestitae. Cellulis funibus subtilis hyalinis iunctis. Cellulae ovoides, ovalis vel sphaericae, 3–12 × 2.5–10 µm. Chloroplastus unicus, parietalis, poculiformis, pyrenoide granis amyli tecto. Reproductio asexualis autosporem ope. A speciebus ceteris generis ordine nucleotidorum in 18S rDNA et ITS differt.

Cells solitary or in 2–4 celled colonies, or in compact aggregations, covered by a gelatinous envelope, planktonic. Cells connected by hyaline stalks. Cells ovoid, oval or spherical, 3–12 × 2.5–10 µm. Single cup-shaped chloroplast with starch-covered pyrenoid. Asexual reproduction by autospore. Differs from other species of the genus by the order of the nucleotides in SSU and ITS rRNA gene sequences.

Holotype: material of the authentic strain CCAP 222/5 is cryopreserved in metabolic inactive state at the Culture Collection of Algae and Protozoa, Oban, Scotland.

Isotype: an air-dried as well as a formaldehyde-fixed sample of the authentic strain CCAP 222/5, deposited at the Botanical Museum at Berlin-Dahlem under the designation B40004041.

Type locality: sewage oxidation pond, Nakuru, Kenya.

Etymology: the species is named in honour of Klaus Dohrmann, a microbiologist. He inspired

the first author to study microphytes.

Authentic strain: CCAP 222/5.

Iconotype: Figure 18*

***Masaia* KRIENITZ, C. BOCK, KOTUT et PRÖSCHOLD gen. nov.**

Latin diagnosis: Cellulae viridis, sphaericae, planctonicae. Chloroplastus unicus, parietalis, poculiformis, pyrenoide granis amyli tecto. Reproductio asexualis autosporem ope, reproductio sexualis ignota. Cellulae solitariae vel in coloniis, 2–4–8 cellularis, interdum tegumento gelatinoso vestitae. A generibus ceteris familiae ordine nucleotidorum in 18S rDNA et ITS differt.

Cells green, spherical, planktonic. Single cup-shaped chloroplast with starch-covered pyrenoid. Asexual reproduction by autospore, sexual reproduction not observed. Cells solitary or in colonies of 2–4–8 cells, covered by a gelatinous envelope. Genus differs from other genera of the family by the order of the nucleotides in SSU and ITS rRNA gene sequences.

Typus generis: *Masaia oloidia* KRIENITZ, C. BOCK, KOTUT et PRÖSCHOLD sp. nov.

Etymology: the genus is named after the Masai, a famous tribe living in East Africa

***Masaia oloidia* KRIENITZ, C. BOCK, KOTUT et PRÖSCHOLD sp. nov.**

Latin diagnosis: Cellulae solitariae vel in coloniis, 2–4–8 cellularis, interdum tegumento gelatinoso vestitae, planctonicae. Cellulis funibus subtilis hyalinis iunctis. Cellulae ovoides, ovalis vel sphaericae, 3.5–12 × 3–12 µm. Chloroplastus unicus, parietalis, poculiformis, pyrenoide granis amyli tecto. Reproductio asexualis autosporem ope. A speciebus ceteris generis ordine nucleotidorum in 18S rDNA et ITS differt.

Cells solitary or in 2–4–8 celled colonies, covered by a gelatinous envelope, planktonic. Cells connected by hyaline stalks. Cells ovoid, oval or spherical, 3.5–12 × 3–12 µm. Single cup-shaped chloroplast with starch-covered pyrenoid. Asexual reproduction by autospore. Differs from species of other genera by the order of the nucleotides in SSU and ITS rRNA gene sequences.

Holotype: material of the authentic strain CCAP 222/32 is cryopreserved in metabolic inactive state at the Culture Collection of Algae and Protozoa, Oban, Scotland.

Isotype: an air-dried as well as a formaldehyde-

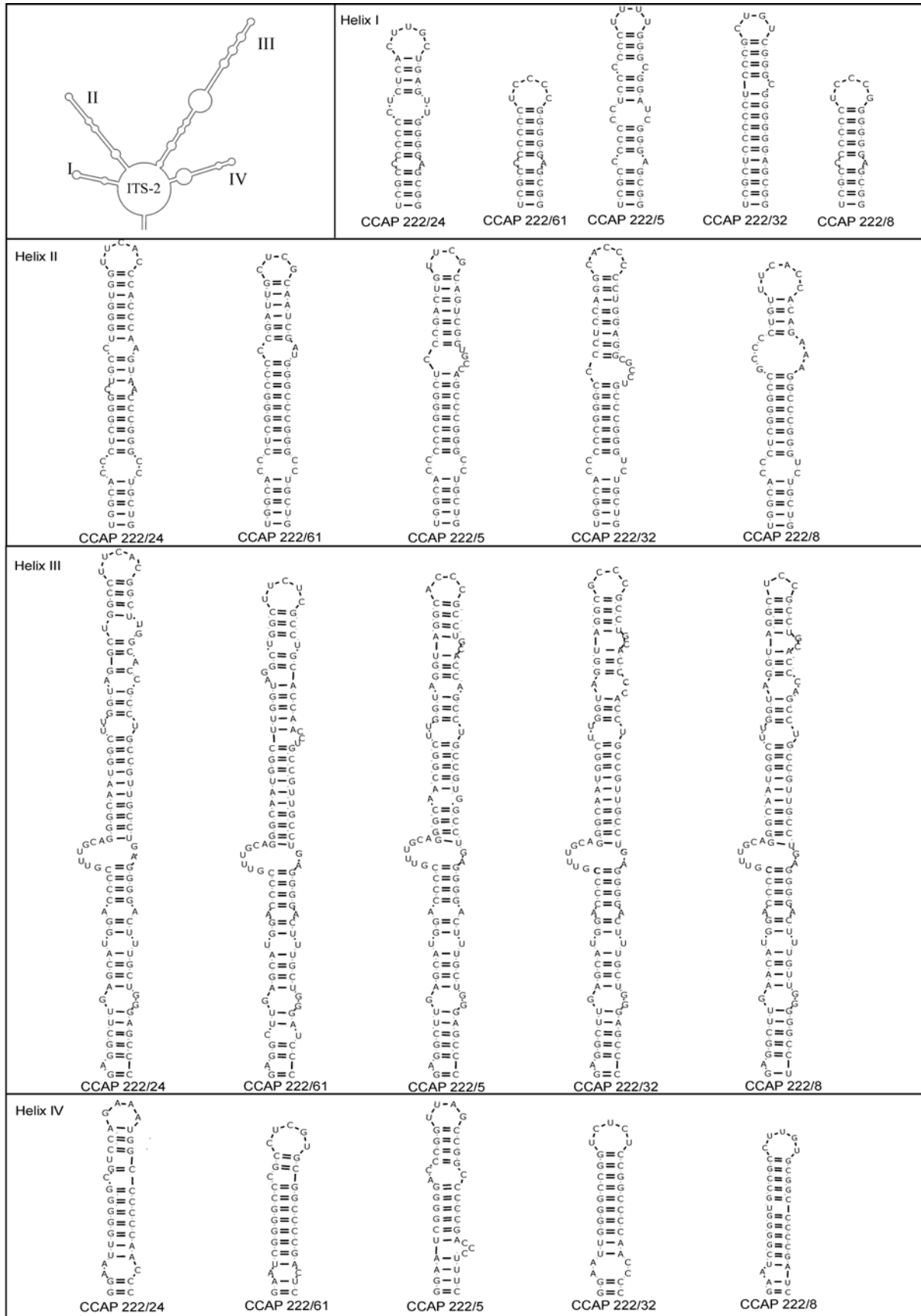
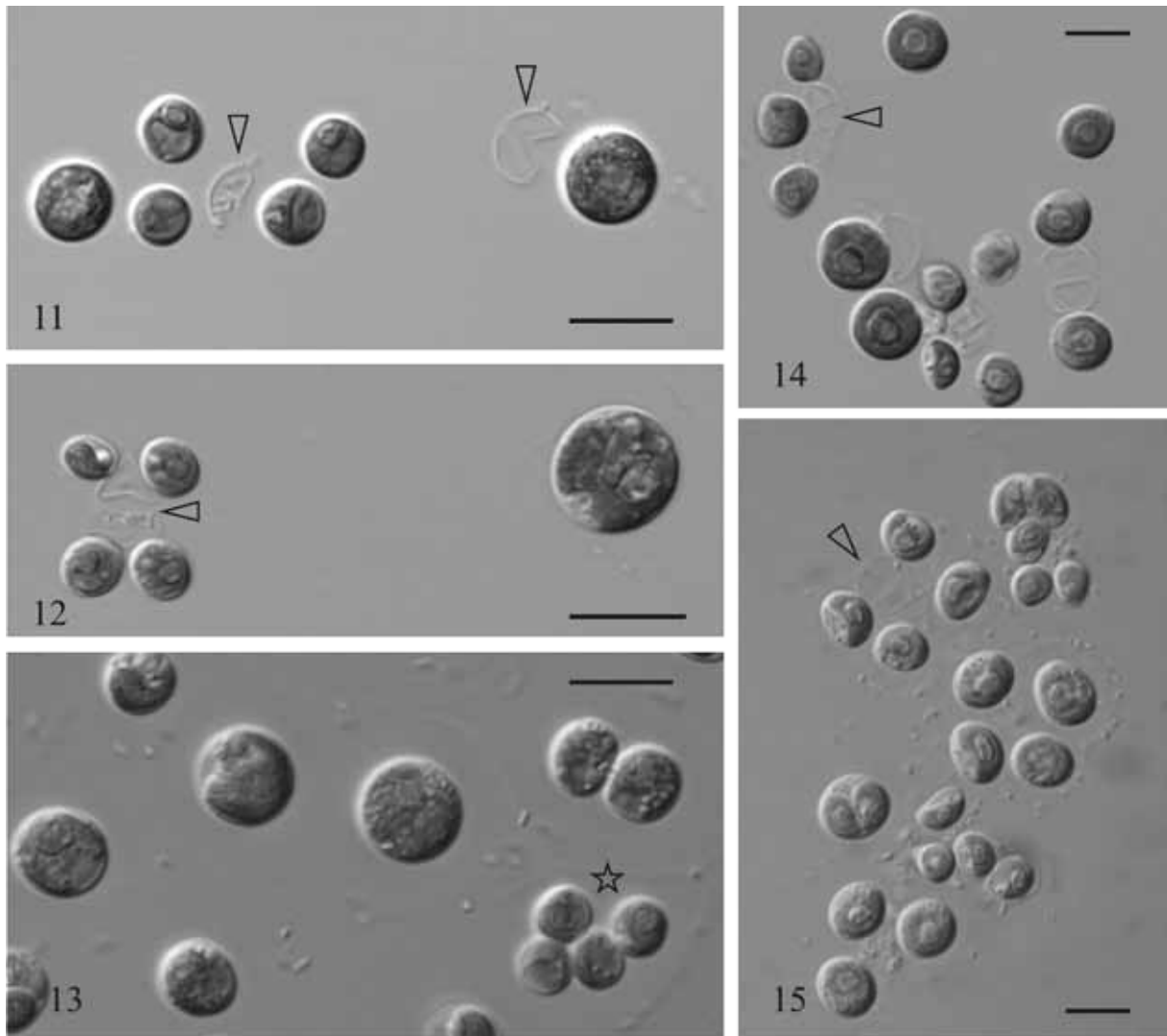


Fig. 10. Comparison of the secondary structure of the helices I – IV of ITS2 rRNA gene of authentic strains of *Marasphaerium gattermannii* (CCAP 222/24), *Compactochlorella kochii* (CCAP 222/61), *Compactochlorella dohrmannii* (CCAP 222/5), *Masaia oloidia* (CCAP 222/32) and *Kalenjinia gelatinosa* (CCAP 222/8). The number of differences is given in Table 2.



Figs 11 – 15. Microphotographs of the newly described genera and species: (11) *Marasphaerium gattermannii*; (12) *Compactochlorella kochii*; (13) *Compactochlorella dohrmannii*; (14) *Masaia oloidia*; (15) *Kalenjinia gelatinosa*. Scale bars 10 μ m.

fixed sample of the authentic strain CCAP 222/32, deposited at the Botanical Museum at Berlin–Dahlem under the designation B40004042.

Type locality: Lake Oloidien, Kenya.

Etymology: the species is named after its the locus classicus, lake Oloidien in Kenya.

Authentic strain: CCAP 222/32.

Iconotype: Figure 19*

***Kalenjinia* KRIENITZ, C. BOCK, KOTUT et PRÖSCHOLD gen. nov.**

Latin diagnosis: Cellulae viridis, sphaericae, planctonicae. Chloroplastus unicus, parietalis, poculiformis, pyrenoide granis amyliis tecto. Reproductio asexualis autosporum ope, reproductio sexualis ignota. Cellulae solitariae vel in coloniis, 2–4 cellularis, interdum tegumento gelatinoso vestitae. A generibus ceteris familiae ordine nucleotidorum in 18S

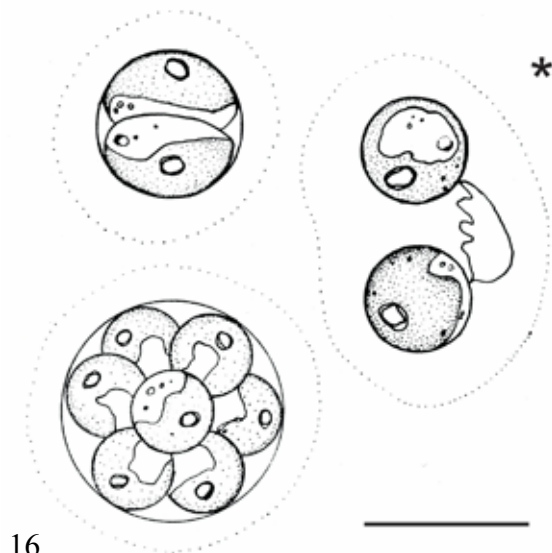
rDNA et ITS differt.

Cells green, spherical, planktonic. Single cup-shaped chloroplast with starch-covered pyrenoid. Asexual reproduction by autosporulation, sexual reproduction not observed. Cells solitary or in colonies of 2–4 cells, covered by a gelatinous envelope. Genus differs from other genera of the family by the order of the nucleotides in SSU and ITS rRNA gene sequences.

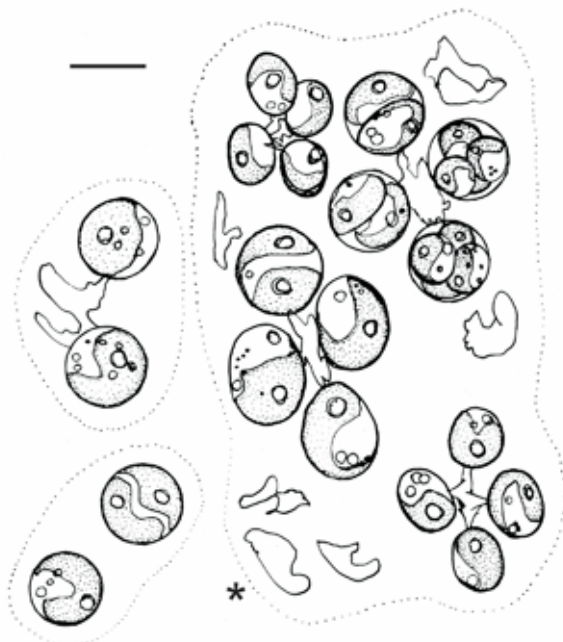
Typus generis: *Kalenjinia gelatinosa* KRIENITZ, C. BOCK, KOTUT et PRÖSCHOLD sp. nov.

Etymology: the genus is named after the Kalenjin, a famous tribe of long distance runners in Kenya.

***Kalenjinia gelatinosa* KRIENITZ, C. BOCK, KOTUT et PRÖSCHOLD sp. nov.**



16



17

Fig 16. Drawings of light microscopy characters of *Marasphaerium gattermannii* in culture. The iconotype is indicated by an asterisk. Scale bar 10 μm .

Fig 17. Drawings of light microscopy characters of *Compactochlorella kochii* in culture. The iconotype is indicated by an asterisk. Scale bar 10 μm .

Latin diagnosis: Cellulae solitariae vel in coloniis, 2–4 cellularis, interdum tegumento gelatinoso vestitae, planctonicae. Cellulis funibus subtilis hyalinis iunctis. Cellulae ovoides, guttae-formis, ovalis vel sphaericae, 4–14 \times 3.5–9 μm . Chloroplastus unicus, parietalis, poculiformis, pyrenoide granis amylicis tecto. Reproductio asexualis autosporum ope. A speciebus ceteris generis ordine nucleotidorum in 18S rDNA et ITS differt.

Cells solitary or in 2–4 celled colonies, covered by a gelatinous envelope, planktonic. Cells connected by hyaline stalks. Cells ovoid, drop-shaped, oval or spherical, 4–14 \times 3.5–9 μm . Single cup-shaped chloroplast with starch-covered pyrenoid. Asexual reproduction by autosporulation. Differs from species of other genera by the order of the nucleotides in SSU and ITS rRNA gene sequences.

Holotype: material of the authentic strain CCAP 222/8 is cryopreserved in metabolic inactive state at the Culture Collection of Algae and Protozoa, Oban, Scotland.

Isotype: an air-dried as well as a formaldehyde-fixed sample of the authentic strain CCAP 222/8, deposited at the Botanical Museum at Berlin-Dahlem under the designation B40004043.

Type locality: sewage oxidation pond, Nakuru, Kenya.

Etymology: the species is named according to the gelatinous envelope which covers the alga.

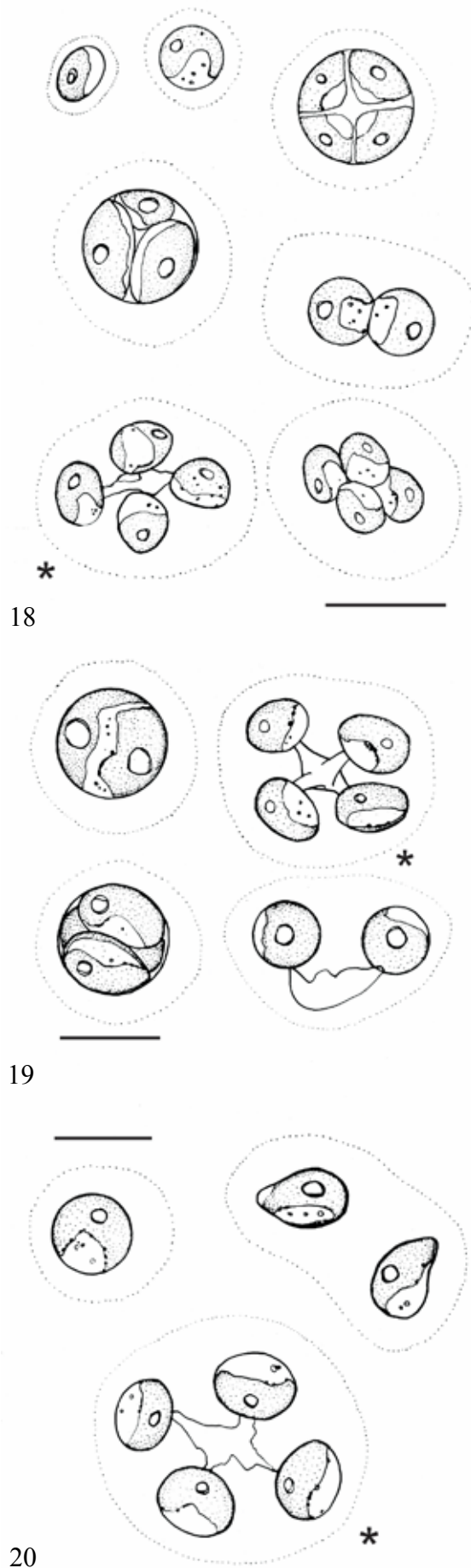
Authentic strain: CCAP 222/8.

Iconotype: Figure 20*.

Discussion

How diverse are coccoid green algae of the *Dictyosphaerium*-morphotype from African inland waters based on morphology and phylogeny?

According to KRIENITZ et al. (2004), the attachment of mucilaginous strands on either the apical or longitudinal side of the cells serves as a morphological criterion that can be used to roughly differentiate between members of the *Chlorella*- and *Parachlorella*-clades respectively. However, very limited phenotypic criteria were given to differentiate between species and genera (see Table 3). In contrast, the results revealed a high genotypic diversity of the *Dictyosphaerium*-like algae. This morphotype of spherical colonial green algae evolved independently within seven different evolutionary lineages of Trebouxiophyceae. Within the *Chlorella*-clade, two species of colony-forming *Hindakia*, *H. tetrachotoma* and *H. fallax*, were established as new combinations by BOCK et al. (2010). Additionally, three solitary African strains were placed directly in the genus *Chlorella* and given new descriptions as *Chlorella rotunda*, *C. singularis* and *C. volutis* C. BOCK, KRIENITZ et PRÖSCHOLD (BOCK et al. 2011a). After



the description of colony-forming members of *Chlorella* by BOCK et al. (2011a) such as *C. colonialis* C. BOCK, KRIENITZ et PRÖSCHOLD, we expected similar species to occur also in our samples. However, we did not find such species in our study.

Based on the careful inventory provided by HUSS et al. (1999), at present, we have to realize step by step that the diversity of the Chlorellaceae is much higher than expected by the authors cited. Already MÜLLER et al. (2005) revealed by amplified fragment length polymorphism (AFLP) analyses of strains of *Chlorella vulgaris* BEIJERINCK from different international strain collections a considerable genomic divergence supporting the existence of cryptic species.

In the *Parachlorella*-clade, six lineages had the *Dictyosphaerium*-morphotypes. The fact that a huge genetic diversity is hidden by a relatively uniform shape is widely reported in Trebouxiophyceae, for example, in ellipsoidal *Chlorella*-like algae (DARIENKO et al. 2010) and spherical *Chlorella*-like algae (BOCK et al. 2011a, PRÖSCHOLD et al. 2011a). This high genetic diversity resulted in the description of five new species placed in four new genera in this study (see Generic and species descriptions). Furthermore, two lineages contained *Dictyosphaerium*-morphospecies, which were not considered in detail in this study. The strain CCAP 222/25 evolved next to the lineages of *Dictyosphaerium* and *Parachlorella* and will be studied and described by Škaloud and co-workers in near future. The strain CCAP 222/43, which is closely related to the needle-shaped *Closteriopsis acicularis* exhibited typical *Dictyosphaerium*-like colonies. We did not describe this strain as a new taxon, because this enigmatic relationship of so different morphotypes needs further investigations including a wider collection of strains.

Our results revealed that the highest diversity of Chlorellaceae occurred in Nakuru town sewage

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Fig 18. Drawings of light microscopy characters of *Compactochlorella dohrmannii* in culture. The iconotype is indicated by an asterisk. Scale bar 10 µm.

Fig 19. Drawings of light microscopy characters of *Masaia oloidia* in culture. The iconotype is indicated by an asterisk. Scale bar 10 µm.

Fig 20. Drawings of light microscopy characters of *Kalenjinia gelatinosa* in culture. The iconotype is indicated by an asterisk. Scale bar 10 µm.

pond, where we found *Chlorella singularis*, *Compactochlorella dohrmannii*, *Masaia oloidea* and *Kalenjinia gelatinosa*. Obviously, such sewage ponds are hot spots for occurrence of the close relatives of *Chlorella* (UHLMANN 1966, 1967). KOTUT et al. (2010) also detected by light microscopy *Hindakia tetrachotoma* (syn. *Dictyosphaerium tetrachotomum* PRINTZ). In our survey, we were not successful in the isolation of *Hindakia* from Nakuru sewage pond. However, we collected it from Lake Baringo.

The high diversity of the *Dictyosphaerium*-morphotype is also shown outside the Trebouxiophyceae. Within the Chlorophyceae a clade evolved a cluster containing solitary and colonial species of the genus *Mychonastes* (KRIENITZ et al. 2011). Previously, the colonial *Dictyosphaerium*-like species were considered as members of the genus *Pseudodictyosphaerium* (HINDÁK 1988, KRIENITZ et al. 1999). In African inland waters five different *Mychonastes*-species were found, four of them established colonies: *M. afer*, *M. ovahimbae*, *M. racemosus* and *M. timauensis* KRIENITZ, C. BOCK, DADHEECH et PRÖSCHOLD (KRIENITZ et al. 2011).

Are the tropical *Dictyosphaerium*-like algae genotypically identical with those from the temperate climatic region?

We did not find a simple answer to this question. On one hand, strains from both the temperate region and the tropics were found in one clade. For example, the strains of the type species *D. ehrenbergianum* from UK and Germany (temperate) were similar to those from Kenya and Tunisia (tropics). This was also the case for the filing strains of the members of the genus *Hindakia* and *Compactochlorella* from Africa and Europe. In contrast to the above, the clusters of *Mucidosphaerium* comprised exclusively of strains from Europe while those of *Masaia* contained only East African strains. These findings are in close agreement with findings of earlier studies on the geographic distribution of algae. Using a morphological approach, KOMÁREK (1983) compared the coccoid green algae from Cuba with species commonly found in the temperate zones. His study revealed that out of total of 109 taxa from this tropical island, 53 taxa were identical to species from the temperate latitudes. The remaining taxa were found exclusively in the tropics, with 21 taxa known only from Cuba. A study on diatoms by HILLEBRANDT et al. (2001) revealed a decreasing

similarity of species composition with increasing geographic distance. COLEMAN (2001) found local adaptation and endemism in phytoflagellates of the genera *Pandorina* and *Volvulina*. A study using phenotypic and genotypic criteria (VANORMELINGEN et al. 2008) established a distribution range for diatoms ranging from a global to a narrow endemic distribution range. Molecular analyses of a marine, picoplanktonic morphospecies-complex *Micromonas pusilla* BUTCHER revealed genotypes of global oceanic distribution and genotypes with a more restricted distribution (SLAPETA et al. 2006). All these findings contradict the conception of universal distribution of microorganisms accentuated by FENCHEL et al. (1997), FINLEY (2002) and FENCHEL & FINLEY (2004). Recently, more and more arguments questioning the validity of the universal distribution of microorganisms have been generated (COLEMAN 2002; LOGARES 2006; FOISSNER 2008).

Evidently, phycogeographical considerations and designation of phyco-floral regions are of great interest. PADISÁK (2009) explained that different geographic distribution pattern of algal taxa are as a result of a difference in the balance between the speed of dispersal and the evolution rate: If the dispersal rate is faster than the evolution rate, the taxon has a wide or 'subcosmopolitan' distribution (species occurring throughout the world but only in appropriate habitats). In contrast, if the rate of dispersal is slower than the rate of evolution, floristic regionality is supported. In a study focusing on desmids, a morphologically well studied algal group, COESEL (1996) established the following distinct phycogeographic regions: Indo-Malaysia/Northern Australia, Equatorial Africa, Tropical South and Central America, North America, Extratropical South America, Eastern Asia, Southern Australia and New Zealand, South Africa, Temperate Eurasia and finally the circumpolar and high mountain regions. Further studies should reveal the extent to which these phycogeographic regions apply to the coccoid green algae and other algal groups with a high level of ubiquity.

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References

- BAAS BECKING, L.G.M. (1934): Geobiologie of inleiding tot de milieukunde Diligentia Wetenschappen, Ser. 18/19. – Van Stockum's Gravenhange, 263 pp.
- BOCK, C., PRÖSCHOLD, T. & KRIENITZ, L. (2010): Two new *Dictyosphaerium*–morphotype lineages of the Chlorellaceae (Trebouxiophyceae): *Heynigia* gen. nov. and *Hindakia* gen. nov. – Europ. J. Phycol. 45: 267–277.
- BOCK, C., KRIENITZ, L. & PRÖSCHOLD, T. (2011a): Taxonomic reassessment of the genus *Chlorella* (Trebouxiophyceae) using molecular signatures (barcodes), including description of seven new species. – Fottea 11: 293–312.
- BOCK, C., PRÖSCHOLD, T. & KRIENITZ, L. (2011b): Updating the genus *Dictyosphaerium* and description of *Mucidosphaerium* gen. nov. (Trebouxiophyceae) based on morphological and molecular data. – J. Phycol. 47: 638–652.
- BYUN, Y. & HAN, K. (2006): PseudoViewer: web application and web service for visualizing RNA pseudoknots and secondary structures. – Nucl. Acids Res. 34: W416–W422.
- COCQUYT, C., VYVERMAN, W. & COMPÈRE, P. (1993): A checklist of the algal flora of the East African Great Lakes. – Script. Bot. Belg. 8: 1–55.
- COESEL, P.F.M. (1996): Biogeography of desmids. – Hydrobiologia 336: 41–53.
- COESEL, P.F.M. & KRIENITZ, L. (2008): Diversity and geographic distribution of desmids and other coccoid green algae. – Biodiv. Conserv. 17: 381–392.
- COLEMAN, A.W. (2001): Biogeography and speciation in the *Pandorina/Volvulina* (Chlorophyta) superclade. – J. Phycol. 37: 836–851.
- COLEMAN, A.W. (2002): Microbial eukaryote species. – Science 297: 337.
- COLEMAN, A.W. (2003): ITS2 is a double–edged tool for eukaryote evolutionary comparisons. – Trends Genet. 19: 370–375.
- COLEMAN, A. W. (2007): Pan–eukaryote ITS2 homologies revealed by RNA secondary structure. – Nucl. Acids Res. 35: 3322–3329.
- DARIENKO, T., GUSTAVS, L., MUDIMU, O., RAD MENENDEZ, C., SCHUMANN, R., KARSTEN, U., FRIEDL, T. & PRÖSCHOLD, T. (2010): *Chloroidium*, a common terrestrial coccoid green alga previously assigned to *Chlorella* (Trebouxiophyceae, Chlorophyta). – Eur. J. Phycol. 45: 79–95.
- DE WIT, R. & BOUVIER, T. (2006): ‘Everything is everywhere, but, the environment selects’; what did Baas Becking and Beijerinck really say? – Environ. Microbiol. 8: 755–758.
- FAWLEY, M.W., DEAN, M.L., DIMMER, S.K. & FAWLEY, K.P. (2005): Evaluating the morphospecies concept in the Selenastraceae (Chlorophyceae, Chlorophyta). – J. Phycol. 42: 142–154.
- FAWLEY, M.W., FAWLEY, K.P. & BUCHHEIM, M.A. (2004): Molecular diversity among communities of freshwater microchlorophytes. – Microb. Ecol. 48: 489–499.
- FENCHEL, T., ESTEBAN, G.F. & FINLAY, B.J. (1997): Local versus global diversity of microorganisms: cryptic diversity of ciliated protozoa. – Oikos 80: 220–225.
- FENCHEL, T. & FINLAY, B.J. (2004): The ubiquity of small species: patterns of local and global diversity. – BioScience 54: 777–784.
- FINLAY, B.J. (2002): Global dispersal of free–living microbial eukaryote species. – Science 296: 1061–1063.
- FINLAY, B.J. & FENCHEL, T. (2002): Microbial eukaryote species. *Science* 297: 337.
- FOISSNER, W. (2006): Biogeography and dispersal of micro–organisms: A review emphasizing protists. – Acta Protozool. 45: 111–136.
- FOISSNER, W. (2008): Protist diversity and distribution: some basic considerations. – Biodiv. Conserv. 17: 235–242.
- HEGEWALD, E., KRIENITZ, L. & SCHNEPF, E. (1994): Studies on *Scenedesmus costato–granulatus* Skuja. – Nova Hedwigia 59: 97–127.
- HEPPERLE, D. & KRIENITZ, L. (2001): Systematics and ecology of chlorophyte picoplankton in German inland waters along a nutrient gradient. – Internat. Rev. Hydrobiol. 86: 269–284.
- HILLEBRANDT, H., WATERMANN, F., KAREZ, R. & BERNINGER, U. (2001): Differences in species richness patterns between unicellular and multicellular organisms. – Oecologia 126: 114–124.
- HINDÁK, F. (1988): Studies on the chlorococcal algae (Chlorophyceae). IV. – Biologické Práce 34: 1–263.
- HOSHINA, R., KATO, Y., KAMAKO, S.I., & IMAMURA, N. (2005): Genetic evidence of ‘American’ and ‘European’ type symbiotic algae of *Paramecium bursaria* Ehrenberg. – Plant Biol. 7: 526–532.
- HUBER–PESTALOZZI, G. (1929): Das Plankton natürlicher und künstlicher Seebecken Südafrikas. – Verh.

- Internat. Ges. Limnol. 4: 343–390.
- HUSS, V. A. R., FRANK, C., HARTMANN, E. C., HIRMER, M., KLOBOUCEK, A., SEIDEL, B. M., WENZELER, P. & KESSLER, E. (1999): Biochemical taxonomy and molecular phylogeny of the genus *Chlorella* sensu lato (Chlorophyta). – J. Phycol. 35: 587–598.
- HUELSENBECK, J.P. & RONQUIST, F. (2001): MrBayes: Bayesian inference of phylogenetic trees. – Bioinformatics 17: 754–755.
- JOB, G. (2008): Treefinder, version of October 2008. Munich, Germany. Distributed by the author at www.treefinder.de.
- KOMÁREK, J. (1983): Contribution to the chlorococcal algae of Cuba. – Nova Hedwigia 37: 65–180.
- KOMÁREK, J. & FOTT, B. (1983): Chlorophyceae (Grünalgen) Ordnung: Chlorococcales. – In: HUBER–PESTALOZZI, G. (ed.) Das Phytoplankton des Süßwassers 7. Teil, 1. Hälfte. – 1044 pp., Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller), Stuttgart.
- KOMÁREK, J. & PERMAN, J. (1978): Review of the genus *Dictyosphaerium* (Chlorococcales). – Arch. Hydrobiol. 51/Algological Studies 20: 233–297.
- KOTUT, K., BALLOT, A., WIEGAND, C. & KRIENITZ, L. (2010): Toxic cyanobacteria at Nakuru sewage oxidation ponds – A potential threat to wildlife. – Limnologia 40: 47–53.
- KRIENITZ, L. & BOCK, C. (2011): *Elongatocystis ecballocystiformis* gen. et comb. nov., and some reflections on systematics of Oocystaceae (Trebouxiophyceae, Chlorophyta). – Fottea 11: 271–278.
- KRIENITZ, L., BOCK, C., LUO W. & PRÖSCHOLD, T. (2010): Polyphyletic origin of the *Dictyosphaerium*-morphotype within Chlorellaceae (Trebouxiophyceae). – J. Phycol. 46: 559–563.
- KRIENITZ, L., BOCK, C., DADHECH, P.K. & PRÖSCHOLD, T. (2011): Taxonomic reassessment of the genus *Mychonastes* (Chlorophyceae, Chlorophyta) including the description of eight new species. – Phycologia 50: 89–106.
- KRIENITZ, L., HEGEWALD, E.H., HEPPERLE, D., HUSS, V.A.R., ROHR, T. & WOLF, M. (2004): Phylogenetic relationship of *Chlorella* and *Parachlorella* gen. nov. (Chlorophyta, Trebouxiophyceae). – Phycologia 43: 529–542.
- KRIENITZ, L., TAKEDA, H. & HEPPERLE, D. (1999): Ultrastructure, cell wall composition and phylogenetic position of *Pseudodictyosphaerium jurisii* (Chlorococcales, Chlorophyta) including a comparison with other picoplanktonic green algae. – Phycologia 38: 100–107.
- KRIENITZ, L. & WIRTH, M. (2006): The high content of polyunsaturated fatty acids in *Nannochloropsis limnetica* (Eustigmatophyceae) and its implication for food web interactions, freshwater aquaculture and biotechnology. – Limnologia 36: 204–210.
- LOGARES, R.E. (2006): Does the global microbiota consist of a few cosmopolitan species? – Ecol. Austral. 16: 85–90.
- 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.
- LUO, W., PRÖSCHOLD, T., BOCK, C. & KRIENITZ, L. (2010): Generic concept in *Chlorella*-related coccoid green algae (Chlorophyta, Trebouxiophyceae). – Plant Biol. 12: 545–553.
- 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.
- MÜLLER, J., FRIEDL, T., HEPPERLE, D., LORENZ, M. & DAY, J.G. (2005): Distinction between multiple isolates of *Chlorella vulgaris* (Chlorophyta, Trebouxiophyceae) and testing for conspecificity using amplified fragment length polymorphism and its ITS rDNA sequences. – J. Phycol. 41: 1236–1247.
- NORTON, T.A., MELKONIAN, M. & ANDERSEN, R.A. (1996): Algal biodiversity. – Phycologia 35: 308–326.
- PADISÁK, J. (2009): The phycogeography of freshwater algae. – In: LIKENS, G. (ed.) Encyclopedia of Inland Waters, Vol. I. Elsevier Inc. Oxford, pp 219–223.
- POTTER, D., LAJEUNESSE, T.C., SAUNDERS, G.W. & ANDERSON, R.A. (1997): Convergent evolution masks extensive biodiversity among marine coccoid picoplankton. – Biodiv. Conserv. 6: 99–107.
- PRÖSCHOLD, T., DARIENKO, T., SILVA, P.C., REISSER, W. & KRIENITZ, L. (2011): The systematics of „*Zoochlorella*“ revisited employing an integrative approach. – Environ. Microbiol. 13: 350–364.
- PRÖSCHOLD, T. & LELIAERT, F. (2007): Systematics of the green algae: Conflict of classic and modern approaches. – In: BRODIE, J. & LEWIS, J. (eds.) Unravelling the Algae: The Past, Present, and Future of the Algae Systematics. Taylor and Francis, London, pp. 123–153.
- RINDI, F., ALLALI, H.A., LAM, D.W. & LÓPEZ–BAUTISTA, J.M. (2010): An overview of the biodiversity and biogeography of terrestrial green algae. – In: RESCIGNO, V. & MALETTA, S. (eds.) Biodiversity Hotspots. Nova Science Publishers, pp. 105–122.
- SLAPETA, J., LÓPEZ–GARCÍA, P. & MOREIRA, D. (2006): Global dispersal and ancient cryptic species in

- the smallest marine eukaryotes. – *Mol. Biol. Evol.* 23: 23–29.
- SWOFFORD, DL (2002): PAUP* Phylogenetic analysis using parsimony (* and other methods). Version 4.0b10. – Sinauer Associates, Sunderland.
- TALLING, J.F. (1987): The phytoplankton of Lake Victoria (East Africa). – *Arch. Hydrobiol., Beihefte Ergebn. Limnol.* 25: 229–256.
- UHLMANN, D. (1966): Beitrag zur Limnologie extrem nährstoffreicher Flachgewässer. II. Plankton–Massenwechsel. – *Wiss. Z. KMU Leipzig, Math.–nat. Reihe* 15: 373–423.
- UHLMANN, D. (1967): Beitrag zur Limnologie saprotropher Flachgewässer. – *Arch. Hydrobiol.* 63: 1–85.
- VANORMELINGEN, P., VERLEYEN, E. & VYVERMAN, W. (2008): The diversity and distribution of diatoms: from cosmopolitanism to narrow endemism. – *Biodiv. Conserv.* 17: 393–405.

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