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POLYPHYLY OF *CHAETOPHORA* AND *STIGEOCLONIUM* WITHIN THE CHAETOPHORALES (CHLOROPHYCEAE), REVEALED BY SEQUENCE COMPARISONS OF NUCLEAR-ENCODED SSU rRNA GENES[1]

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Previously published molecular phylogenetic analyses of the Chaetophorales (Chlorophyceae) suffered from limited taxon sampling (six genera with only a single species per genus). To test the monophyly of species-rich genera, and to analyze the phylogenetic relationships among families and genera in the Chaetophorales, we determined nuclear-encoded SSU rDNA sequences from 30 strains of Chaetophorales, performed phylogenetic analyses using various methods, and screened clades for support by unique molecular synapomorphies in the SSU rRNA secondary structure. The Schizomeridaceae and the weakly supported Aphanochaetaceae were recovered as basal lineages. The derived family Chaetophoraceae diverged into two clades: the "*Uronema* clade" containing unbranched filaments, and a sister clade designated as "branched Chaetophoraceae" comprising *Chaetophora*, *Stigeoclonium*, *Draparnaldia*, *Caespitella*, and *Fritschiella*. Although some terminal clades corresponded to genera described (e.g., *Caespitella* and *Draparnaldia*), other clades were in conflict with traditional taxonomic designations. Especially, the genera *Stigeoclonium* and *Chaetophora* were shown to be polyphyletic. The globose species *Chaetophora elegans* was unrelated to lobate *Chaetophora* spp. (e.g., *Chaetophora lobata*). Since the original description of *Chaetophora* referred to a lobate thallus organization, the latter clade represented *Chaetophora* sensu stricto. In consequence, *C. lobata* was designated as lectotype of *Chaetophora*. Two *Stigeoclonium* species, *Stigeoclonium farctum* Berthold and *Stigeoclonium* '*Longipilus*', diverged independently from the type species of *Stigeoclonium*, *Stigeoclonium tenue* (C. Agardh) Kütz. These results indicated that some commonly used taxonomic characters are either homoplasious or plesiomorphic and call for a reevaluation of the systematics of the Chaetophorales using novel morphological and molecular approaches.

Key index words: **18S rDNA*; Aphanochaete; Caespitella; Chaetophora*; Chaetophorales*; Draparnaldia*; *Fritschiella;* phylogeny; polyphyly; *Stigeoclonium***

Abbreviations: **CBC, compensatory base change; CRW, comparative RNA Web site and project; ERRD, the European Ribosomal RNA Database; ICBN, International Code of Botanical Nomenclature; INA, Index Nominum Algarum; MCMC, Markov chain Monte Carlo; ML, maximum likelihood; MP, maximum parsimony; NHS, nonhomoplasious synapomorphy; NJ, neighbor joining; OCC, Oedogoniales, Chaetopeltidales, and Chaetophorales; RAxML, randomized accelerated maximum likelihood; *rbc*L, gene encoding RUBISCO LSU; UTC, Ulvophyceae, Trebouxiophyceae, and Chlorophyceae**

The order Chaetophorales (Chlorophyceae) comprises predominantly freshwater taxa, and only a few genera are known from terrestrial habitats (Berthold 1878, Aziz and Islam 1962, Ettl and Gärtner 1995). Most members of the Chaetophorales have been described from temperate and tropical regions (Saxena 1962, Islam 1963, Printz 1964, Bourrelly 1972, Tupa 1974, Prescott 1979, Sarma 1986).

Traditionally, unbranched and branched filamentous as well as parenchymatous green algae containing a single parietal chloroplast per cell were

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classified within the order Chaetophorales sensu Wille (1901). Some authors (e.g., Hazen 1902, West and Fritsch 1927) considered unbranched genera as members of the Ulotrichales Borzi, regarding only branched taxa as genuine Chaetophorales. On the basis of ultrastructural analyses of mitosis-cytokinesis (summarized by Stewart and Mattox 1975) and motile cell ultrastructure (Manton 1964, Melkonian 1975, Floyd et al. 1980) in selected taxa, Silva (1982) redefined the order Chaetophorales to include unbranched or branched filaments (occasionally parenchymatous) with the ultrastructural characteristics of the Chlorophyceae (i.e., a collapsing telophase spindle, phycoplast, and cruciate microtubular flagellar root system with basal bodies exhibiting slight clockwise displacement without overlap) recognizing three families (Chaetophoraceae, Aphanochaetaceae, Schizomeridaceae). With this restricted circumscription, many of the traditional genera of the Chaetophorales (e.g., *Coleochaete*, *Chaetosphaeridium*, *Microthamnion*, and *Trentepohlia*) were transferred to other green algal orders or classes (summarized by Mattox and Stewart 1984, O'Kelly and Floyd 1984, Melkonian 1990). Molecular phylogenetic analyses performed since the 1990s corroborated these conclusions (Friedl and Zeltner 1994, O'Kelly et al. 1994; reviews by Melkonian and Surek 1995, Lewis and McCourt 2004, and Pröschold and Leliaert 2007).

Currently, the order Chaetophorales sensu John (1984) includes unbranched and branched filamentous taxa. Cytokinesis involves a phycoplast-associated cell plate; daughter cells remain in contact through plasmodesmata, thus resulting in true multicellularity of the filaments. During cytokinesis, centrioles remain in the position of the former spindle poles (i.e., they are not associated with the division plane). Asexual reproduction is by quadriflagellate zoospores. Of the three families, only the Chaetophoraceae contains several genera with highly differentiated morphologies and life histories (Godward 1942, Forest 1956, Abbas and Godward 1963, Printz 1964, Cox and Bold 1966, Sarma and Jayaraman 1980, John 1984, Michetti et al. 2010).

Within the Chaetophoraceae, definition of genera and species has been traditionally based on characters (e.g., extent of prostrate and upright systems, hair formation, degree of branching) that are known to be sensitive to environmental factors (Vischer 1933, Tupa 1974, Harding and Whitton 1978, Johnstone 1978, Francke and Ten Cate 1980, Gibson and Whitton 1987, Van Beem and Simons 1988, Pawlik-Skowrońska 2003). Some authors even hypothesized that the genera *Schizomeris*, *Caespitella*, and *Draparnaldia* might represent simplified growth forms of different *Stigeoclonium* spp., albeit without conclusive experimental evidence (Uspenskaja 1930, Cox and Bold 1966, Bourrelly 1972, Campbell and Sarafis 1972, Johnstone 1978). Nevertheless, the species-rich genera of the Chaetophoraceae are generally regarded as monophyletic—that is, *Chaetophora* (defined by mucilaginous thalli with long, multicellular hairs), *Stigeoclonium* (presence of prostrate and erect filament systems, the latter terminating in multicellular hairs, with only a thin layer of mucilage), and *Draparnaldia* (differentiation into an erect main axis and subordinate lateral branch systems, again terminating with multicellular hairs). Among these genera, *Stigeoclonium* and *Draparnaldia* contain the largest number of described species (each ~80), although some critical revisions of *Stigeoclonium* (Islam 1963, Cox and Bold 1966, Francke and Simons 1984, Simons et al. 1986) using the morphology of prostrate filaments and type of zoospore germination as major taxonomic characters have reduced the number of species to 23 (Islam 1963) or even only three (Simons et al. 1986).

The first molecular phylogenetic analysis of four genera/species of the Chaetophoraceae, using nuclear-encoded SSU rDNA sequence comparisons, confirmed the monophyly of this family as well as its placement within the class Chlorophyceae (Booton et al. 1998). Buchheim et al. (2001) added *Schizomeris* and *Aphanochaete*, thus ''completing'' the order Chaetophorales and, by analyzing nuclear-encoded SSU + partial LSU rDNA data, provided the first evidence for a sister-group relationship between Chaetophorales and Chaetopeltidales. Recent multigene analyses of partial and complete chloroplast genomes from six Chlorophyceae (including *Stigeoclonium*, *Floydiella*, and *Oedogonium*) gave further evidence for phylogenetic relationships among Oedogoniales, Chaetopeltidales, and Chaetophorales (Brouard et al. 2008, Turmel et al. 2008, 2009).

Although previous molecular phylogenetic analyses have been performed with six chaetophoralean genera, these analyses included only one or two (*Uronema*) representatives of each genus (Booton et al. 1998, Buchheim et al. 2001). Thus, the relationship between the families Schizomeridaceae and Aphanochaetaceae remains unknown, as well as the phylogenetic status of the species-rich genera of the Chaetophoraceae (*Chaetophora*, *Draparnaldia*, and *Stigeoclonium*). In the present contribution, we extended the taxon sampling and determined 30 new nuclear-encoded SSU rDNA sequences from the Chaetophorales (i.e., two strains of *Schizomeris*, five strains of the family Aphanochaetaceae, and 23 strains of the Chaetophoraceae). Molecular phylogenetic analyses unexpectedly revealed polyphyly of well-established genera and challenge the taxonomic value of their definitions based on morphological characters.

MATERIALS AND METHODS

Cultures, DNA isolation, gene amplification, and sequencing. Thirty strains of Chaetophorales were used for determination of new sequence data (Table S1 in the supplementary material; taxa in bold in Fig. 1). Sequence data of five of these strains were already available. However, these sequences

contained ambiguities as well as apparent errors when compared with the conserved 18S rRNA secondary structure and thus had to be resequenced. The following abbreviations were used for strain designations: CCALA, Culture Collection of Autotrophic Organisms, CZ (http://www.butbn.cas.cz/ccala/index.php); CCAP, Culture Collection of Algae and Protozoa (http://www.ccap.ac.uk/); SAG, Sammlung von Algenkulturen, University of Göttingen, Germany (http://www.epsag.uni-goettingen.de/html/sag.html); ACOI, Coimbra Collection of Algae (http://acoi.ci.uc.pt/). Four strains isolated for this study by L. C. (abbr. LC_L; Table S1) were identified using species descriptions of Pascher (1914), Islam (1963), and Starmach (1972); these strains are available through Culture Collection of Algae at the University of Cologne, Germany (CCAC; http://www.ccac.uni-koeln.de/). In addition, all other strains listed in Table S1 were investigated by LM (Olympus BX 51; Olympus, Tokyo, Japan) to ensure their correct identification. Total genomic DNA was extracted with the DNeasy Plant Mini Kit from Qiagen (Hilden, Germany; http://www.qiagen.com), used for gene amplification by PCR, and sequenced as described earlier Marin et al. (2003). Newly determined sequences of 30 strains are available under the accession numbers FN824371–FN824400 (Table S1).

Alignments and phylogenetic analyses. Together with 53 published sequences representing the chlorophyte classes Ulvophyceae, Trebouxiophyceae, and Chlorophyceae (UTC clade), 30 new chaetophoralean SSU rDNA sequences were subjected to manual alignment procedures guided by the conserved secondary structural architecture of the SSU rRNA (see below), using SeaView 4.2 (http://pbil.univ-lyon1.fr/software/seaview.html). BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi) with new SSU rDNAs as Query revealed eight previously published chaetophoralean homologs. Only four of them were integrated into our alignment, whereas the remaining sequences were omitted since we resequenced the same strains—that is, *Schizomeris* SAG 44.84; *C. lobata* CCAP 413/1; and *Stigeoclonium helveticum* CCAP 477/1, SAG 477-2, and CCALA 499 (Table S1 including taxonomic authors). The taxon sampling of nonchaetophoralean green algae for phylogenetic analyses was guided by preanalyses involving >300 Viridiplantae (trees not shown). To represent the diversity within the UTC clade, 49 basal, short-branched divergences of major subclades were selected.

We prepared two data sets: an 83 taxa alignment covering the UTC clade with 1,704 unambiguously aligned nucleotide positions and a smaller alignment containing 40 taxa of Oedogoniales (3), Chaetopeltidales (3), and Chaetophorales (34 taxa) (= OCC clade) with 1,743 unambiguously aligned characters.

Phylogenetic analyses were performed by several methods: randomized accelerated maximum likelihood (RAxML), maximum likelihood (ML), PhyML, distance (neighbor joining, NJ), maximum parsimony (MP), and Bayesian analyses. For ML, PhyML, and NJ analyses, the appropriate model of sequence evolution including model parameters was determined with ModelTest 3.7 (Posada 2008), resulting in GTR + I + G as selected by the Akaike criterion. The same model was used for RAxML and MrBayes with parameters estimated by these programs. Analyses used RAxML 7.0.3. (Stamatakis 2006), PAUP*4.0b10 (for ML, NJ, MP; Swofford 2000), PhyML (Guindon and Gascuel 2003), and MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003).

The tree topology in Figure 1 was obtained by heuristic search under the ML criterion, starting with an NJ tree. ML analyses reached the ''optimal'' tree after ~1,000 rearrangements, and therefore, ML bootstrap analyses (100 replicates, starting trees obtained by randomized sequence addition) could here be constrained toward 4,000 rearrangements per replicate. Distance (NJ, 1,000 replicates) and MP bootstrap analyses (1,000 replicates, each with 10 randomized sequence addition replicates) have not been constrained. For 1,000 RAxML bootstrap replicates, 10 multiple searches per replicate were defined. Bayesian analyses used two Markov chain Monte Carlo (MCMC) chains with 2,000,000 generations, and 640,000 generations were discarded as ''burn-in.'' Bootstrap values <50% and Bayesian posterior probabilities <0.95 were considered as ''no support.'' In Figure 2, the Bayesian analysis used 1,000,000 generations (20,000 = ''burn-in''); for other methods, see above.

Molecular synapomorphies of the order Chaetophorales and nested subclades. The order Chaetophorales was analyzed for molecular ''nonhomoplasious (= unique) synapomorphies'' (NHS) to define clades unambiguously. For this study, an NHS is defined as ''unique within the Viridiplantae.'' Methods to identify NHS using an exhaustive search has been described previously (Marin et al. 2003, 2005). To find NHS of Chaetophorales, a taxon-rich SSU rDNA alignment was used to obtain an MP tree that contained five glaucophytes, 28 rhodophytes, and 1,226 Viridiplantae (tree not shown). Synapomorphies were described by their positions (Fig. 2; Table 1) according to two different rRNA secondary structure models and nomenclature (helix numbering) systems: (i) the European Ribosomal RNA Database (ERRD; http://bioinformatics.psb.ugent.be/webtools/rRNA/) and (ii) the Gutell Lab comparative RNA Web site and project (CRW) site (http://www.rna.ccbb.utexas.edu/). The following ''reference'' secondary structure diagrams may be used to find NHS-type synapomorphies: ERRD, *Chlamydomonas reinhardtii* (http://bioinformatics.psb.ugent.be/webtools/rRNA/secmodel/Crei_SSU.html); CRW, *Escherichia coli* helix numbering system (http://www.rna.ccbb.utexas.edu/CAR/1A/Structures/h.16.b.E.coli.hlxnum.pdf); CRW, *Staurastrum* (http://www.rna.ccbb.utexas.edu/RNA/Structures/d.16.e.Staurastrum.sp.M752.pdf); CRW, *Apis mellifera*, including an *E. coli*–based helix numbering system (Gillespie et al. 2006).

Formalized analysis and ancestral state reconstruction of morphological characters in the Chaetophorales. For 40 taxa (as in Fig. 2), 18 morphological characters with two to five character states were combined within a data matrix in nexus format (Appendix S1 in the supplementary material). The data matrix was first used for a cladistic analysis via heuristic searches under the MP criterion implemented in PAUP, resulting in several equally parsimonious trees. Second, morphological data were mapped upon the 18S rDNA tree topology by loading the ML treefile from Figure 2, with branch lengths reflecting morphological character changes. In both cases, synapomorphy searches were performed as described above for molecular characters. Unique morphological synapomorphies for selected clades were then reanalyzed via ancestral state reconstruction implemented in MrBayes by mapping this morphological character onto Bayesian trees based on molecular data. In practice, morphological as well as molecular data of 40 taxa were combined within a partitioned nexus file (mixed ''standard/DNA'' model; files not shown), with the clade of interest defined by constraint, and used for ancestral state reconstruction as described in the manual of MrBayes. In all cases, both MCMC chains congruently supported only one character state by high probability values. Both probabilities were integrated as mean values (without burn-in generations) in Figure 4.

Topology tests of user-defined trees. User-defined trees were generated by modifying the ML topology of the OCC clade analysis (40 taxa), by (i) running ML analyses with constraints, using PAUP, or (ii) by collapsing or moving single branches through manual edition of the original treefile, using TreeView (Page 1996; http://darwin.zoology.gla.ac.uk/~rpage/treeviewx/). The original (best) treefile and six user-defined trees were compared by the approximately unbiased test, the

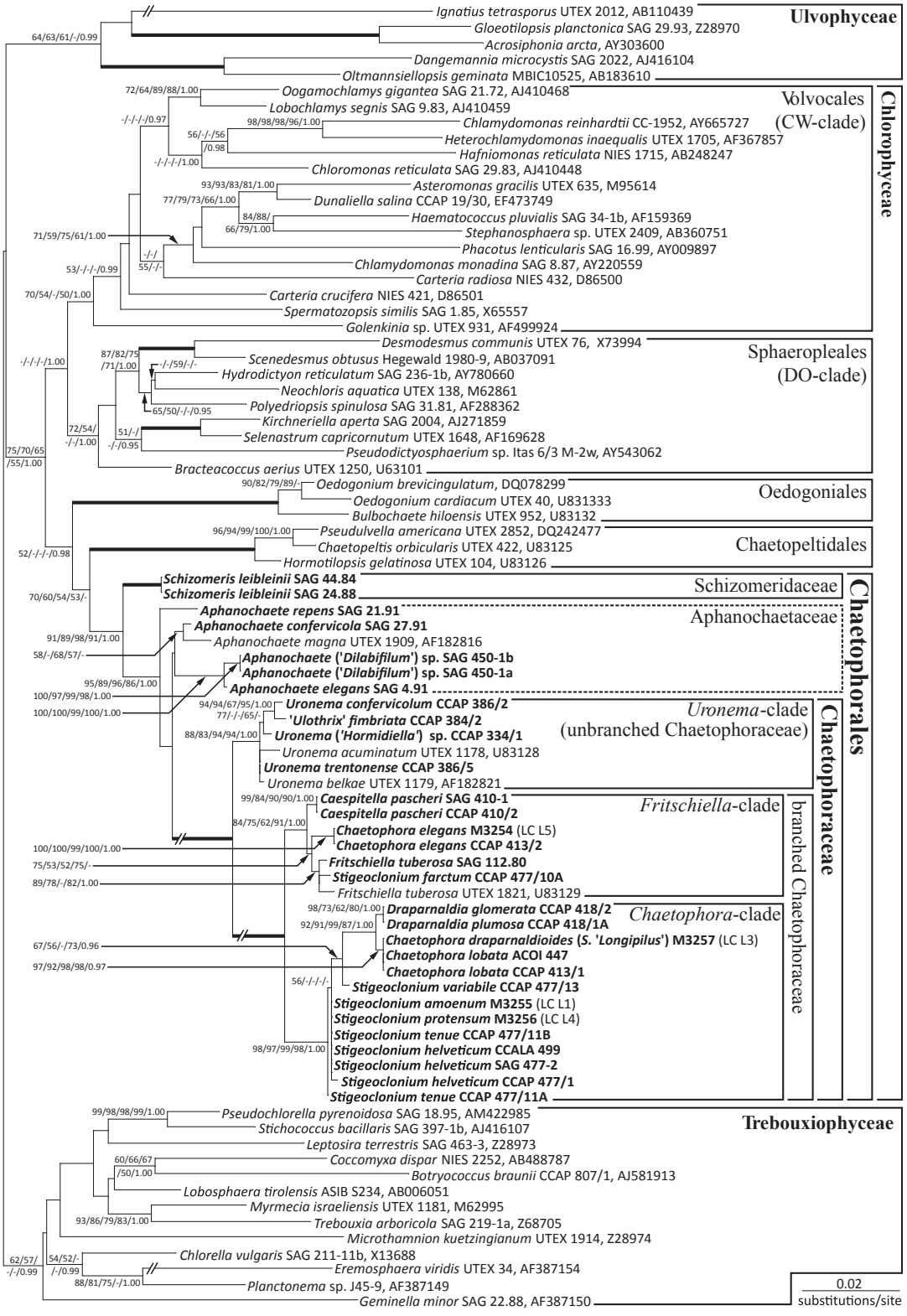


FIG. 1. Maximum-likelihood (ML) phylogeny of 83 Chlorophyta using nuclear-encoded SSU rDNA sequence comparisons; 1,704 aligned characters were used for analyses. Newly determined sequences are in bold (for accession numbers, see Table S1 in the supplementary material); taxon names are combined with strain designations and (for published sequences) accession numbers. Support values at branches are bootstrap partitions from randomized accelerated maximum likelihood (RAxML), ML, neighbor joining (NJ), maximum parsimony (MP), and Bayesian posterior probabilities. Bold branches were maximally supported by all methods (= 100/100/100/100/1.00). Interrupted branches (//) have been shortened to 50% of their original length. The branch separating the Trebouxiophyceae from the remaining classes was defined as root of the tree.

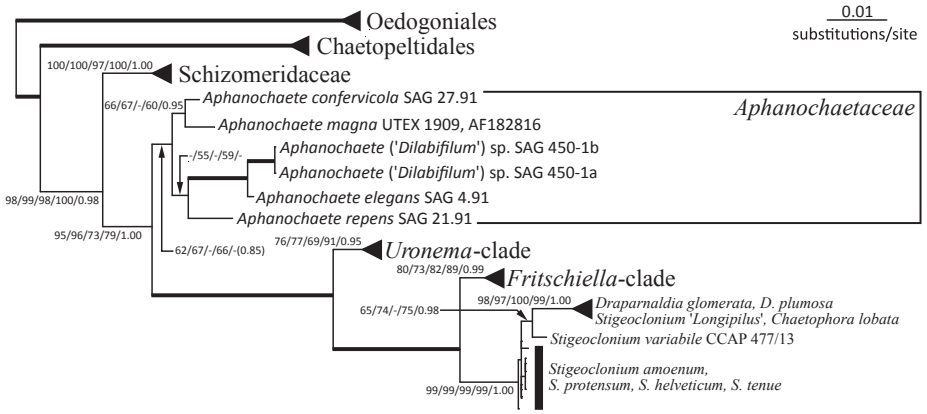


FIG. 2. Maximum-likelihood phylogeny of the Oedogoniales, Chaetopeltidales, and Chaetophorales (OCC clade) using 1,743 aligned characters of the 18S rRNA gene. Significances as in Figure 1. Note that the family Aphanochaetaceae forms a monophyletic divergence, in contrast to Figure 1, albeit with low support. The branching pattern in graphically reduced clades (triangles) is identical to Figure 1.

TABLE 1. Synapomorphy support for derived Chaetophorales and nested subclades.

Taxon/character	Evolutionary change	Characterization
Chaetophoraceae		
Helix 8: bp 4 [H122: bp 5]	A-U ==> G-C	Exceptions: G-C in *Friedlia irregularis* nom. nud. (Trebouxiophyceae)
Helix 24 [H655: bp 672/743]	[U-G ==> U○U]	U = NHS (A in *Dictyochloris* and *Halicoryne*, C in *Heterochlamydomonas*)
Branched Chaetophoraceae		
Spacer Helices 9-10 [spacer H144 -H184a]	8 nt's ==> 9 nt's [6 nt's ==> 7 nt's]	NHS within Chlorophyta
Helix E10_1 [H184b-1]: bp 5	U-G ==> A-U	A = NHS within Chlorophyceae
Internal loop of Helix 13 [H289] (reverse strand): nt 2	A ==> G	G = NHS
Chaetophora clade		
Helix 18 [H441]: bp 1	G-C ==> U-A	U-A = NHS
Draparnaldia, Chaetophora lobata, Chaetophora draparnaldioides (= *Stigeoclonium* '*Longipilus*')		
Spacer Helices 18-19 [H441 -H500]: nt 3	G ==> U	U = NHS within Chlorophyceae
C. lobata, C. draparnaldioides (= *S.* '*Longipilus*')		
Helix 43 [H1118]: sixth from last bp	U-A ==> C-G	C-G = NHS within Chaetophorales
Chaetophora elegans		
Helix 24: last bp [H655: bp 680/736]	A-U ==> U-A	U-A = NHS
Fritschiella tuberosa, Stigeoclonium farctum		
Helix 25 [H673]: bp 2	G-C ==> A-U	Almost unique within Chlorophyceae (A-U in *Chlamydomonas sordida*)

Nomenclature of rRNA secondary structures after (i) the European Ribosomal RNA Database (http://bioinformatics.psb.ugent.be/webtools/rRNA/), and (ii) [nomenclature in brackets] the Gutell Lab comparative RNA Web site and project site (http://www.rna.ccbb.utexas.edu/); for reference structure diagrams, see Materials and Methods. Unique signatures without known parallel changes in other Viridiplantae are flagged as nonhomoplasious synapomorphy (NHS).

Shimodaira–Hasegawa test, and the Kishino–Hasegawa test using CONSEL (Shimodaira and Hasegawa 2001, Shimodaira 2002; http://www.is.titech.ac.jp/~shimo/prog/consel/).

RESULTS

The Chaetophorales in the context of the UTC clade. To analyze the Chaetophorales together with representatives of all derived classes of the Chlorophyta (i.e., the UTC clade), a nuclear-encoded SSU rDNA alignment consisting of 83 sequences (13 Trebouxiophyceae, five Ulvophyceae, and 65 Chlorophyceae, including 34 Chaetophorales) was used for tree reconstructions (Fig. 1). Within the Chlorophyceae, the Chaetophorales showed a sister-group relationship with the Chaetopeltidales, supported by low-to-moderate-bootstrap percentages (70% by RAxML), but without Bayesian support (Fig. 1). These two orders formed a nonsupported assemblage with the Oedogoniales, representing the OCC clade sensu Turmel et al. (2008).

The monophyly of the Chaetophorales was strongly supported, as was the basal divergence of *Schizomeris leibleinii* (= family Schizomeridaceae) as sister of the remaining Chaetophorales (families Aphanochaetaceae and Chaetophoraceae; Fig. 1). The six strains of the genus *Aphanochaete* investigated were not recovered as monophyletic (Fig. 1); instead they diverged paraphyletically as three independent branches whose interrelationships could not be resolved in the global UTC analysis: (i) *Aphanochaete repens*, (ii) *Aphanochaete confervicola* and *Aphanochaete magna*, and (iii) *Aphanochaete elegans* together with two strains (SAG 450-1a, SAG 450-1b) previously labeled ''*Dilabifilum* sp.'' (Fig. 1,

Table S1). In contrast, the family Chaetophoraceae gained maximal support by all methods applied and showed a basal split into the well-supported ''*Uronema* clade'' (consisting of unbranched filamentous taxa) and the remaining genera (5) of the Chaetophoraceae (termed ''branched Chaetophoraceae'' in Fig. 1), which gained maximal support. Relationships within the ''*Uronema* clade'' (six strains investigated) remained largely unresolved, and only the monophyly of ''*Ulothrix*'' *fimbriata* and *Uronema confervicolum* (the type species of *Uronema*) was strongly supported (Fig. 1). The ''branched Chaetophoraceae'' diverged into two well-supported sister clades. One was named the ''*Chaetophora* clade'' since it contained *C. lobata*, the lectotype species of *Chaetophora* (designated here). The other clade was labeled the ''*Fritschiella* clade'' because it included the type species of *Fritschiella*, *Fritschiella tuberosa* (Fig. 1). Interestingly, the two most common and well-known genera of the Chaetophorales, *Stigeoclonium* and *Chaetophora*, were dispersed over these two clades, intermixed with the remaining genera, and therefore, both represented polyphyletic taxa (Fig. 1). More specifically, two *Stigeoclonium* spp., *S. farctum* and *S.* '*Longipilus*' (an illegitimate name, synonymous to *Chaetophora draparnaldioides*; see below), were clearly separated from the type species of the genus (*S. tenue*), and two strains of *C. elegans* belonged to the *Fritschiella* clade and were thus unrelated to *C. lobata* (Fig. 1).

Seven strains of *Stigeoclonium*, representing four species (*S. tenue*, *Stigeoclonium amoenum*, *S. helveticum*, *Stigeoclonium protensum*), diverged at the base of the ''*Chaetophora* clade,'' albeit without synapomorphic signals in the SSU rDNA and thus without a common branch. Therefore, we cannot presently recognize a ''*Stigeoclonium* s. str. clade'' (*S. tenue* is the type species). Although sequence diversity among these strains was low, *S. helveticum* CCAP 477/1 differed from two putatively identical strains (SAG 477-2, CCALA 499, the same isolate from Vischer; Table S1) by three substitutions in the SSU rRNA, two of which represented a single autapomorphic compensatory base change (CBC) in a conserved base pair of Helix 29 (the next to last pair, usually U-A) toward C-G (not shown). The well-supported ''crown'' lineage of the ''*Chaetophora* clade'' comprised *Draparnaldia*, *S.* '*Longipilus*' (= *C. draparnaldioides*), and *C. lobata* (Fig. 1). *S.* '*Longipilus*' and both strains of *C. lobata* studied had identical SSU rDNA sequences; however, it should be noted that the morphology of *S.* '*Longipilus*' strain M3257 corresponded to its description in the literature and therefore differed from *C. lobata* (results not shown). Although *S.* '*Longipilus*' belonged to the earliest species of *Stigeoclonium* described (Kützing 1843, 1845), its original name was *C. draparnaldioides*, also described by Kützing (1834) 9 years before he established the genus *Stigeoclonium* (http://ucjeps.berkeley.edu/INA.html). In light of its phylogenetic position and due to nomenclatural reasons (unnecessary change of the epithet by Kützing), we consider *S.* '*Longipilus*' to be a synonym of the earlier described *C. draparnaldioides* (Fig. 1). Another species of *Stigeoclonium* (*Stigeoclonium variabile*) branched in an intermediate position between the basal *Stigeoclonium* polytomy, and the ''crown'' lineage, though this position received only moderate support (Fig. 1).

The ''*Fritschiella* clade'' comprised *Caespitella pascheri*, *C. elegans*, both represented by two strains with identical SSU rDNA sequences, and a subclade consisting of *F. tuberosa* and *S. farctum*. The moderate sequence diversity in the latter subclade (five positions differed between CCAP 477/10A and SAG 112.80) is reflected in the considerably different morphology of *F. tuberosa* and *S. farctum* (results not shown).

Phylogenetic analysis of the OCC clade. Thirty-four Chaetophorales, together with their closest relatives (Chaetopeltidales, Oedogoniales), were analyzed without other green algae to increase the number of aligned characters (1,743 vs. 1,704 in the global analysis) and to test the robustness of chaetophoralean clades in a modified taxonomic environment (i.e., OCC clade only) (Fig. 2). Tree topology and support values were largely congruent (compare Figs. 1 and 2); however, we encountered one prominent difference to the global analysis, concerning *Aphanochaete* (= family Aphanochaetaceae). Whereas the six strains of *Aphanochaete* were not resolved as monophyletic in the global analysis (Fig. 1), they formed a weakly supported clade in the OCC analysis (≥62% support by RAxML, ML, and MP; Fig. 2), thus rendering the family Aphanochaetaceae monophyletic. When the OCC analysis was repeated with only 1,704 characters (as in Fig. 1), *Aphanochaete* again collapsed and formed paraphyletic subclades (tree not shown).

Synapomorphy support for clades. To further substantiate chaetophoralean clades and to present molecular characters for future taxonomic revisions, all clades/branches of the Chaetophorales were analyzed for presence of unique (= nonhomoplasious) synapomorphies in the nuclear-encoded SSU rRNA molecule. The order Chaetophorales as well as the basally branching families Schizomeridaceae and Aphanochaetaceae gained no support by molecular synapomorphies. However, the derived family Chaetophoraceae and six of its subclades could be unambiguously characterized by NHS, as summarized in Table 1 and Figure 3. Only two subclades lacked NHS support, the ''*Uronema* clade,'' and the ''*Fritschiella* clade.'' Most synapomorphies listed in Table 1 represented CBCs in rRNA helices, characterizing six clades unambiguously. Three unique synapomorphies occurred in single-stranded parts of the rRNA molecule, that is, substitutions in internal loop and spacer regions, or an insertion (Table 1).

The polyphyly of the genera *Chaetophora* and *Stigeoclonium* was clearly mirrored by several unique synapomorphies. Among several branches that

separate *C. lobata* from *C. elegans*, four were not only supported by high bootstrap values/posterior probabilities (Fig. 1), but also by unique synapomorphies (Table 1). *C. lobata* together with closely (*C. draparnaldioides*) and more distantly related taxa (*Draparnaldia*) as well as the whole ''*Chaetophora* clade'' all gained NHS support to the exclusion of *C. elegans* (Table 1, Fig. 3). Moreover, both strains of *C. elegans* displayed a unique CBC in Helix 24, representing an NHS to the exclusion of all Viridiplantae including *C. lobata* (Fig. 3). Similarly, the type species of *Stigeoclonium* (*S. tenue*) was clearly differentiated from *S.* '*Longipilus*' (both members of the ''*Chaetophora* clade'') and also from *S. farctum* (within the ''*Fritschiella* clade'') by NHS (Table 1, Fig. 3). Synapomorphy analyses of user-defined trees that enforced either *Chaetophora* or *Stigeoclonium* as monophyletic (see below) revealed absence of any nuclear-encoded SSU rRNA synapomorphies that would support these artificially generated clades.

Evolution of morphological characters within the Chaetophorales. Comparing results from cladistic analyses of morphological characters alone (Fig. 4A) with the tree topology that was favored by molecular data (Figs. 2 and 4B) revealed several clades, which were congruently recovered by both data sets (1–8 in Fig. 4). However, several branches of the cladistic tree were in conflict with 18S rDNA analyses (dashed lines in Fig. 4A). Only three clades, which were highly supported by 18S rDNA data (dashed lines in Fig. 4B), showed incongruence with the morphological tree. First, the Chaetophoraceae (clade 9 in Fig. 4) gained no support by cladistic analyses (Fig. 4A), although pyrenoid structure was revealed as a unique morphological synapomorphy of this clade (Fig. 4C). Second, the clade comprising *Draparnaldia* and two species of *Chaetophora* (excluding *C. elegans*; clade 10 in Fig. 4B), suggesting two independent origins of thick mucilage within the Chaetophoraceae, was in conflict with the cladistic analysis (Fig. 4A), which favored monophyly of all *Chaetophora* species and a unique gain of thick mucilage. Third, the *Fritschiella*/*S. farctum* clade (no. 11 in Fig. 4B), displaying secondarily reduced branching of filaments, formed two independent lineages at the base of the ''branched Chaetophoraceae'' clade in Figure 4A. It should be noted that both analyses (Fig. 4, A and B) recovered *Stigeoclonium* as nonmonophyletic, in contrast to the conflicting case of *Chaetophora*. Bayesian ancestral state reconstruction of morphological synapomorphies of selected clades (4, 9, 10, and 11 in Fig. 4) largely confirmed interpretations that resulted from cladistic synapomorphy searches (summarized in Fig. 4C) by high probability values ($P > 0.94$), except for lower probabilities for the only synapomorphy of the Chaetophoraceae (clade 9). In addition, the ''mucilage'' character was investigated by Bayesian ancestral state reconstruction for the common ancestor of the ''branched Chaetophoraceae'' lineage, resulting in high support ($P = 0.99/0.99$) for the character state ''thin mucilage,'' which often was used for defining a single genus (i.e., *Stigeoclonium*; see Discussion).

Topology tests of user-defined trees. To reevaluate the major results of this study by ML-based topology tests, we generated artificial (user-defined) trees, which addressed alternative hypotheses to our results. Various topology tests (au, kh, sh) were applied to estimate whether user-defined trees are ''significantly worse'' than the ''best'' tree at the significance level 0.05 (Table 2).

Two user-defined trees were not significantly worse than the original tree and thus could not be rejected by statistical comparisons using the SSU rDNA data: (i) the tree with the common branch of *Aphanochaete* collapsed, and (ii) the tree ''*Stigeoclonium* monophyletic A'' with an enforced clade comprising the four ''main'' *Stigeoclonium* species together with *S. variabile* (Table 2). In contrast, trees enforcing a monophyletic genus *Stigeoclonium* including the more-distant species (*S. farctum* and/or *S.* '*Longipilus*'), as well as trees enforcing a monophyletic genus *Chaetophora* (*C. lobata*, *C. draparnaldioides* and *C. elegans*), were rejected by nearly all topology tests with *P*-values well below 0.05 (Table 2). Therefore, topology tests clearly confirmed the nonmonophyly of the genera *Stigeoclonium* and *Chaetophora*.

Taxonomic revision.

Chaetophora Schrank 1783, Naturforscher (Halle) 19: p. 125.

Lectotype (designated here): Chaetophora lobata Schrank 1783, Naturforscher (Halle) 19: p. 125, tab. VII, figs. 2, 3.

Synonyms: Chaetophora incrassata (Hudson) Hazen 1902, Mem. Torrey Bot. Club 11: p. 214 (Basionym: *Ulva incrassata* Hudson 1778, Flora Anglica, Tomus II: p. 572; non *U. incrassata* O. F. Müller 1775, Flora Danica 4, fasc. II: p. 7, tab. DCLIII); *Chaetophora endiviifolia* (Roth) Agardh 1812, Dispositio Algarum Sueciae p. 42 [Basionym: *Rivularia endiviaefolia* Roth 1798, in Roemer (ed) Archiv für die Botanik 1/3: p. 51]; *Chaetophora cornudamae* (Roth) Bory de Saint-Vincent 1823, Dict. Class. Hist. Nat. 3: p. 431 (Basionym: *Rivularia cornudamae* Roth 1797, Catalecta Botanica 1: p. 212, tab. VI, fig. 2).

DISCUSSION

In the present study, we demonstrated that the most common chaetophoralean genera, *Chaetophora* and *Stigeoclonium*, as currently circumscribed, are polyphyletic taxa. Evidence for this conclusion included not only branching patterns and support values in phylogenetic trees but also unique synapomorphies in the SSU rRNA molecule. In addition, user-defined trees generated to restore monophyly of *Chaetophora* and *Stigeoclonium* were significantly worse than the best tree confirming polyphyly of these taxa based on topology tests. Although the

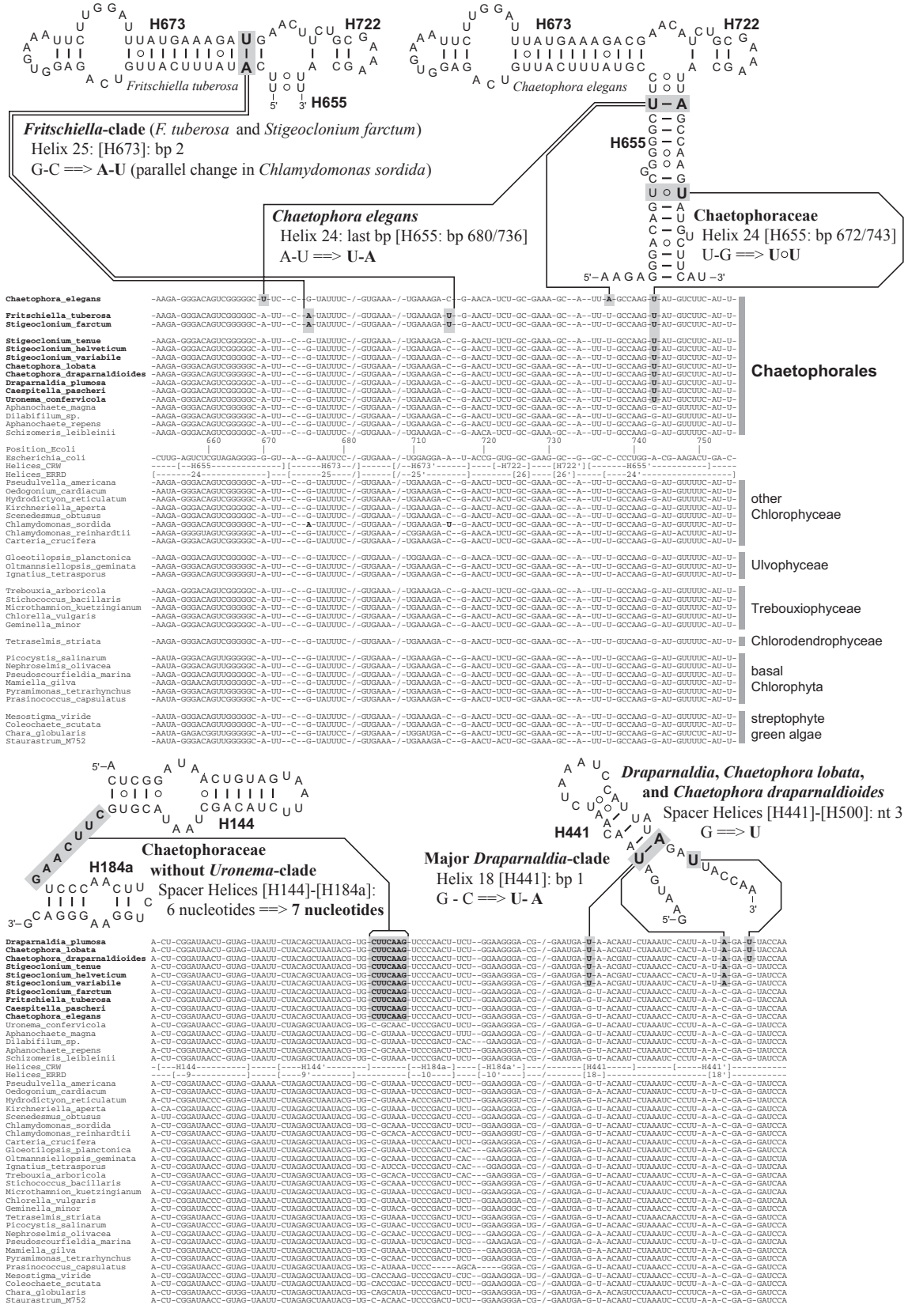


FIG. 3. Evidence for the polyphyly of *Chaetophora* and *Stigeoclonium* by unique molecular synapomorphies characterizing selected clades within the Chaetophoraceae. Secondary-structure diagrams based upon the first taxon intercepted by black lines. For details, see Table 1.

TABLE 2. Topology tests upon six user-defined trees derived from Figure 2 (= best tree), with a focus on the genera *Aphanochaete*, *Chaetophora*, and *Stigeoclonium*.

	Method	Observation	au	kh	sh
Best tree (Fig. 2)		−2.7	0.857	0.844	0.986
Aphanochaete not monophyletic[a]	TreeView	2.7	0.170	0.156	0.801
Stigeoclonium monophyletic A[b]	PAUP	3.2	0.316	0.278	0.791
Stigeoclonium monophyletic B[c]	PAUP	29.7	0.016*	0.018*	0.103
Stigeoclonium monophyletic C[d]	PAUP	129.6	<0.000*	0*	0*
Chaetophora monophyletic A[e]	PAUP	95.0	<0.000*	0*	0*
Chaetophora monophyletic B[f]	TreeView	108.0	<0.000*	0*	0*

User-defined trees were either generated manually (TreeView), or by running maximum-likelihood (ML) analyses with constraints, using PAUP (see Materials and Methods). Trees are characterized by the observed difference in −ln Lik (obs), and *P*-values of the approximately unbiased test (au), Kishino–Hasegawa test (kh), and the Shimodaira–Hasegawa test (sh). *P*-values <0.05, indicating significant rejection at the 5% level, are flagged with an asterisk (*).

[a] Common branch of *Aphanochaete* collapsed.

[b] ''Main'' *Stigeoclonium* (= *Stigeoclonium tenue*, *amoenum*, *helveticum*, *protensum*) plus *Stigeoclonium variabile*.

[c] ''Main'' *Stigeoclonium* plus *S. variabile* and *Stigeoclonium* '*Longipilus*'.

[d] ''Main'' *Stigeoclonium* plus *S. variabile*, *S.* '*Longipilus*', and *Stigeoclonium farctum*.

[e] *Chaetophora elegans* sister of *Chaetophora lobata/draparnaldioides* in the *Chaetophora* clade.

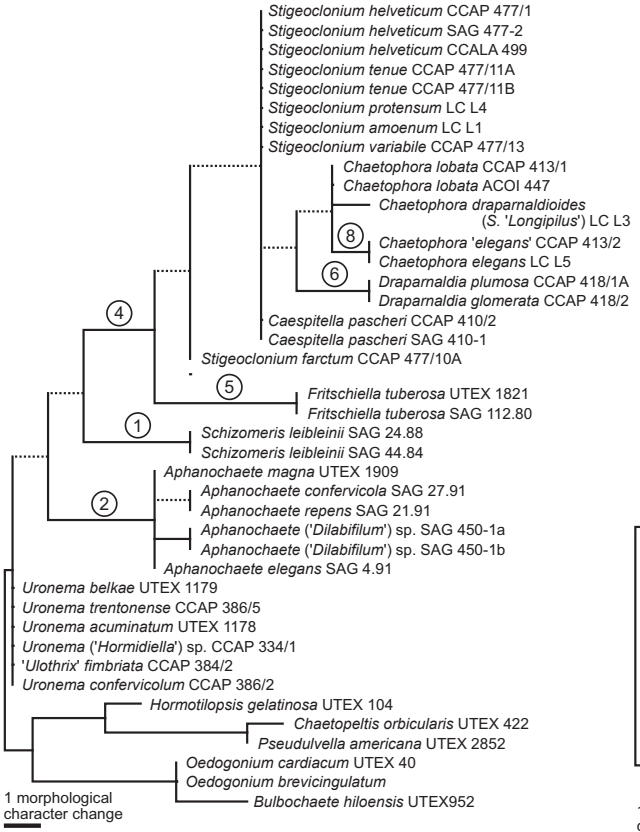
[f] *Chaetophora elegans* sister of *C. lobata/draparnaldioides* in the *Fritschiella* clade.

Chaetophorales have been studied for almost two centuries, the concepts and definitions of ''old'' genera (*Chaetophora*, *Draparnaldia*, and *Stigeoclonium*), established by Schrank (1783), Bory de Saint-Vincent (1808), and Kützing (1843), respectively, have remained virtually unchanged since about 1850. In particular, *Chaetophora* and *Stigeoclonium* have generally been regarded as two separate taxonomic units, and we found almost no reference for the notion that either of these genera could be para- or polyphyletic and may need revision at the genus level (see, however, Forest 1956). Notably, species of *Chaetophora* have not been reassigned to other genera, except for the transfer of marine species to phaeophyte and rhodophyte genera—for example, *Chaetophora maritima* to *Ectocarpus* (Rosenvinge 1910) and *Kolderupia* (Lund 1959), *C. marina* to *Leathesia* (Decaisne 1842), *Chaetophora lumbricalis* to *Nemalion* (Bornet 1892), and *Chaetophora pellita* to *Cruoria* (Fries 1835). Newly discovered taxa with a *Stigeoclonium*-like morphology were generally attributed to *Stigeoclonium*. Two genus descriptions by Pascher (1905) and Vischer (1933) represent the only exceptions. Pascher (1905) transferred *Stigeoclonium terrestre* to his new genus *Iwanoffia*, due to its terrestrial lifestyle and biflagellate zoospores; unfortunately, the phylogenetic position of *Iwanoffia* cannot be investigated since no cultures are available. Vischer (1933) discovered and isolated a new *Stigeoclonium*-like taxon with unusual grasslike growth and, rather than describing it as a new species of *Stigeoclonium*, created the new genus *Caespitella* (type: *C. pascheri*). Interestingly, this genus was not accepted by later authors (Fritsch 1935, Cox and Bold 1966, Shyam and Sarma 1980) and merged with *Stigeoclonium*, the species designated as *S. pascheri* by Cox and Bold (1966). Fortunately, we could investigate the authentic strain of *C. pascheri* (i.e., the culture established by Vischer). Our results based on molecular synapomorphies as well as phylogenetic reconstructions clearly favor Vischer's concept of *Caespitella* as a separate genus, independent of *Stigeoclonium*.

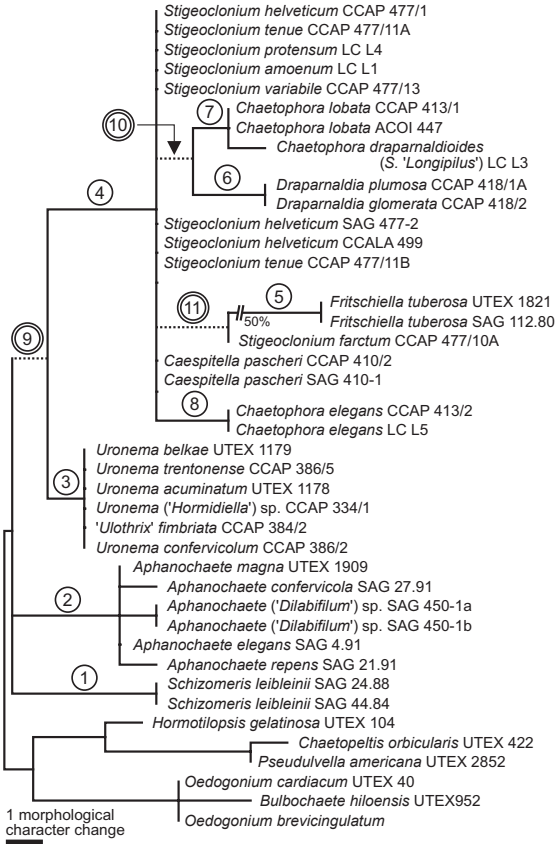
Polyphyly of Chaetophora *and its taxonomic history.* The polyphyly of ''old'' genera in their broad meaning (sensu lato; s. l.) is often related to the choice of plesiomorphic or homoplasious diagnostic characters (e.g., in *Pleurastrum*, *Chamydomonas*, *Chloromonas*; Friedl 1996, Pröschold et al. 2001). When polyphyly of a genus is demonstrated, it is necessary to select one clade as ''genus sensu stricto'' (s. str.) by (i) identifying the type species of the genus in question and (ii) analyzing morphological characters that were used in the original diagnosis. In the present study, we established *Chaetophora* as polyphyletic, diverging into one clade with *C. elegans* forming globose thalli, and another clade containing *C. lobata* in which the thallus has an irregularly lobed shape (Schrank 1783, Hazen 1902).

The taxonomic history of *Chaetophora* is very confused. Currently, it is even unclear which *Chaetophora* species must be regarded as type, and three databases (Index Nominum Algarum [INA], http://ucjeps.berkeley.edu/INA.html; Index Nominum Genericorum, http://botany.si.edu/ing/; Algaebase, http://www.algaebase.org/) yield three different results. When *Chaetophora* was established (Schrank 1783), the genus contained only two species, *Chaetophora globosa* and *C. lobata*, without designation of a type species. However, the first of these species resulted from transferring *Conferva stellaris* Müller to *Chaetophora* as *C. globosa* (as an illegitimate change of the epithet—the valid name would have been ''*Chaetophora stellaris*''; Art. 11.4. in International Code of Botanical Nomenclature [ICBN]; http://ibot.sav.sk/icbn/main.htm). Hazen (1902) identified *C. globosa* as lectotype species of *Chaetophora*, thereby following the ''American Code,'' that is,

A.) One of 50 most parsimonious trees resulting from MP analyses of 18 morphological characters (total tree length = 40 character changes)



B.) ML tree topology resulting from 18S rDNA analyses (see Fig. 2) with branch lengths estimated upon 18 morphological characters (total tree length = 44 character changes)



C.)

clade 1 (*Schizomeris*):
- **filament organization**: uniseriate ==> multiseriate erect system
- **pyrenoid structure**: matrix with cytoplasmic channels ==> traversed by several undulating thylakoids
- **flagellar root system**: cruciate with 5-2-5-2 microtubules ==> 5-5-5-5 microtubules

clade 2 (*Aphanochaete*):
- **vegetative growth form**: only with erect filaments ==> predominantly prostrate
- **pyrenoid structure**: matrix with cytoplasmic channels ==> penetrated shallowly by a few thylakoids
- **hairs**: absent ==> unicellular hairs, basally swollen (parallel: *Bulbochaete*)

clade 3 (*Uronema*-clade):
- **branching**: branched (with primary branches) ==> unbranched (parallel: *Hormotilopsis*, *Oedogonium*)

clade 4 (branched Chaetophoraceae):
- **vegetative growth form**: only with erect filaments ==> well developed erect and prostrate systems (p = 0.95 / 0.96)
- **branching**: branched (with primary branches) ==> with more than two levels of branching (changed: *Fritschiella*-clade) (p = 0.94 / 0.94)

clade 5 (*Fritschiella*):
- **filament organization**: uniseriate ==> usually uniseriate, but occasonally with multiseriate prostrate system
- **mucilage**: thin mucilage ==> without mucilage
- **orange/red secondary carotenoids**: absent ==> present
- **habitat**: attached in freshwater ==> also subaerial on soil

clade 6 (*Draparnaldia*):
- **chloroplasts**: parietal with straight edge ==> parietal with serrate edge
- **cell shape in the main axis of filaments**: cylindrical ==> barrel-shaped

clade 7 (*Chaetophora lobata* / *draparnaldioides*):
- **colony shape**: no solid macroscopic colonies ==> solid lobate colonies

clade 8 (*Chaetophora elegans*):
- **colony shape**: no solid macroscopic colonies ==> solid globose colonies

clade 9 (Chaetophoraceae):
- **pyrenoid structure**: matrix with a few to several thylakoids ==> matrix bounded by one peripheral thylakoid (p = 0.71 / 0.75)

clade 10 (*C. lobata*, *C. draparnaldioides*, *Draparnaldia*):
- **mucilage**: thin mucilage ==> always with thick mucilage (p = 0.97 / 0.96) (thick mucilage covering only older thalli in *C. draparnaldioides*) (parallel change: *C. elegans*)

clade 11 (*Fritschiella*, *Stigeoclonium farctum*):
- **branching**: with more than two levels of branching ==> with primary and secondary branches (p = 0.97 / 0.98) (parallel change: *Aphanochaete* strains SAG 450-1a, SAG 450-1b)

FIG. 4. Cladistic analysis of morphological character evolution in the Chaetophorales. (A) Maximum-parsimony (MP) analysis of morphological characters using PAUP. (B) Morphological data matrix (Appendix S1 in the supplementary material) mapped upon the branching pattern that resulted from maximum-likelihood (ML) analyses of 18S rDNA data (treefile of Fig. 2). Note that all divergences collapsed that displayed no morphological character changes (e.g., the *Fritschiella* clade and the *Chaetophora* clade resolved in Fig. 2). Dashed lines in the morphological tree indicate noncongruence with the molecular tree and vice versa. Encircled numbers 1–9 indicate clades supported by both data sets; clades 9–11 (double circled) were recovered only by 18S rDNA data. (C) Morphological character changes (ancestral ==> derived character states) of 11 clades labeled in trees. Selected character states were analyzed by Bayesian ancestral state reconstruction and probabilities for both Markov chain Monte Carlo chains (P = chain1/chain2).

selecting the first-mentioned species in Schrank's original description (see Canon 15 in the American Code of Botanical Nomenclature [1907]; *Torrey Botanical Club Bulletin* 34:167–78; http://www.jstor.org/stable/2479237). This method of lectotype selection is now considered as ''largely mechanical'' and must not be followed (Art. 10.5 in ICBN). As Schrank's (1783) conception of the new genus *Chaetophora* was largely based on his own observations on *C. lobata*, we herein designated *C. lobata* as lectotype of this genus. *C. lobata* was briefly described as ''This species is lobed...the color is grass-green'' (translated from German), and illustrated (Schrank 1783, figs. 2 and 3, tab. VII) as a deeply bilobed thallus with long, radiating hairs, growing on submerged *Ceratophyllum*. Schrank's (1783, p. 125) generic diagnosis (''Muscus frondibus setas longissimas ferentibus'') can be translated as ''a moss with fronds, carrying extremely long hairs.'' Clearly, Schrank's (1783) circumscription of *Chaetophora* was based upon species with distinctly lobed thalli (''frondibus''), characterizing not only *C. lobata* but also later described taxa (e.g., *C. incrassata*, *Chaetophora atra*, *C. cornudamae*, and *C. endiviifolia*). Later authors (Hazen 1902, Starmach 1972) regarded *C. endiviifolia*, *C. cornudamae*, and *C. incrassata* as synonymous. Hazen (1902) also listed Schrank's (1783) *C. lobata* as synonym of *C. incrassata*, albeit contrary to the current rule of priority (Art. 11.4. in ICBN; http://ibot.sav.sk/icbn/main.htm). Moreover, for the new combination *C. incrassata*, she selected *U. incrassata* Hudson (1778) as basionym, which is an invalid homonym of *U. incrassata* Müller (1775). We therefore listed *C. incrassata*, *C. cornudamae*, and *C. endiviifolia* as synonyms of *C. lobata*. In our phylogenetic analyses, one of the two unrelated *Chaetophora* lineages combined the lobate species, *C. lobata*, with *C. draparnaldioides*, which is characterized by hemispherical lobes (''Fronde hemisphaerica''; Kützing 1834). Therefore, we regard the subclade containing these lobate species as *Chaetophora* sensu stricto.

Chaetophora elegans, a species with globose thalli, branched independently from *Chaetophora* s. str. in our phylogenetic trees. *C. elegans* was the first globose species described in this genus (Agardh 1812) and was followed by others (e.g., *C. attenuata*, *C. pisiformis*, *C. punctiformis*, *C. tuberculosa*). To circumscribe the ''extended'' genus *Chaetophora*, inclusive of lobate forms, Kützing (1843, p. 325) introduced an emended diagnosis in which the shape of the thallus was no longer specified (''heteromorphis''). Instead, he placed emphasis on the presence of a mucilaginous envelope (''Phycoma gelatinosum... mucosa involutis'') surrounding the filaments to distinguish *Chaetophora* from its relatives (*Stigeoclonium*, *Draparnaldia*). This relaxed diagnosis of *Chaetophora* defined a polyphyletic genus, using a homoplasious character, that is, the copious production of mucilage, which apparently evolved independently in two lineages within the Chaetophorales (see Fig. 4).

Polyphyly of Stigeoclonium. The genus *Stigeoclonium* s. l. represents the most species-rich genus of the Chaetophorales, together with *Draparnaldia* (both ~80 species; *Chaetophora*: ~60 species; http://ucjeps.berkeley.edu/INA.html). One of the five original species introduced by Kützing (1843), *S. tenue*, is the currently accepted type species (ICBN) and should thus define *Stigeoclonium* s. str. (this is deferred to a later publication). Although our analyses included two strains of *S. tenue*, their SSU rDNA sequences were almost identical to those of three other species, which together formed a polytomy at the base of the *Chaetophora* clade. Other molecular markers (e.g., *rbc*L, internal transcribed spacer regions) will have to be analyzed in addition to rDNA genes to resolve the basal branches of the ''*Chaetophora* clade'' and to define *Stigeoclonium* s. str. unambiguously. However, our SSU rDNA phylogenies as well as formalized cladistic analyses of morphological characters resolved two additional species of *Stigeoclonium* as members of two lineages, which are clearly unrelated to each other and also to *S. tenue* (i.e., *S.* '*Longipilus*' and *S. farctum*). Therefore, *Stigeoclonium* s. l. is polyphyletic, and it is reasonable to assume that the original description of the genus by Kützing (1843) was also based on homoplasious characters. In the diagnosis of *Stigeoclonium*, Kützing (1843, p. 253) listed several features, which also apply to *Chaetophora* but regarded the presence of a very thin layer of mucilage surrounding filaments (''cellulae gelineae tenuissimae'') as characteristic for *Stigeoclonium*. For *S. tenue*, Kützing (1843) described and illustrated zoosporangia in all filaments (main axis and lateral branches) and pointed out a difference to *Chaetophora* and *Draparnaldia* in which zoosporangia are confined to lateral branches. The presence of a thin layer of mucilage and the type of zoospore formation are still used to define this genus and apply to all *Stigeoclonium* species studied here as well as to *C. pascheri*. In molecular phylogenetic trees, these *Stigeoclonium*-like taxa represent mainly the basal divergences of both the ''*Fritschiella* clade'' and the ''*Chaetophora* clade'' but also occupy some derived positions (e.g., *S. farctum*). We conclude that the traditional diagnostic characters used to define *Stigeoclonium* represent plesiomorphies of the entire ''branched Chaetophoraceae'' clade and have been inherited from the last common ancestor of this major lineage of Chaetophorales (this view was confirmed by Bayesian ancestral state reconstruction; see Results). The same likely applies to other characters added later to descriptions of *Stigeoclonium* (e.g., intercalary vegetative cell division) (Islam 1963).

The divergent position of *S.* '*Longipilus*' as closest relative of *C. lobata*, though initially unexpected, gains support by selected morphological features

and is accordingly supported by the cladistic analysis performed here (Fig. 4). In contrast to ''adult'' filaments, ''young'' stages of *S.* '*Longipilus*' have been described as ''surrounded by mucilage matrix as with *Chaetophora*'' (Islam 1963) and forming small tufts, again resembling *Chaetophora* (our own unpublished observations; see also fig. 104 in Pascher 1914), corroborating its placement in the phylogenetic analyses. Moreover, the short, cylindrical to globose cells, deeply constricted at the partition wall, which are characteristic of *S.* '*Longipilus*', differ from most other *Stigeoclonium* species (Islam 1963). *S.* '*Longipilus*' has initially been described under the name *C. draparnaldioides* (Kützing 1834), and our phylogenetic analyses provide clear support for its original designation.

Phylogeny of Draparnaldia. The present paper contributed the first released nuclear-encoded SSU rDNA sequence data of *Draparnaldia* (i.e., of *D. glomerata* and *D. plumosa*). Phylogenetic analyses recovered *Draparnaldia* as a monophyletic sister clade of *Chaetophora* s. str. However, the monophyly of *Draparnaldia* still needs to be confirmed by addition of more species. Monophyly of *Draparnaldia* may reflect the fact that this genus appears well delimited from *Chaetophora* s. str. as well as from *Stigeoclonium* by differentiation into a large-celled main axis of unlimited growth, which never forms zoospores, and lateral filaments of limited growth, composed of smaller cells, capable of zoospore differentiation (Pascher 1914, Forest 1956).

Character evolution in the Chaetophorales. Based on the present phylogenetic analyses, a first attempt can be made to trace the evolution of morphological characters during the diversification of the Chaetophorales (see Fig. 4). First, the following characters likely represent the ancestral state (plesiomorphies) within the order: multicellular, branched filaments consisting of uninucleate cells with a single, parietal chloroplast; filaments attached to a substrate; and reproduction by quadriflagellate zoospores. Only few morphological characters can presently be regarded as synapomorphies of the Chaetophorales to the exclusion of other Chlorophyceae—for example, features of the flagellar apparatus (upper and lower pairs of basal bodies in a clockwise [1/7 o'clock] arrangement, peripheral and terminal fibers between adjacent basal bodies; Melkonian 1975, Watanabe and Floyd 1989, Lewis and McCourt 2004) and the mode of cell division (closed mitosis, cytokinesis by formation of a phycoplast-associated cell plate with several plasmodesmata; Stewart et al. 1973, Mattox and Stewart 1984), which, however, has not yet been studied in detail in the Chaetopeltidales. Both basal chaetophoralean lineages, the Schizomeridaceae and Aphanochaetaceae, show divergent traits: *Schizomeris* developed a unique multiseriate filament system lacking hairs and a prostrate system of ''creeping'' filaments. In contrast, the thallus of *Aphanochaete* is dominated by prostrate filaments with colorless hairs often present, usually unicellular, unsheathed, with a bulbous base. The derived family Chaetophoraceae shows a basal divergence into unbranched (*Uronema* clade) and branched morphotypes and thus cannot yet be circumscribed by morphological synapomorphies, except pyrenoid ultrastructure (pyrenoids bounded, but not traversed by thylakoids; Stewart and Mattox 1975, John 1984). Unbranched Chaetophoraceae mainly display ''loss of'' characters, such as branching, a prostrate filament system, and hairs. Branched Chaetophoraceae are well distinguished by differentiation into prostrate and upright filament systems, usually terminating with gradually tapering, multicellular hairs. Altogether, these synapomorphies may characterize a *Stigeoclonium*-like morphology (see above). The ''branched Chaetophoraceae'' lineage likely evolved toward mucilaginous (*Chaetophora*) and highly differentiated thalli (*Fritschiella* and *Draparnaldia*), which are traditionally also regarded as the most derived Chaetophorales (Forest 1956, Islam 1963). Rather than a single evolutionary series, molecular phylogenies suggest two independent, parallel progressions from *Stigeoclonium*-like ancestors toward more structurally complex genera such as *Fritschiella* and *Draparnaldia*.

Our study clearly revealed that several morphological characters previously used for genus and species descriptions are insufficient to determine taxonomic and evolutionary relationships in the Chaetophorales. As a next step, it seems necessary to combine more detailed molecular phylogenetic analyses, using a more taxon-rich multigene approach, in combination with careful reevaluation of morphological traits under both natural and controlled conditions.

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Supplementary Material

The following supplementary material is available for this article:

Table S1. Strain designations and origins for 30 strains of Chaetophorales examined in this study and accession numbers of newly determined nuclear-encoded SSU rRNA genes.

Appendix S1. Data matrix of 18 morphological characters in the Chaetophorales used for cladistic analyses and ancestral state reconstructions in Figure 4.

This material is available as part of the online article.