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Rindifilum ramosum gen. nov., sp. nov., a new freshwater genus within the Ulvales (Ulvophyceae, Chlorophyta)

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ABSTRACT
The present paper provides a phylogenetic and morphological study of two strains that turn out to represent a new genus and species, *Rindifilum ramosum* gen. nov., sp. nov., within the family Ctenocladiaceae (Ulvales). *Rindifilum ramosum* gen. nov., sp. nov. grows in association with the lichenized ascomycetes genus *Verrucaria* Schrader. Phylogenetic reconstructions based on the *rbc*L, 18S rRNA and *tuf*A genes showed that the investigated strains belonged to a lineage distinct from those sequenced so far. Moreover, comparisons based on morphological observations revealed no differences between the two strains. The newly genus *Rindifilum* gen. nov. exhibits a unique combination of morphological features, as the "pear-shaped" cells that develop directly into a "hammer-shaped filament", making it distinct from all other green algae described so far.

KEY WORDS
Green algae,
Chlorophyta,
Ulvophyceae,
new genus,
new species.

RÉSUMÉ
Rindifilum ramosum *gen. nov., sp. nov., un nouveau genre d'eau douce au sein des Ulvales (Ulvophyceae, Chlorophyta).*
Le présent article fournit une étude phylogénétique et morphologique de deux souches qui s'avèrent représenter un genre nouveau et une espèce nouvelle, *Rindifilum ramosum* gen. nov., sp. nov., au sein de la famille des Ctenocladiaceae (Ulvales). *Rindifilum ramosum* gen. nov., sp. nov. se développe en association avec le genre *Verrucaria* Schrader, un ascomycète lichénisé. Les reconstructions phylogénétiques basées sur les gènes *rbc*L, 18S rRNA et *tuf*A ont montré que les souches étudiées appartiennent à une lignée distincte de celles séquencées jusqu'à présent. De plus, les comparaisons basées sur les observations morphologiques n'ont révélé aucune différence entre les deux souches. Le nouveau genre *Rindifilum* gen. nov. présente une combinaison unique de caractéristiques morphologiques, comme les cellules en forme de « poire » qui se développent directement en un « filament en forme de marteau », ce qui le distingue de toutes les autres algues vertes décrites jusqu'à présent.

MOTS CLÉS
Algues vertes,
Chlorophyta,
Ulvophyceae,
genre nouveau,
espèce nouvelle.

INTRODUCTION

The class Ulvophyceae K.R.Mattox & K.D.Stewart was first described in Mattox & Stewart (1984) and is one of the main groups of green algae that summons a great variety living in a broad scale of habitats worldwide. The Ulvophycean order Ulvales Blackman & Tansley contains morphologically diverse algae with both macroscopic and microscopic thalli, generally possessing a single parietal chloroplast with one to several pyrenoids. The majority of Ulvales species inhabit marine ecosystems, where they often function as key distributors of nutrients and life environment for numerous invertebrates and vertebrates. However, several species also colonize a plethora of freshwater and terrestrial habitats. Interestingly, Ulvales includes many euryhaline species, i.e., those organisms having a very broad range of tolerance to the salinity gradient (Škaloud *et al.* 2018). However, research of these algae is often scanty although a great diversity can be discovered in these environments (Darienko *et al.* 2009; Škaloud *et al.* 2013). Recently, many non-marine members of orders Ulvales and Ulotrichales Borzì have been taxonomically revised (Darienko & Pröschold 2017; Škaloud *et al.* 2018).

Following the latter publication, we studied two isolates, SAG 2039 and SAG 2052. According to Škaloud *et al.* (2018), these two strains are molecularly identical and represent a fully supported lineage within the Ctenocladiaceae (Ulvales).

Both strains were obtained from the SAG (Sammlung von Algenkulturen Göttingen), under the name *Dilabifilum* sp. (Table 1). Both strains were isolated from two species belonging to the *Verrucaria* Schrader genus. Verrucariaceae Zenker (Verrucariales, Ascomycota) is a group of mainly lichenized ascomycetes comprising widely diverse habits (Gueidan *et al.* 2007). Half of all ascomycetes are lichenized (Singh *et al.* 2015) and are found in every terrestrial habitat capable of supporting photosynthesis. In the family Verrucariaceae, a remarkable number of algal genera can be found (Thüs *et al.* 2011).

The genus *Dilabifilum* Tschermak-Woess was erected in 1970. However, *Dilabifilum* has been recently synonymised with the genus *Pseudendoclonium* Wille, forming a lineage distinct from the SAG 2039 and SAG 2052 strains investigated herein (Darienko & Pröschold 2017). Therefore, these strains represent a separate entity for which we propose the new genus and species, *Rindifilum ramosum* gen. nov., sp. nov.

MATERIAL AND METHODS

Culture conditions and light microscopy

The algae SAG 2039 and SAG 2052 strains were phototrophically cultivated at 25°C under 12/12 light-dark illumination of 60-80 µmol photons m^{-2} s^{-1} (Light meter Delta OHM HD2302.0) white light in modified WARIS-H culture medium (McFadden & Melkonian 1986). Morphological parameters were investigated using light microscope Olympus CKX41 (Olympus, Tokyo, Japan) inverted light microscope or by an optical light microscopy Leica microsystems DM750 (Switzerland). Microphotographs were taken with digital camera Canon EOS 1100D adapted to the microscope or by a digital colour camera (EC3, Leica Microsystems, Switzerland) equipped with LAS EZ 3.2.1 software (Leica microsystems, Switzerland). The most important aspects such as cell dimensions, presence and shape of pyrenoid, branching pattern, shape of cell colonies, etc., were documented. The series of optical sections were collected to reconstruct the several stages of the thallus.

Table 1. — Culture strains information.

Culture strain number	Sampling locality
SAG 2039	June 2000, Germany, Südschwarzwald, St. Wilhelmer Talbach, *c.* 700 m a.s.l., isolated from lichen *Verrucaria scabra* Vezda.
SAG 2052	July 2003, Switzerland, Davos Valley, bank of brook Drusatschabächel, 1570 m a.s.l., isolated from lichen *Verrucaria margacea* (Wahlenb.) Wahlenb.

DNA extraction, PCR amplification and DNA sequencing

Total genomic DNA was isolated using the Instagene Matrix (Bio-Rad Laboratories, Hercules, CA, USA). Sequences of nuclear 18S rDNA and chloroplast *tuf*A genes were obtained by PCR amplification using the primers 18SF (5'-AAC CTG GTT GAT CCT GCC AGT-3') and 18SR (5'-TGA TCC TTC TGC AGG TTC ACC TAC G-3'; from Katana *et al.* 2001), and *tuf*GF4 (5'-GGN GCN GCN CAA ATG GAY GG-3') and *tuf*AR (5'-CCT TCN CGA ATM GCR AAW CGC-3'; from Fama *et al.* 2002). Each 20 µl reaction contained: 13 µl of sterile Milli-Q water, 2.2 µl of $MgCl_2$ (Bioline LAB MARK), 2 µl of Gold™ buffer (Bioline LAB MARK), 0.6 µl of Enhancer (Bioline LAB MARK), 0.4 µl of dNTP (Bioline LAB MARK), 0.3 µl each of the forward and reverse primer (25nM), 0.2 µl of Gold DNA polymerase (5U/µl, Bioline LAB MARK) and 1 µl DNA (~10 ng $µl^{-1}$). To amplify 18S rDNA gene, PCR amplification was performed using a thermal cycler Eppendorf Mastercycler ep S with the following protocol: denaturation at 95°C for seven minutes; 35 cycles of denaturation at 94°C for 30 seconds, primer annealing at 50°C for one minute and elongation at 72°C for 2.5 minutes; final elongation at 72°C for 10 minutes. PCR amplification of the *tuf*A gene was performed using the same cycler as follows: denaturation at 94°C for four minutes; 38 cycles of denaturation at 94°C for one minute, primer annealing at 45°C for 30 seconds and elongation at 72°C for one minute; final elongation at 72°C for seven minutes. The quality and yield of the PCR products was checked in UV light using 1 % agarose gel containing ethidium bromide. Amplified PCR products were purified using the MinElute PCR Purification Kit (Qiagen, Crawley, UK). Sequencing was carried out by Macrogen, Inc (Europe, Meibergdreef 31, 1105, AZ).

TABLE 2. — List of sequences analysed in this study. Classification, strain numbers, and GenBank accession numbers are provided.

			GenBank accession numbers		
Order/Family	**Taxon name**	**Strain number**	**18S rDNA**	***tuf*A**	***rbc*L**
Kornmanniaceae	*Halofilum ramosum* Darienko & Pröschold	SAG 2050	MF000571	MF000589	–
	Blidingia dawsonii (Hollenberg & I.A.Abbott) S.C.Lindstrom, L.A.Hanic & L.Golden	UBC A84927	DQ001138	–	–
	Tellamia contorta Batters	–	AF499663	–	AF499679
	Kornmannia leptoderma (Kjellman) Bliding	–	AF499661	–	AF499677
	Paulbroadya prostrata (Broady & Ingerfeld) Darienko & Pröschold	SAG 23.92	FR865752	MF000590	–
	Pseudendoclonium commune Darienko & Pröschold	SAG 2051	MF000572	MF000591	–
	Pseudendoclonium incrustans Darienko & Pröschold	CCAP 415/1	FR865750	–	–
	Lithotrichon pulchrum Darienko & Proeschold	SAG 2038	MF034614	–	–
Bolbocoleonaceae	*Bolbocoleon piliferum* Pringsheim	WA2-9b2a	AY303598	AY454421	–
Phaeophilaceae	*Phaeophila dendroides* (P.L. Crouan & H.M. Crouan) Batters	WA1.15D	AY454432	AY454414	–
	Phaeophila dendroides	FR1.2a2	AY454430	AY454415	–
Cloniophoraceae	*Cloniophora spicata* (Schmidle) Islam	SAG 7.97	JF680949	JF680963	–
Ulvellaceae	*Ulvella tongshanensis* H.Zhu & G.Liu	FACHB 1780	KM226211	KM226208	KM226206
	Ulvella viridis (Reinke) R.Nielsen, C.J.O'Kelly & B.Wysor	MA1.2a1	AY303594	AY454407	–
	Ulvella endozoica (Goldberg, Makemson & Colley) R.Nielsen, C.J.O'Kelly & B. Wysor	UTEX 2352	AY205327	AY454412	–
Ulvaceae	*Ulva shanxiensis* Ulva shanxiensis L.Chen, J.Feng & S.L.Xie	SAS 06035	KJ617035	KJ617036	–
	Ulva californica Wille	FH 3.2	AY303586	AY454401	–
	Ulva limnetica Ichihara et Shimada	P36	AB425959	–	AB425968
	Percursaria percursa (C.Agardh) Rosenvinge	UTEX 1423	AY303589	AY454403	–
	Ochlochaete hystrix Thwaites	MA1.8d1	AY454428	AY454406	–
	Ruthnielsenia tenuis (Kylin) O'Kelly, Wysor & Bellows	Ma2.6a1	AY454426	AY454405	–
Ctenocladiaceae	*Pseudopleurococcus printzii* J.Snow	SAG 467-1	MF000573	MF000592	–
	Rindifilum ramosum gen. nov., sp. nov.	SAG 2039	MF000574	MF000593	–
	Rindifilum ramosum gen. nov., sp. nov.	SAG 2052	MF000575	MF000594	–
	Ctenocladus circinnatus Borzì	TB2014012	KU362724	KU362726	–
	Ctenocladus circinnatus	KZ-26-3	MK231274	–	–
	Ctenocladus circinnatus	CCMP 2158	MF034603	–	–
Chlorocystidales	*Halochlorococcum moorei* (N.L.Gardner) Kornmann & Sahling ex Guiry	Wa14B	AY198122	AY454417	–
	Desmochloris halophila (Guillard, Bold & McEntee) Watanabe, Kuroda & Maiwa	CCAP 6006/1	FM882216	–	–
	Chlorocystis sp.	CCAP 233/1	FR865693	–	–
Ulotrichaceae	*Pseudoneochloris marina* S.Watanabe, A.Himizu, L.A.Lewis, G.L.Floyd & P.A.Fuerst	UTEX 1445	U41102	AY454422	AF499682
	Pseudoneochloris sp.	NKY372003	LC505539	–	–
Ulotrichales	*Ulothrix zonata* F.Weber & D.Mohr) Kütz.	UTEX 745	KU865575	AY454424	–
	Tupiella akineta (Tupa) Darienko & Pröschold	UTEX 1912	DQ011230	AY835431	AY835431
	Sarcinofilum mucosum (Broady) Darienko & Pröschold	SAG 24.93	KM020139	MF000597	–
Scotinosphaerales	*Scotinosphaera gibberosa* (Vodenicarov & Benderliev) Wujek & R.H.Thompson	CAUP H 5301	HE860255	–	HE860267
	Scotinosphaera lemnae (Puncochárová) Wujek & R.H.Thompson	CAUP H 5303a	HE860257	–	HE860269

ALIGNMENT AND PHYLOGENETIC ANALYSES

Multiple alignments of 18S rDNA, *tuf*A and *rbc*L genes were analysed to infer the phylogenetic position of the SAG 2039 and SAG 2052 strains within the Ulvales. In addition to the most closely related sequences and the representatives of particular Ulvales families, the sequences of Chlorocystidales Kornmann & Sahling, Ulotrichales, *Pseudoneochloris* Watanabe, Himizu, Lewis, Floyd & Fuerst clade and Scotino-

sphaerales Škaloud, Kalina, Nemjová, De Clerck & Leliaert were included into the alignments (Table 2). Concerning the phylogenetic analysis, there is only a limited number of available *rbc*L sequences for taxa belonging to *Rindifilum* gen. nov. and related taxa. Accordingly, our dataset has included 37 18S and 24 *tuf*A, but only 8 *rbc*L sequences. The three genes are only available for three species (*Ulvella tongshanensis* H.Zhu & G.Liu, *Pseudoneochloris marina* S.Watanabe, A.Himizu, L.A.Lewis, G.L.Floyd & P.A.Fuerst, and *Tupiella akineta* (Tupa) Darienko & Proeschold). However, it is usual that in studies analysing concatenated alignments consisting of several loci partitions, not all partitions are completely sequenced due to missing data in published repositories or technical difficulties to obtain sequences of these loci. Usually, it is impossible to get fully completed datasets. Moreover, the usage of a concatenated dataset with missing data is suitable as shown by Verbruggen *et al.* (2010: fig. 1). In addition, we have compared the phylogenies inferred separately for each of three genes sequenced. The resulting trees were congruent in both the position and relationships of taxa (data not shown), warranting the use of concatenated alignment even with the high proportion of missing data in the *rbc*L dataset.

18S rDNA sequence alignment was compiled using MAFFT 7.429 (Katoh *et al.* 2002). The sequence of *Pseudoneochloris marina* (U41102) was improved by correcting obvious sequencing errors at the end of the sequence. First, we replaced ambiguous bases in conserved SSU rDNA regions by the conserved Ulvales bases; second, we corrected several erroneous bases in the position 1523-1626, using the sequence of *Pseudoneochloris* sp. LC505539 as a guide.

Sequences of the chloroplast genes were aligned manually. The substitution models were evaluated using the jModelTest (Guindon & Gascuel 2003; Darriba *et al.* 2012), identifying the GTR+I+Γ as the most appropriate model for all three gene partitions. Bayesian inference of the concatenated dataset of 18S rDNA, *rbc*L and *tuf*A genes was inferred with MrBayes 3.2.6 (Ronquist *et al.* 2012). Two parallel Monte Carlo Markov chains runs were carried out for six million generations each with one cold and three heated chains. Trees and parameters were sampled every 100th generation. Convergence of the two runs was assessed during the run by calculating the average standard deviation of split frequencies. The "burn-in" was specified at the value 1000 using the "sump" command. The maximum likelihood (ML) analysis was performed using RAxML 8.1.20 on the concatenated dataset partitoned to individual genes. The evolutionary model used was the default GTR+Γ. Bootstrap analysis was performed with the rapid bootstrapping procedure, using 100 pseudoreplicates. All analyses were run at the Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal (http://www.phylo.org/sub_sections/portal; Miller *et al.* 2010).

RESULTS

Phylogenetic analyses

The two strains SAG 2039 and SAG 2052 with similar morphology were subjected to genetic examination and the near full-length 18S rRNA gene sequences were identical. Results of the phylogenetic relationships of the concatenated dataset of 18S rDNA, *rbc*L and *tuf*A sequences (Fig. 1) placed these strains in a distinct clade within the family Ctenocladiaceae. The new genotypes were distinct from the available environmental sequences (Fig. 1). To evaluate the phylogenetic positions of the newly sequenced strains, we performed phylogenetic analyses with all major taxa in the order Ulvales using Chlorocystidales, Ulotrichales, Scotinosphaerales and *Pseudoneochloris* clade as the outgroups. A phylogenetic analysis performed using a concatenated genes alignment supported seven distinct families in the Ulvales. The genus *Rindifilum* gen. nov. is a sister to the rest of Ctenocladiaceae and to the Phaeophilaceae D.F. Chappell, C.J. O'Kelly, L.W. Wilcox & G.L. Floyd group. Bayesian and ML analyses inferred the phylogenetic trees with identical topologies.

Morphological observations

Thalli up to 0.5 mm consist of prostrate and erected/upright filaments (Fig. 2A). The vegetative cells of prostrate system are composed by rounded/spherical (5.5-13.5 µm in diameter) or pear-shaped to ovoid cells (6.2-13.2 µm long and 4.2-10.6 µm wide), either solitary or forming short easily disintegrating filaments (Fig. 2B-G). The chloroplast is parietal with an oval pyrenoid 1.9-3 µm long 1.7-2.5 µm wide (Fig. 2B, E). Our observations suggest that the young cells are rounded/spherical and during the cell cycle, they become ovoid to pear-shaped. These globose cells forming irregularly branched filaments are typically more densely pigmented (Fig. 2H). To produce the filament, the pyriform cells of prostrate system developed into flask-shaped cells (Fig. 2I) finally developing into characteristic "hammer-shaped cells" 11-77.6 µm long and 7.3-33 µm wide (Fig. 2J-L). The cells of the filament measured up to 147 µm long and 4 µm wide (Fig. 2M). Cells asexually reproduced by forming sporangia with usually four autospores (Fig. 2N-P). Neither sexual reproduction nor zoospores have been observed. Akinetes and akinete-like structures have been formed. In the strain SAG 2052, we observed the formation of codiolum-like unicells which did not show further development (Fig. 2Q, S). Akinetes were spherical to ovoid with thick cell wall (Fig. 2R). Above-mentioned genetic investigation, as well as detailed morphological analyses of all the studied *Rindifilum* strains, revealed the existence of a new genus and its type species. Description is provided below.

TAXONOMY

Phylum CHLOROPHYTA Reichenbach
Class ULVOPHYCEAE K.R.Mattox & K.D.Stewart
Order ULVALES Blackman & Tansley
Family Ctenocladiaceae Borzì

Rindifilum gen. nov.

Algae typically have a heterotrichous thallus. Most of the thallus are prostrate and the erect or semi-erect system remains poorly developed.

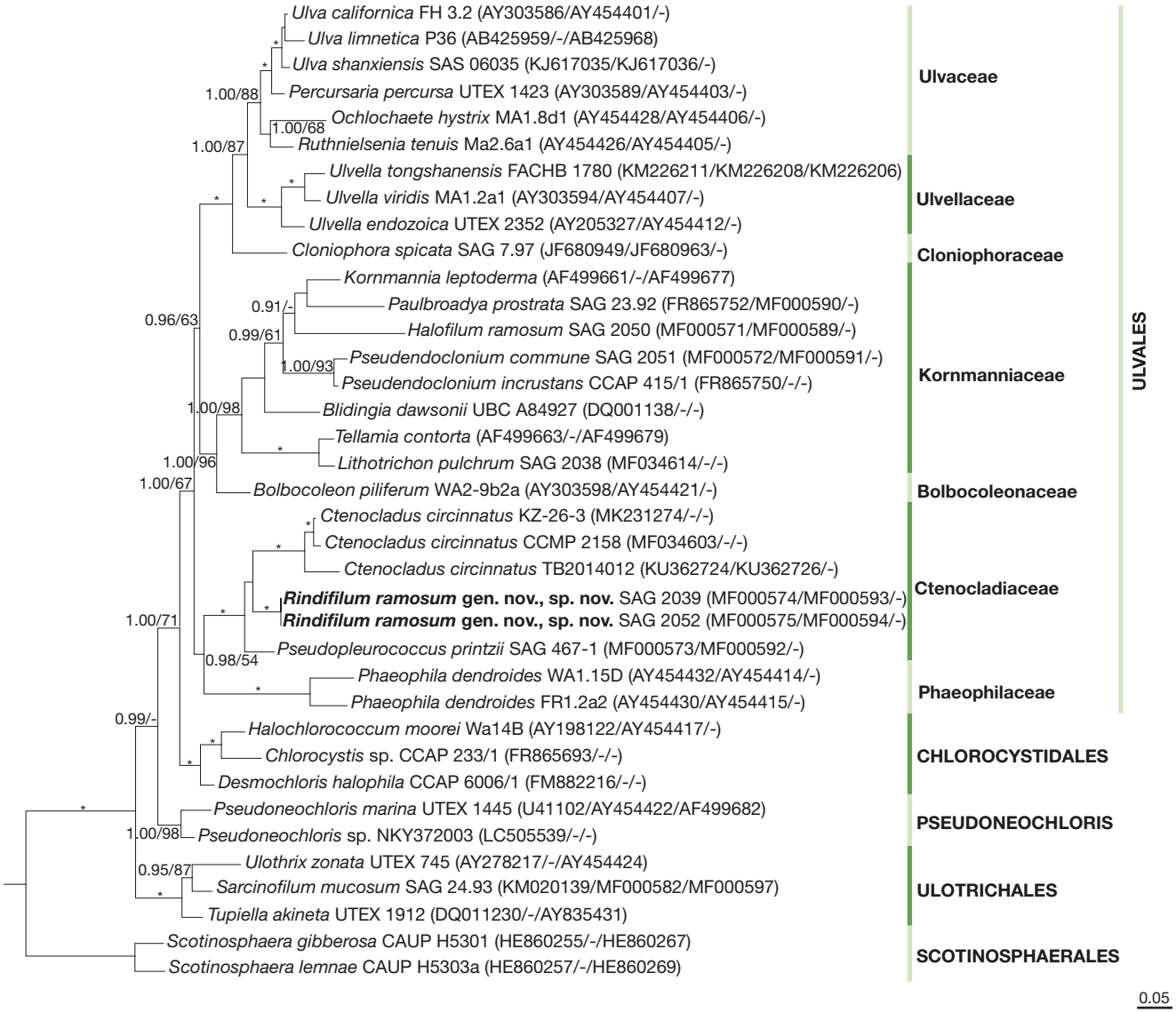

Fig. 1. — The phylogenetic position of *Rindifilum ramosum* gen. nov., sp. nov., obtained by a Bayesian inference analysis of the concatenated and partitioned 18S rDNA, *tuf*A, and *rbc*L dataset. **Asterisks** indicate the highest support values obtained by all three inference methods. GenBank accession numbers for the concatenated sequences (18S rDNA, *tuf*A and *rbc*L, respectively) accompany each species name. Newly obtained sequences are given in **bold**. Scale bar shows the estimated number of substitutions per site.

Both the prostrate and upright structures are distinctly irregular in shape. A prostrate very dense system of branched filaments gives rise to an upright system of thinner, also much branched, uniseriate filaments. Prostrate system is formed from rounded/spherical cells often gathered into cell packages. Cells are uninucleate, possess a parietal chloroplast and one pyrenoid. Asexual reproduction by two or four autospores. Sexual reproduction was not observed. Differs from other genera by 18S rRNA and *tuf*A sequences. Moreover, this genus differs in morphology by the combination of features as the "pear-shaped cells" that develop directly into a "hammer-shaped filament" (Fig. 2I-L).

Type species. — *Rindifilum ramosum* sp. nov.

Etymology. — This genus is named in honour of Dr Fabio Rindi, who contributed to the knowledge of green algae, including the order Ulvales.

Rindifilum ramosum gen. nov., sp. nov.

Rindifilum ramosum gen. nov., sp. nov. with the features of the genus. Thalli up to 0.5 mm consist of prostrate and erected/upright filaments. The vegetative cells of prostrate system are composed by rounded/spherical, pear-shaped or ovoid cells, up to 13.5 µm wide and 13.2 µm long. The pyriform cells of the prostrate system first develop into characteristic "hammer-shaped cells", up to 77.6 µm long and 33 µm wide. Later on, filaments up to 147 µm long and 4 µm wide are produced. The chloroplast is parietal, usually filling the cell, with a spherical pyrenoid.

Holotype. — Strain SAG 2052 permanently cryopreserved in a metabolically inactive state (cryopreservation in liquid nitrogen) in the Culture Collection of Algae of the Charles University in Prague (CAUP) as the item CAUP J 1701. Living cultures of the alga are maintained at the SAG, University of Göttingen, Germany, with

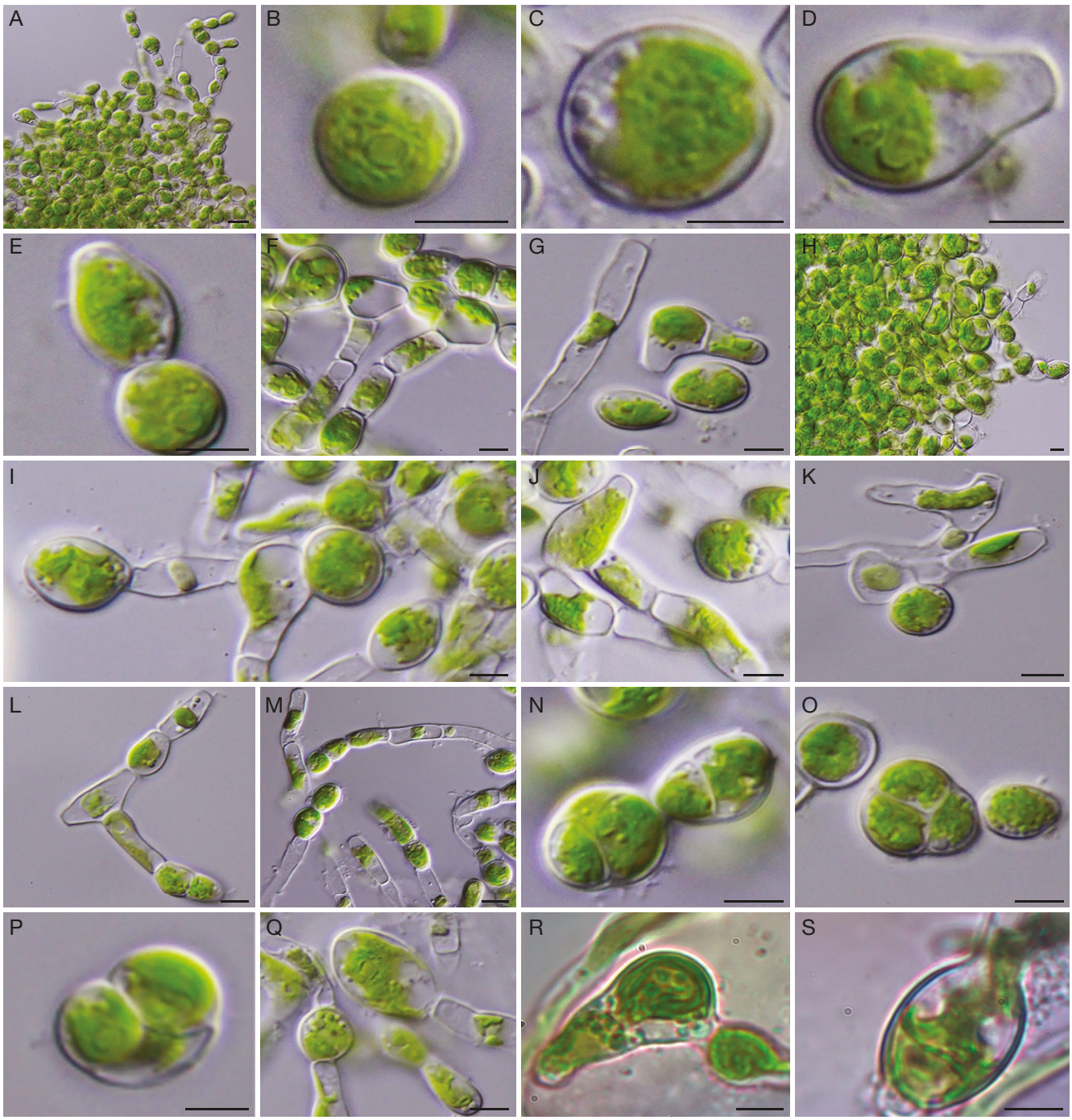

Fig. 2. — Light microscopic morphology of *Rindifilum ramosum* gen. nov., sp. nov. **A**-**Q**, SAG 2052; **R**, **S**, SAG 2039. Scale bars: 5 µm.

the strain code SAG 2052 (ex-type culture). Illustrations of the holotype are provided in Figure 3.

Type locality. — **Switzerland**. Davos Valley, bank of brook Drusatschabächel, 1570 m a.s.l., 46°49'18"N, 9°51'36"E (1000 m).

Habitat. — Photobiont from lichen *Verrucaria margacea* (Wahlenb.) Wahlenb.

Etymology. — L. neut. adj. *ramosum*, branching, referring to the morphology of the cells.

DISCUSSION

In recent molecular studies, a substantial amount of taxonomical work has been done. Many genera of the orders Ulvales and Ulotrichales have been reassessed (Darienko & Pröschold 2017; Škaloud *et al.* 2018) and both orders have been revised.

Within these orders, unrelated species may possess the similar morphology of cell packages more or less dispatching branched filaments (*Pseudopleurococcus* J.Snow; *Pseudendoclonium*; *Hazenia* H.C.Bold, etc.). This heterotrichous habit

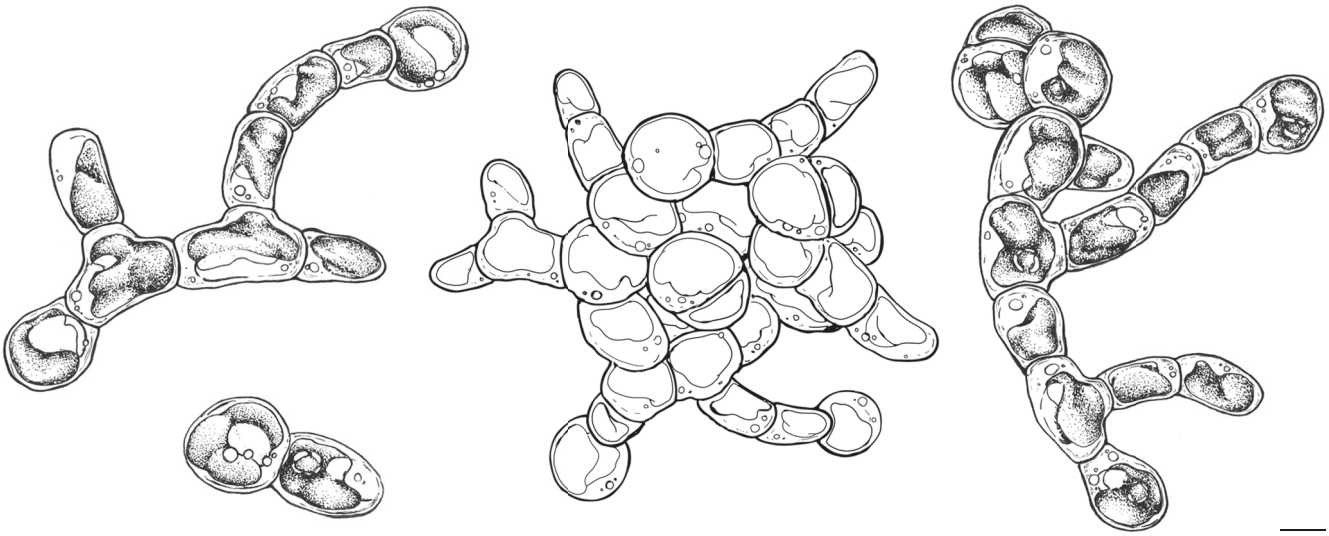

Fig. 3. — *Rindifilum ramosum* gen. nov., sp. nov. (SAG 2052). Scale bar: 5 µm.

of the thalli with irregularly branching vertical filaments arising from a prostrate basal disc of cells some of which are rhizoid-like in appearance and function may indeed represent an ancient evolutionary advantage in green algae (Mullins 2007). As such, this morphology apparently became a subject to convergent evolution and occurred in several lineages of ulvalean and ulotrichalean algae. This inevitably led to problems in recognizing and describing species showing the aforementioned (*Pseudendoclonium*-like) kind of morphology.

This problem is apparent also in the Ctenocladiaceae family. Until now, this family includes three genera: *Ctenocladus* Borzì, *Pseudopleurococcus* and *Spongioplastidium* Vischer (Škaloud *et al*. 2018). *Pseudopleurococcus printzii* (Vischer) Bourrelly was described in Vischer 1933. In 1970, Tschermak-Woess transferred this species to *Dilabifilum*. However, two years later, Bourrelly (1972) suggested its transfer to *Pseudendoclonium*. Then, Darienko & Pröschold (2017) assigned this species to the genus *Ctenocladus* despite morphological dissimilarity of these two species. Finally, Škaloud *et al*. (2018) restored its original status, *Pseudopleurococcus printzii*, based on the facts *Ctenocladus* and *Pseudopleurococcus* form two distinct clades within the family, and they are morphologically well distinguishable by branching pattern and akinete formation. *Ctenocladus circinnatus* Borzì was described by Borzì (1883) and it is known as a rare species. According to Darienko & Pröschold (2017), its taxonomical status remains unresolved due to morphological similarities with *Lochmiopsis* Woronichin & Popova and *Pseudopleurococcus printzii*, and due to lack of authentic material. However, the genus *Lochmiopsis* differs by the thallus form, which is attached to the base of a root-shaped cellular callus and present a branched turf around 0.2-10 mm in diameter (Woronichin & Popova 1929). In contrast, *Pseudopleurococcus* forms richly branched filaments, forming dense, radiating clusters 0.5-1 mm in diameter (Vischer 1933).

To date, no sequence data are available for *Spongioplastidium*. However, this genus is included in the Ctenocladiaceae based on morphological observations of Vischer (1933), who pointed to its similarity with the genus *Pseudopleurococcus*, considering the overall thallus appearance and the absence of flagellate reproductive cells.

The two SAG strains, SAG 2039 and 2052, originally described as *Dilabifilum* sp., are genetically distinct from the known taxa of Ctenocladiaceae. In addition, they are morphologically well discernible from molecularly yet uncharacterized *Spongioplastidium*, forming spongiose chloroplast with indistinct pyrenoid. Therefore, we are hereby proposing a new generic and specific name for these two strains: *Rindifilum ramosum* gen. nov., sp. nov. Although the morphological distinction of ulvalean and ulotrichalean filamentous algae is extremely difficult by a morphological similarity of several unrelated lineages and a high morphological plasticity of several species, the newly proposed genus *Rindifilum* gen. nov. exhibits a unique combination of morphological features making it distinct from all other green algae described so far. However, it is worth mentioning that each of these discriminating traits alone (e.g. pear-shaped cells and hammer-shaped filaments) was previously observed in morphologically similar genera. For example, Liu *et al*. (2019) have observed some cells similar of our "flask-shaped cells" in the *Lithotrichon* Darienko & Pröschold genus. They called these structure "enlarged cells" and described them as akinetes detached from the threads in the germination phase. Furthermore, a similar "hammer-shaped" filament observed for *Rindifilum* was detected also by Hodač *et al*. (2015) in a strain of *Pseudopleurococcus printzii* isolated from calcified biofilms of karstic streams. Moreover, the transformation of *Rindifilum* gen. nov. coccoid cells into sarcinoid cell packets later developing into sporangia was already observed in several *Pseudendoclonium* isolates by Johnson & John (1990). Finally, an analogous structure of *Codiolum*-stage was observed and described by O'Kelly *et al*. (2004) for a *Collinsiella tuberculata* Setchell & N.L.Gardner (ulotrichalean taxa) and others Ulvophyceae (Darienko & Pröschold 2017).

CONCLUSIONS

It is and will remain to be one of the goals of taxonomy to reduce the number of cryptic taxa which are phenotypically indistinguishable. Indeed, species delineation within the order Ulvales, is often difficult due to the lack of distinguishing morphological features. Furthermore, unrelated taxa share similar morphological futures, e.g. many species showing the *Pseudendoclonium*-like morphology will probably have to be revised in the future. This study deals with the taxonomic description of a new genus and species, *Rindifilum ramosum* gen. nov., sp. nov., belonging to the Ctenocladiaceae family. More culture studies, more sampling, and sequence data will supply important additional insights into the biology of *Rindifilum* gen. nov. Moreover, future studies likely will reveal additional new species of this genus.

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Authors' contributions
V.M. obtained the morphological data, assembled figure panels, and drafted parts of the manuscript and revised it. M.K. obtained and processed sequence data, and drafted parts of the manuscript. A.L. drafted parts of the manuscript, and revised it. P.S. conceived the study, performed the final phylogenetic analyses, and drafted parts of the manuscript. All authors read and approved the final manuscript.

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