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A re-classification of the Acrochaetiales based on molecular and morphological data, and establishment of the Colaconematales *ord. nov.* (Florideophyceae, Rhodophyta)

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Systematics of the red algal order Acrochaetiales and related taxa was investigated using combined small- and large-subunit nuclear ribosomal DNA (SSU and LSU rDNA, respectively) sequence data. These data were subjected to distance, parsimony and maximum likelihood analyses. The resulting phylogenies were congruent with previously published SSU results in that all included orders (Balbianiales, Batrachospermales, Nemaliales, Palmariales and Thoreales) were resolved as monophyletic except the Acrochaetiales, which consisted of two lineages (Acrochaetiales I and II). The Batrachospermales and Thoreales occupied equivocal positions as early diverging lineages, while the Balbianiales generally resolved as sister to an Acrochaetiales-Nemaliales-Palmariales (ANP) complex. Relationships among the four lineages of the ANP complex were not completely resolved, but detailed analyses weakly positioned Acrochaetiales II as sister to the Nemaliales, whereas Acrochaetiales I displayed a moderate to strong affiliation with the Palmariales. Acrochaetiales I included representatives of the genera Acrochaetium, Audouinella and Rhodochorton, whereas Acrochaetiales II had a number of acrochaetioid species including a representative of the genus Colaconema. Compared with published SSU phylogenies, bootstrap values within the two Acrochaetiales lineages increased substantially in combined SSU/LSU analyses. Based on these results, emended generic descriptions are provided for Acrochaetium, Audouinella, Colaconema and Rhodochorton, and a new family of acrochaetioid algae is described, the Colaconemataceae J. T. Harper et G. W. Saunders. The Acrochaetiaceae now includes the genera Acrochaetium, Audouinella and Rhodochorton, while the Colaconemataceae is considered monogeneric at this time. It is quite likely that additional genera will be recognized within the Colaconemataceae pending further investigation. Acrochaetiaceae is retained as the sole family of the Acrochaetiales, although the tenuous recognition of the Palmariales as distinct from this order is discussed, whereas Colaconemataceae is transferred to the new order Colaconematales J. T. Harper et G. W. Saunders.

Key words: Acrochaetiaceae, Acrochaetiales, Colaconemataceae fam. nov., Colaconematales ord. nov., Florideophyceae, large-subunit rDNA, molecular systematics, phylogeny, Rhodophyta, small-subunit rDNA

Introduction

Recent molecular systematic investigations have resolved four higher-level lineages within the red algal class Florideophyceae (Saunders & Bailey, 1997, 1999; Saunders & Kraft, 1997; Harper & Saunders, 2001*b*). Of these, Lineage 2 includes those members possessing two caps on the cytoplasmic faces of the pit plugs, with or without a membrane. This lineage includes the orders Acrochaetiales, Balbianiales, Balliales, Batrachospermales, Corallinales, Nemaliales, Palmariales, Rhodogorgonales and Thoreales *nom. int.* (cf. Sheath *et al.*, 2000).

Until recently, there were few molecular systematic investigations focused on the members of this lineage. However, studies using small-subunit nuclear ribosomal DNA (SSU rDNA) data have resolved many novel relationships among the included taxa. Saunders *et al.* (1995) illustrated the close relationship between members of the Acrochaetiales, Nemaliales and Palmariales, whereas Saunders & Bailey (1997) provided the first molecular evidence for an association between the Corallinales and Rhodogorgonales. Vis *et al.* (1998) examined the systematics of the Batrachospermales, and highlighted the unusual position of the Thoreaceae with respect to other members of the

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order. Harper & Saunders (1998) provided the first detailed molecular study of the Acrochaetiales and discovered two relatively divergent lineages (Acrochaetiales I and II) within the order which seemed. at least morphologically, homogeneous. Sheath & Müller (1999) reported molecular evidence for the recognition of the Balbianiales as a distinct clade within Lineage 2, while the Balliales was proposed by Choi et al. (2000) to accommodate a few red algal species previously classified in the Ceramiales. Lastly, Sheath et al. (2000) have proposed recognition of the Thoreales for the batrachospermalean Thoreaceae, which had consistently occupied a relatively distinct position in rbcL- and SSUbased phylogenies (Harper & Saunders, 1998; Vis et al., 1998).

In spite of these taxonomic advances, a number of relationships within Lineage 2 remain unresolved. In particular: (1) the relative positions of the Balbianiales, Batrachospermales and Thoreales; and (2) the relationships between the Acro-chaetiales, Nemaliales and Palmariales.

The Acrochaetiales presents an interesting problem for red algal systematists. Compared with the remainder of the Florideophyceae, members of the Acrochaetiales possess simple vegetative (generally heterotrichous, simple or branched filaments) and reproductive (absence of a carpogonial branch, reduced postfertilization development) attributes. It is this apparent simplicity that has led to arguably one of the most chaotic taxonomic histories in red algal systematics. Since first proposed by Feldmann (1953), ordinal status for the Acrochaetiales has been a topic of debate (Dixon, 1961, 1973; Feldmann, 1962; Papenfuss, 1966; Garbary, 1978). However, recent molecular investigations have supported its recognition (Saunders et al., 1995; Harper & Saunders, 1998). Taxonomic confusion remains an issue at the subordinal level, a direct consequence of the numerous generic and specific delineations within the order. The number of species recognized within the complex is bewildering (400+) and, as Garbary (1978) noted, 'Attempts at identifying [species] ... leads one into a maze of literature and specific epithets in which it is often easier to describe new taxa than to compare specimens with descriptions in monographs or herbarium material.' Over 90 new species have been described during the last 50 years, each delimited primarily on progressively finer divisions of preexisting criteria, creating a large number of interrelated taxa.

Though hard to imagine, the confusion surrounding generic concepts within the Acrochaetiales is almost equally chaotic. Twenty generic names have been used in association with acrochaetioid algae: *Acrochaetium, Audouinella, Balbiania, Byssus, Callithamnion, Ceramium, Chantransia, Chantran* siella, Chromastrum, Colaconema, Conferva, Grania, Kylinia, Liagorophila, Pseudacrochaetium, Pseudochantransia, Rhodochorton, Rhodothamniella, Thamnidium and Trentepohlia. Of these, only seven are currently available for use with species of this order: Acrochaetium, Audouinella, Colaconema, Grania, Kylinia, Liagorophila and Rhodochorton (for more detailed accounts see Woelkerling, 1971, 1983). At least 24 different classification schemes involving taxa of generic and higher rank have been proposed and these concepts have ranged from recognizing a single genus (Drew, 1928; Dixon &

proposed and these concepts have ranged from recognizing a single genus (Drew, 1928; Dixon & Irvine, 1977; Garbary, 1978) to as many as eight genera (Feldmann, 1962). These discrepancies in the literature have led to multiple concepts associated with each genus. Such an abundance of generic concepts has led to,

and then been fuelled by, a lack of consensus as to which characters are useful for taxonomy at this rank. In 1928, Drew advocated a monogeneric Acrochaetiaceae and placed all marine and freshwater acrochaetioid taxa within Rhodochorton. Thus, this genus included plants in which the chloroplasts vary from one to many per cell, and are parietal, stellate, ribbon-shaped, discoid or have radiating lobes. Papenfuss (1945) and Feldmann (1962), however, recognized multiple genera within the Acrochaetiales and had a restricted concept of Rhodochorton: asexual marine plants with cells possessing few to many discoid chloroplasts. The other included genera, notably Acrochaetium and Audouinella, have suffered similarly owing to multiple concepts. To further complicate matters, there are a number of discrepancies in the literature as to the importance of particular characters in species and generic delineation. Some studies have shown that the occurrence of and number of pyrenoids per plastid can vary with respect to light intensity (Stegenga & Vroman, 1976, 1977) and life history stage (Garbary & Rueness, 1980), whereas others have concluded that the number of pyrenoids appears to be a stable character for species and genus delineation (Woelkerling, 1971, 1973; Stegenga, 1985; Stegenga et al., 1997).

Harper & Saunders (1998) used SSU data in a systematic investigation of the Acrochaetiales to provide a framework for addressing the taxonomic conundrums surrounding this order. Although their study unequivocally recognized two divergent lineages of acrochaetioid taxa, they failed to resolve relationships between these two lineages and the closely allied Nemaliales and Palmariales, and further failed to resolve affinities among species within the acrochaetioid lines. They suggested that an alternative molecular marker might provide better resolution at both these taxonomic levels.

Sequences of the nuclear ribosomal large subunit (LSU) have been used with increasing frequency for

phylogenetic inference (Leffers et al., 1987; Baroin et al., 1988; Cedergren et al., 1988; Qu et al., 1988; Gouy & Li, 1989a, b; Lenaers et al., 1989, 1991; Preparata et al., 1989; Kuzoff et al., 1998). In contrast to the SSU, the LSU is larger (~ 3500 base pairs (bp) versus ~ 1800 bp) and possesses more extensive regions of greater sequence divergence (Harper & Saunders, 2001 a). Recent work using the LSU for molecular investigations within the Rhodophyta (Freshwater & Bailey, 1998; Freshwater et al., 1999; Harper & Saunders, 2001 a, b) has illustrated the potential of these data for assessing phylogenies within and among red algal orders. Given the chaotic state of taxonomy within the Acrochaetiales, as well as the equivocal SSU-based relationships between the two divergent lineages, our objectives were to use combined LSU and SSU sequence data to resolve relationships within and between the two Acrochaetiales lineages, among the Acrochaetiales and closely related orders, and to ascertain which traditional characters may be useful in delineating genera in light of these molecular phylogenies.

Materials and methods

Species included in this study are presented in Table 1 (sample collection information and isolate numbers can be obtained from the authors). Names of species within Acrochaetiales I and II follow Harper & Saunders (1998); samples that are representative of genera are referred to these genera (Acrochaetium secundatum, Audouinella hermannii, Colaconema asparagopsis, Rhodochorton *purpureum* and *R. tenue*), whereas all other species within these lineages are listed as species of 'Audouinella'. Following the procedures outlined in Saunders (1993), samples were processed and genomic DNA isolated. Approximately 85% of the LSU was PCR-amplified as three or four overlapping fragments for 9 taxa (Table 1) using oligonucleotide PCR primers as described in Harper & Saunders (2001 a, b). The Taq DNA polymerase PCR kit (Life Technologies, Gibco BRL) was used to amplify LSU fragments and the reaction profiles are given in Harper & Saunders (2001a). The Wizard (TM) PCR Preps DNA purification system (Promega, Madison, WI) was used to purify PCR products. Both strands were sequenced using the Taq Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems (ABI), division of Perkin Elmer, Wellesley, MA), the PCR primers and additional internal sequencing primers

Table 1.	Sources	of SSU	and	LSU	sequence	data	used	in	the	multiple	alignme	ent
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Classification	Species and authority	SSU GenBank	LSU GenBank/ Reference
ACROCHAETIALES	Acrochaetium secundatum (Lyngbye) Nägeli	AF079784	AF528044
	'Audouinella' amphiroae (Drew) Garbary	AF079785	AF528045
	'Audouinella' caespitosa (J. Agardh) Dixon	AF079787	AF528046
	'Audouinella' dasyae (Collins) Woelkerling	L26181	AF419100
	'Audouinella' daviesii (Dillwyn) Woelkerling	AF079788	AF528047
	'Audouinella' endophytica (Batters) Dixon	AF079789	AF419101
	Audouinella hermannii (Roth) Duby	AF026040	AF419102
	'Audouinella' pectinata (Kylin) Papenfuss	AF079790	AF528048
	'Audouinella' proskaueri (West) Garbary	AF079791	AF528049
	'Audouinella' rhizoidea (Drew) Garbary	AF079792	AF528050
	'Audouinella' tetraspora Garbary et Rueness	AF079793	AF528051
	Colaconema asparagopsis Chemin	AF079795	AF421125
	Rhodochorton purpureum (Lightfoot) Rosenvinge	U23816	AF419103
	Rhodochorton tenue Kylin	AF079796	AF421126
BALBIANIALES	Balbiania investiens (Lenormand in Kützing) Sirodot	AF132294	AF421124
BALLIALES BATRACHOSPERMALES	Ballia callitricha (C. Agardh) Kützing	AF236790	AF419106
Batrachospermaceae	Batrachospermum boryanum Sirodot	AF026044	AF419108
*	Batrachospermum turfosum Bory de Saint-Vincent	AF026049	AY056020
	Sirodotia suecica Kylin	AF026053	AY056021
Lemaneaceae NEMALIALES	Lemanea fluviatilis (Linnaeus) C. Agardh	AF026051	AF419110
Galaxauraceae	Galaxaura marginata (Ellis et Solander) Lamouroux	AF006090	AF419138
Liagoraceae	Cumagloia andersonii (Farlow) Setchell et Gardner	AF097878	AF419137
PALMARIALES	,		
Palmariaceae	Halosaccion glandiforme (S.G. Gmelin) Ruprecht	L26193	AF528052
	Palmaria palmata (Linnaeus) Kuntze	Z14142	Y11506
Rhodophysemataceae	Rhodophysema elegans (P. et H. Crouan ex J. Agardh) Dixon	U23817	AF419140
Rhodothamniellaceae	Camontagnea oxyclada (Montagne) Pujals	AF079794	AF419139
THOREALES			
Thoreaceae	<i>Thorea</i> sp.	AF420253	AF419145
	Thorea violacea Bory de Saint-Vincent	AF026042	AF419146



Figs 1–4. Phylogenetic analyses of Align1 with and without *Camontagnea*. The outgroup, *Ballia*, has been pruned from the base of the trees in these figures. Fig. 1. ML tree with *Camontagnea* included. Figs 2–4. Simplified representations of the distance, unweighted parsimony and weighted parsimony bootstrap results, respectively. Numbers above and below branches indicate bootstrap values for the alignment with (above) and without (below) *Camontagnea* included. Asterisk denotes a node that was fully supported (100%) in all analyses; +indicates < 50% support. AC, Acrochaetiales; BL, Balbianiales; BT, Batrachospermales; NM, Nemaliales; PL, Palmariales; and TH, Thoreales.

(Harper & Saunders, 2001*a*,*b*). LSU sequences were edited by eye using the SeqEd DNA sequence editor (ABI) software package. Edited sequences were aligned relative to one another using the SeqPup multiple alignment program (Gilbert, 1995). The sequences obtained herein were added to an alignment including 19 previously determined red algal LSU sequences (Table 1). Corresponding SSU sequences for all taxa were added to the alignment, unalignable and ambiguous regions were removed, and the terminal 5' and 3' ends of the LSU and SSU corresponding to the PCR primers were excluded.

Initial analyses were performed on a phylogenetically broad data set (Align1) of combined SSU/LSU sequences with Ballia callitricha chosen as the outgroup (Harper & Saunders, 1998; Choi et al., 2000). The alignment had 28 taxa and 4192 sites. To include more of the variable sequence regions in subsequent analyses, and to remove homoplasy introduced by distant outgroups (Lyons-Weiler et al., 1998), three additional alignments were assessed: Align2-Batrachospermales and Balbianiales included as outgroups; Align3 - Balbianiales as outgroup; Align4-ingroup only (Acrochaetiales, Nemaliales and Palmariales - unrooted trees). Each alignment was also analysed with Camontagnea (Rhodothamniellaceae, Palmariales) removed (long branch relative to other ingroup taxa) so that a total of eight alignments were analysed. All analyses were completed using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b8a Altivec (Swofford, 2001). Each alignment was assessed for an optimal model prior to analyses using the program MODELTEST 3.06 (Posada & Crandall, 1998; individual parameters varied slightly but a general time reversible model with gamma was identified for all alignments). Maximum likelihood analysis was performed (10 random sequence addition replicates) using a heuristic search with stepwise addition and tree bisectionreconnection (TBR) branch swapping. Under distance analysis, the general time reversible model was used to convert the sequence alignment into a distance matrix and the neighbor-joining algorithm was used (assuming equal rates at all sites) to construct a phylogenetic tree (Saitou & Nei, 1987). Unweighted parsimony analysis was completed (50 random sequence additions, gaps treated as missing data) using a heuristic search with steepest descent and TBR branch swapping (Swofford, 2001). In a second round of parsimony, transversions were weighted two-to-one over transitions (herein referred to as 'weighted' parsimony). To estimate the robustness of internal nodes, bootstrap resampling was completed (2000 replicates for distance and parsimony (10 random sequence addition replicates) and 100 replicates, unless otherwise indicated, for maximum likelihood (5 random sequence additions) (Felsenstein, 1985)).

Results

Fig. 1 presents the maximum likelihood (ML) result for Align1 (-Ln likelihood = 13994·00785) for the combined SSU/LSU analyses. ML analysis of this alignment excluding *Camontagnea* (-Ln likelihood = 13480·37994) gave a tree of virtually identical topology (result not shown). The Batrachospermales was positioned as the earliest divergence followed by the Thoreales and then the Balbianiales.



Fig. 5. Simplified representations for ML analyses of Align2 (Batrachospermales and Balbianiales included as outgroups), Align3 (Balbianiales as outgroup) and Align4 (ingroup only) with and without Camontagnea. Values at internal branches indicate bootstrap support for ML, distance, unweighted parsimony and weighted parsimony (top to bottom). Asterisk denotes a node that was fully supported (100%) in all analyses; + indicates < 50%support; and N indicates not determined. Align2 (+ Camontagnea): #Nemaliales was sister to Acrochaetiales I, Acrochaetiales II and Palmariales in distance analyses (53% support). Align2 (-*Camontagnea*): Only 50 replicates completed for ML bootstrap. #Nemaliales was sister to Acrochaetiales I, Acrochaetiales II and Palmariales in distance analyses (53% support). Align3 (+ Camontagnea): # Acrochaetiales II joined Nemaliales in unweighted parsimony (52% support). AC, Acrochaetiales; BL, Balbianiales; BT, Batrachospermales; NM, Nemaliales; PL, Palmariales; TH, Thoreales.

Acrochaetiales II was positioned as sister to the Palmariales with the Nemaliales allied to this lineage and Acrochaetiales I at the base of the ANP (Acrochaetiales-Nemaliales-Palmariales) complex (Fig. 1). The distance, unweighted parsimony (three trees, length = 1532, consistency index = 0.646, retention index = 0.704) and weighted parsimony (one tree, length = 2085, consistency index = 0.649, retention index = 0.708) all differed from the ML result with respect to the relative positions of the Balbianiales, Batrachospermales and Thoreales (trees not shown) but these three lineages were solidly (99–100% bootstrap support) positioned as basal divergences relative to the four lineages of the ANP complex in bootstrap analyses with these same methods (Figs 2, 3 and 4, respectively). Within the ANP complex all methods provided full support for monophyly of the four key lineages, whereas

relationships among these same lineages varied in support and pattern. With the exception of distance (Fig. 2), removal of *Camontagnea* prior to analysis actually decreased support for resolved affinities among the four lineages of the ANP complex (Figs 3, 4), indicating that inclusion of *Camontagnea* in combination with distant outgroups may be artificially supporting incorrect relationships.

In a series of subsequent analyses aimed at reducing homoplasy by removing distant outgroups and allowing more of the variable regions of the alignment to be included in analyses, a definite pattern started to emerge (Fig. 5). Analyses gave unequivocal support for Acrochaetiales I, Acrochaetiales II, Nemaliales and Palmariales as monophyletic lineages. Moving from Align2, through Align3 and Align4 (Fig. 5) there was an increase in support for an association between Acrochaetiales I and the Palmariales, an association which gained greater support for all types of analyses and for all alignments when Camontagnea was excluded (Fig. 5). Similarly, there was a weak to moderately supported trend for an association between Acrochaetiales II and the Nemaliales.

Fig. 6 presents an enlarged view of the ML result for the Acrochaetiales I and II clades based on Align4 (minus *Camontagnea*). Similar results were obtained among these species for all types of analyses in all the other alignments studied, although bootstrap support was generally lower with increasingly distant outgroups included (trees not shown). Within Acrochaetiales I (Fig. 6), the two species of *Rhodochorton* (*R. purpureum* and *R. tenue*) grouped with full support. The position of Acrochaetium as sister to an Audouinella/ Rhodochorton clade received weak to moderate support under ML and distance analyses (70%, 82%), whereas Audouinella occupied a basal position under unweighted (81%) and weighted (77%) parsimony. Many of the relationships within Acrochaetiales II (Fig. 6) received moderate to strong bootstrap support, the one notable exception being the equivocal position of 'Audouinella' endophytica. Two clades within Acrochaetiales II were resolved. The first grouped 'Audouinella' pectinata and "Audouinella' proskaueri with full support, with Colaconema asparagopsis positioned as sister (85% ML, 97% distance, 65% unweighted and 69% weighted parsimony). The second clade contained the remaining Acrochaetiales II taxa with moderate to strong support under all analyses (Fig. 6). Within this lineage a fully supported clade for 'A'. caespitosa and 'A'. rhizoidea was resolved. In a second moderately supported group relationships among 'Audouinella' daviesii and 'Audouinella' amphiroae were equivocal relative to one another and to a fully supported grouping of 'Audouinella' dasyae and 'Audouinella' tetraspora.



Fig. 6. Detail of relationships resolved under ML for Acrochaetiales I and II with Align4 (*– Camontagnea*). Values at internal branches indicate bootstrap support for ML, distance, unweighted parsimony and weighted parsimony, respectively (values in parentheses for Acrochaetiales I supported *Audouinella* as basal to *Acrochaetium* and *Rhodochorton*). Asterisk denotes a node that was fully supported (100%) in all analyses; +indicates < 50% support. # 'A'. *amphiroae* joined 'A'. *daviesii* in distance analyses (65% support); @ 'A'. *endophytica* resolved at the base of Acrochaetiales II in distance, unweighted parsimony and weighted parsimony (77%, 87% and 59% support, respectively).

Discussion

Balbianiales, Batrachospermales and Thoreales

Recent rbcL- and SSU-based molecular investigations have explored the phylogenetic relationships among the red algal orders Balbianiales, Batrachospermales and Thoreales (Vis et al., 1998; Sheath & Müller, 1999; Pueschel et al., 2000; Sheath et al., 2000). Results herein are congruent with these previous studies. As reported by Vis et al. (1998), our phylogenies support the monophyly of the Batrachospermales sensu stricto and are consistent with polyphyly for the family Batrachospermaceae and the genus *Batrachospermum*. The phylogenetic affinities of the Batrachospermales within Lineage 2 were not resolved using rbcL and SSU data (Vis et al., 1998; Pueschel et al., 2000) and our phylogenies did not offer any further resolution. In all molecular results to date, the Batrachospermales has occupied an equivocal position ancestral to the Balbianiales and the ANP complex. For our phylogenetically broad alignment (Align1) only unweighted parsimony, albeit with no support, contradicted this result (Fig. 3).

A similar situation is true for the newly proposed Thoreales (Sheath *et al.*, 2000). The placement of *Thorea* spp. in our trees supports the previous molecular investigations that have illustrated its unique position separate from the Batrachospermales, the order in which it traditionally has been placed (Harper & Saunders, 1998; Vis *et al.*, 1998; Sheath & Müller, 1999).

The phylogenetic relationships of the Balbianiales have only recently been investigated. Sheath et al. (1994) described the freshwater red alga Rhododraparnaldia oregonica Sheath, Whittick et Cole as possessing morphological, anatomical and ultrastructural features seemingly intermediate between the Acrochaetiales and Batrachospermales. The phylogenetic analyses of Vis et al. (1998) supported this hypothesis and this taxon was placed in an unresolved position in this region of their rbcL- and SSU-based trees. Sheath & Müller (1999) found that the freshwater red alga Balbiania investiens shared many features in common with R. oregonica and that the two species grouped together with full support in their molecular phylogenies. As members of the newly described order Balbianiales (Sheath & Müller, 1999), Balbiania was placed with moderate support as the sister lineage to the ANP complex, congruent with our ML, distance and weighted parsimony results (Figs 1, 2 and 4).

ANP complex and monophyly of the Acrochaetiales

A close relationship between the Acrochaetiales, Nemaliales and Palmariales has been posited in alpha systematic literature since the inception of the Palmariales (cf. Saunders, 2002) and elucidated in every molecular systematic investigation examining these taxa to date (e.g. Saunders *et al.*, 1995; Harper & Saunders, 1998; Sheath & Müller, 1999). Results from the current analyses support this close association. Phylogenies based on SSU (Harper & Saunders, 1998) clearly resolved two divergent lineages within the Acrochaetiales, but whether or not they would eventually form a monophyletic order was uncertain because relationships between these two lineages and the Nemaliales and Palmariales were unresolved in that study. Harper & Saunders (1998) considered that the lack of resolution provided by SSU-based trees within and among Acrochaetiales I and II precluded making any formal taxonomic changes. The order was left with a possibly polyphyletic origin and a framework was provided for future delineation of genera. Combined SSU/LSU analyses give substantially increased support for many of the nodes among lineages of the ANP complex, as well as within Acrochaetiales I and II. In light of this, taxonomic revisions that adequately reflect the included diversity must now be formally addressed.

Our analyses indicate that Acrochaetiales I and the Palmariales are close allies, whereas Acrochaetiales II is a distant relative of these previous two lineages showing some affinities with the Nemaliales. It is no longer tenable to retain the two Acrochaetiales lineages within a single order (one which is clearly polyphyletic) and ignore the association of its component lineages with the Nemaliales and Palmariales.

At the two extremes are recognition of four separate orders for the lineages of the ANP complex, or the merger of all four into an expanded Nemaliales. There will undoubtedly be proponents of both options. We argue that neither of these proposals is entirely satisfactory – an intermediate solution perhaps being best in the long term – but that the former is the more conservative interim step and will generate the least taxonomic confusion in the literature until more data are generated.

To start at the least controversial level, we consider that the two lineages of Acrochaetiales are clearly distinct at the familial level: an emended Acrochaetiaceae for Acrochaetiales I; and Colaconemataceae *fam. nov.* for Acrochaetiales II. The ordinal assignments of these two families raise greater complexity.

Our molecular results indicate a weak affiliation of Colaconemataceae with the Nemaliales. However, the inclusion of the Colaconemataceae in the Nemaliales is challenged by the filamentous nature of the colaconematacean gametophyte, as well as the absence of a carpogonial branch and of sterile gametophytic tissue associated with the developing carposporophyte – such features granted significant weight in traditional florideophyte systematics (cf. Saunders & Kraft, 1997). Furthermore, the Colaconemataceae is virtually as distinct at the molecular level (based on SSU and LSU) from the Nemaliales as both these taxa are from Acrochaetiales I and Palmariales. The last-mentioned point provides justification for recognition of the Colaconemataceae at the ordinal level even if future molecular analyses were to resolve it weakly as sister to the Acrochaetiales I/Palmariales lineage. Short of sinking all four lineages into a single order, a proposal difficult to accept when the substantial differences between the groups are considered (Saunders & Kraft, 1997; Saunders, 2002), it is an inevitable conclusion that the Colaconemataceae requires ordinal status. A more difficult issue to address concerns the distinctness of the Palmariales from Acrochaetiales I.

Recognition of a close association between the Acrochaetiales and Palmariales has been a recurrent theme in recent literature (cf. Saunders, 2002) and was highlighted by the transfer of acrochaetioid-like taxa to the Palmariales (Saunders et al., 1995). In retrospect, a close association between Acrochaetiales I and the Palmariales is perhaps intuitive when the features of these two groups are considered. Features include: lack of monosporangia and carposporophyte generation in Rhodochorton and all species of the Palmariales; B-phycoerythrin in Acrochaetium and in Rhodophysemataceae; and the presence of stellate plastids with central pyrenoids, B-phycoerythrin, and gametophytes with unicellular bases in Acrochaetium and the Rhodothamniellaceae – taxa which probably represent the earliest divergences within their respective lineages (Figs 1, 6). Many of these features are rare or absent in Colaconemataceae and Nemaliales. and in some cases among all other Florideophyceae (e.g. stellate plastids with central pyrenoids and B-phycoerythrin). It can reasonably be argued that there is a stronger justification for merging the Palmariales into the Acrochaetiales, generating an order of four families (Acrochaetiaceae, Palmariaceae, Rhodophysemataceae, Rhodothamniellaceae), than for resisting ordinal status for Colaconemataceae and including it in the Nemaliales. In fact the only feature remaining to distinguish the Palmariales is the complete absence of gonimoblast development following fertilization – a feature which is open to some interpretation for Rhodochorton, Acrochaetiales I (Saunders et al., 1995). Nonetheless, we resist such a merger for the time being because Acrochaetiales I and the Palmariales are unequivocally monophyletic groups in all our analyses, and their association relative to the Nemaliales and Colaconemataceae should stand the test of further phylogenetic scrutiny (notably increased taxon sampling for Nemaliales and Palmariales) prior to a potentially superfluous synonymy which would only add unnecessary confusion to the taxonomic literature. Thus we continue to recognize the Palmariales including its three component families, restrict the Acrochaetiales to include only an emended Acrochaetiaceae (discussed below), and recognize Acrochaetiales II at the familial and ordinal level (Colaconemataceae, Colaconematales).

Taxonomic treatment

Acrochaetiales Feldmann emend. J. T. Harper *et* G. W. Saunders

Algae composed of monosiphonous simple or branched filaments, fitting one of the three following sets of characteristics: (1) marine acrochaetioid algae; multiple parietal discoid to band-shaped plastids per cell, lacking pyrenoids; monosporangia unknown, apparently reproducing primarily by asexual tetrasporangia; where known, sexual life histories biphasic with reduced gametophytes; (2) freshwater algae of acrochaetioid morphology; cells possessing multiple parietal lobed to discoid plastids, lacking pyrenoids; plants reproducing primarily by monosporangia, but where known, possessing a triphasic life history with morphologically similar gametophytes and tetrasporophytes; and (3) marine acrochaetioid algae; cells possessing a single axile stellate chloroplast, each with a single central pyrenoid; plants reproducing primarily by monosporangia, but where known, possessing a triphasic life history.

TYPE FAMILY: Acrochaetiaceae Fritsch emend. J. T. Harper *et* G. W. Saunders

Acrochaetiaceae Fritsch emend. J. T. Harper *et* G. W. Saunders

Characters as for the order.

TYPE GENUS: *Audouinella* Bory de Saint-Vincent (1823: 340).

The SSU-based trees of Harper & Saunders (1998) and our SSU/LSU results have positioned type species of three acrochaetioid genera within the Acrochaetiaceae: Acrochaetium (A. secundatum), Audouinella (A. hermannii) and Rhodochorton (R. purpureum). The divergence between the included members in terms of habitat, plastid morphology and life history details has prompted some authors to propose recognition of separate acrochaetioid families for each of the genera (Volvosek *et al.*, 2000). While there are differences between these taxa, recognizing three families for a clade of organisms that are so closely related to one another at the molecular level seems unwarranted.

Although the molecular divergence among these taxa is comparatively low, the three included genera

differ from each other with respect to habitat, morphology and anatomy. Based on these characteristics, and as the original concepts of these genera are no longer tenable, the following emendations are presented.

Acrochaetium Nägeli emend. J. T. Harper *et* G. W. Saunders

Marine algae composed of monosiphonous simple or branched filaments; cells possessing a single axile stellate chloroplast, each with a single central pyrenoid; plants reproducing primarily by monosporangia, but where sexual reproduction is known, possessing a triphasic life history.

TYPE SPECIES: Acrochaetium secundatum (Lyngbye) Nägeli in Nägeli et Cramer (1858: 532).

BASIONYM: Callithamnion daviesii var. secundatum Lyngbye (1819: 129).

SYNONYMS: *Colaconema secundata* (Lyngbye) Woelkerling (1973: 575) and others listed in this source.

As is the case with virtually every other genus that has been placed within the Acrochaetiales, Acrochaetium has been variously circumscribed to include a spectrum of different species. These range from all acrochaetioid algae known to produce monospores (Nägeli, 1861), all asexual acrochaetioid algae (Bornet, 1904; Collins, 1906), all marine acrochaetioids (Bøergesen, 1915), and those acrochaetioid taxa with a single parietal chloroplast per cell (Papenfuss, 1945). Originally, the generally cited lectotype species of Acrochaetium was Ac. daviesii. However, Woelkerling (1983) designated Ac. secundatum as the lectotype, characterizing Acrochaetium by the presence of cells with a single stellate chloroplast with a central pyrenoid. This delineation was followed by Stegenga (1985) in his treatment of the South African Acrochaetiaceae.

Harper & Saunders (1998) included 'Audouinella' arcuata (Drew) Garbary, Hansen et Scagel in their SSU-based molecular analyses of the Acrochaetiales. The plastid features of this species are the same as those for Acrochaetium secundatum, and both grouped with high bootstrap support in all SSU trees. Therefore, this taxon is more correctly referred to the genus Acrochaetium as Ac. arcuatum (Drew) Tseng.

The genus *Liagorophila* was described by Yamada (1944) for an endophytic acrochaetioid alga – *Liagorophila endophytica* Yamada. Novel carpogonial branch structure and post-fertilization development led Yamada to regard this taxon as deserving of generic designation. Garbary (1980) questioned using such characteristics for generic circumscription within a group where the sexual stages are not known for the majority of the

Acrochaetiales taxonomy

included taxa. Garbary (1980) therefore synonymized *Liagorophila* with the genus *Audouinella*, proposing the combination *Au. yamadae* Garbary. Regardless, Bula-Meyer (1986) and Lee *et al.* (1986) both retained *Liagorophila* as a distinct genus. Given that the cells of the marine *L. endophytica* each possess a single stellate plastid with a central pyrenoid, we propose the following new combination in *Acrochaetium*:

Acrochaetium yamadae (Garbary) J. T. Harper *et* G. W. Saunders, *comb. nov*.

BASIONYM: *Liagorophila endophytica* Yamada (1944: 16).

SYNONYM: *Audouinella yamadae* Garbary (1980: 68).

Liagorophila is thus synonymized with *Acro- chaetium*.

Audouinella Bory de Saint-Vincent emend. J. T. Harper *et* G. W. Saunders

Freshwater algae composed of monosiphonous simple or branched filaments; cells possessing multiple parietal lobed to discoid plastids, lacking pyrenoids; plants reproducing primarily by monosporangia but, where sexual reproduction is known, possessing a triphasic life history with morphologically similar gametophytes and tetrasporophytes.

TYPE SPECIES: *Audouinella hermannii* (Roth) Duby *in* De Candolle (1830: 972).

BASIONYM: Conferva hermannii Roth (1806: 180).

SYNONYMS: *Chantransia hermannii* (Roth) Desvaux (1809: 310).

Audouinella is the type genus for the order Acrochaetiales and as such has been the epithet used most often as the taxonomic 'umbrella' to accommodate species in monogeneric treatments (e.g. Dixon & Irvine, 1977; Garbary, 1978). Few detailed studies of freshwater Acrochaetiales are available and therefore, with respect to the other genera in this family, there are now comparatively few species of true Audouinella (cf. Jiménez & Necchi, 2001, and references therein). With the above generic delineation, separating Audouinella from other acrochaetioid algae is simplified, as the genus is composed thus far of only freshwater taxa.

Rhodochorton Nägeli emend. J. T. Harper *et* G. W. Saunders

Marine algae composed of monosiphonous simple or branched filaments; multiple parietal discoid to band-shaped plastids per cell, lacking pyrenoids; monosporangia unknown, plants reproducing primarily by apparently asexual tetrasporangia; where known, sexual life histories are biphasic, and heteromorphic with reduced gametophytes.

TYPE SPECIES: *Rhodochorton purpureum* (Lightfoot) Rosenvinge (1900: 75).

BASIONYM: Byssus purpurea Lightfoot (1777: 1000).

SYNONYMS: *Audouinella purpurea* (Lightfoot) Woelkerling (1973: 536) and others listed in this source.

The concept of *Rhodochorton* has varied tremendously since Nägeli described the genus for acrochaetioid algae reproducing via tetraspores. Drew (1928, 1935) concluded that the genus should include virtually all described acrochaetioid algae while Feldmann (1962) followed the original description of Nägeli (1861). Within the Acrochaetiaceae, this genus is easily distinguished from *Acrochaetium* in lacking monosporangia and stellate plastids, and from *Audouinella* in being marine and lacking monosporangia.

Colaconematales J. T. Harper *et* G. W. Saunders, *ord. nov.*

DIAGNOSIS: Algae rubrae marinae pusillulae ex simplicibus vel ramosis filis monosiphonibus constantes; unaquaeque cellula unum usque ad pluros chloroplastos parietales continet quae varias figuras habent (ita ut lobatas, vel spirales vel etiam irregulares sed numquam stellatas), sunt cum pyrenoidibus sive non; reproductio asexualis primum per monosporangiam; curriculum vitae triphasicum est.

Marine algae composed of monosiphonous simple or branched filaments; cells containing one to several parietal plastids of varying shape (lobed to spiral to irregular, but never stellate), with or without pyrenoids; asexual reproduction primarily by monosporangia; possessing a triphasic life history.

TYPE FAMILY: Colaconemataceae J. T. Harper et G. W. Saunders, *fam. nov*.

Colaconemataceae J. T. Harper *et* G. W. Saunders, *fam. nov*.

DIAGNOSIS: Characteres ut ordine. Characters as for the order.

TYPE GENUS: Colaconema Batters (1896: 8).

At present, the Colaconemataceae includes a single genus, *Colaconema*, but will most certainly be

considered to include a number of genera pending future research. The level at which generic delineations will be established will depend on what features (morphological and/or molecular) are deemed to be phylogenetically informative.

The task of coping with the assignment of species to generic epithets and concepts within the Colaconemataceae is much more problematic than in the Acrochaetiaceae. Unlike the genera within the Acrochaetiaceae, the most accessible characters for delineation of genera within this complex – plastid and pyrenoid details – do not show meaningful diagnostic variation within this group. In addition, our dataset lacks representatives of the type species for the remaining valid generic names for acrochaetioid algae – *Colaconema, Grania* and *Kylinia* – species of which are most likely included in the Colaconemataceae.

The genus *Kylinia* was initially established by Rosenvinge (1909) for acrochaetioid algae possessing stalked spermatangia. While a number of authors have restricted and limited the concept of this genus based on holdfast and plastid characteristics, Kylinia has either been treated as doubtfully distinct or synonymized within a broadly defined Audouinella (Dixon & Irvine, 1977) or *Rhodochorton* (Drew, 1928). The plastid features of the type species K. rosulata Rosenvinge are similar to those of some species in the Colaconemataceae (each cell possessing a single lobed, parietal plastid with pyrenoids reportedly lacking). Stegenga & Van Wissen (1979) noted that the tetrasporophyte of K. rosulata was in actuality 'Audouinella' stricta (Rosenvinge) South *et* Tittley, thus confirming the placement of the genus Kylinia within the Acrochaetiales. Subsequently, however, Stegenga (1985) noted that the identification of K. rosulata in the cultures of Stegenga & Van Wissen (1979) may have been erroneous, and the status of Kylinia was again uncertain.

To date, stalked spermatangia have been found in two other red algae – *Balbiania investiens* and *Rhododraparnaldia oregonica* – both of the newly described order of freshwater red algae, the Balbianiales (Sheath & Müller, 1999). If stalked spermatangia are diagnostic of this order, then *Kylinia* may well be a marine representative of the Balbianiales. Although other researchers have treated *Kylinia* in more broadly based generic concepts than its original description, the affinities of this genus with the Acrochaetiales and/or Balbianiales must be determined before it can be used as an epithet for acrochaetioid taxa.

The original description of the subgenus *Grania* (Rosenvinge, 1909) was to accommodate acrochaetioid algae that possessed ribbon-shaped plastids, terminal and intercalary carpogonia, and seriate carposporangia (cf. Woelkerling, 1971). In

1944, Kylin elevated *Grania* to generic rank, with Grania efflorescens (J. Agardh) Kylin as the type species. The shape of the plastids, interpreted variously as ribbon-shaped or spiral, is quite diagnostic for this species. Of those species included in our study, only 'Au'. pectinata has been reported to possess plastids of similar morphology (West, 1969). Rosenvinge (1909) noted the apparent close relationship between 'Au'. pectinata and G. efflorescens and Woelkerling (1971) raised concerns about the need for clarification of the relationship between these two species. Plants intermediate between these two species with respect to cell size and sporangial arrangement have been reported (West, 1969; Woelkerling, 1971), but these similarities alone are not sufficient for delineating the genus Grania for the 'Au'. pectinata/'Au'. proskaueri clade in our trees. Detailed morphological and anatomical observations of all three species are needed and sequence data must be acquired for G. efflorescens to understand fully the relationships between these taxa prior to reinstatement of Grania.

The last remaining generic epithet for members of the Colaconemataceae is Colaconema, which was established by Batters (1896) for acrochaetioid algae with prostrate, irregular filaments, and monosporangia formed on terminal and intercalary cuplike cells. Woelkerling (1971) noted that because no sexual details of the species originally included in the genus have been elucidated, the systematic position of the genus is uncertain. However, a number of authors have recognized Colaconema as an acrochaetioid genus, although, not surprisingly, with dramatically different delineations. Woelkerling (1971) used Colaconema as a form genus for all asexual taxa of uncertain systematic position in his treatment of South Australian Acrochaetiales. Stegenga (1985) treated 'Audouinella' daviesii as the type representative of Colaconema and, accordingly, Colaconema comprised those acrochaetioid algae with one or more plastids per cell, each with a single pyrenoid. 'Au.' daviesii, however, is not the type of Colaconema. Rather, as Papenfuss (1945) noted, Batters (1896) included three species in his initial description of Colaconema: C. bonnemaisoniae, C. chylocladiae and C. reticulatum. Of these, only C. bonnemaisoniae was found to possess the characteristics upon which the genus was based: cup-like cells subtending the monosporangia. Thus, C. bonnemaisoniae should be regarded as the type species for this genus and Stegenga's (1985) typification and concept are therefore invalid (cf. Woelkerling, 1971).

As stated previously, our data set includes only one certain representative of the genus *Colaconema*: *C. asparagopsis*. The relationship between *C. asparagopsis* and *C. bonnemaisoniae* has been the subject of considerable discussion (Woelkerling, 1971; Dixon & Irvine, 1977; Magne, 1977; Garbary et al., 1982). Most authors agree that the two are exceedingly similar in almost every respect, and the relationships between them and other closely related species (C. americana Jao, C. simplex Inagaki and C. delisea (Levring) Garbary) require further investigation. Our material of C. asparagopsis agrees with the detailed description provided by Magne (1977), but differs from that of Dixon & Irvine (1977) in that our plants possess a single parietal plastid with one pyrenoid (as opposed to multiple small discoid plastids with no pyrenoids). Given these similarities, we consider our sample to be representative of the genus Colaconema and we herein refer all species within the Colaconemataceae to the genus *Colaconema* as an interim step toward improving taxonomy in the Acrochaetiales sensu lato.

Since *Colaconema* now includes species which do not possess the cup-like monosporangia-bearing cells from the original description, the following is an emendation to the generic delineation of the genus and a list of the included taxa, including new combinations.

Colaconema Batters emend. J. T. Harper *et* G. W. Saunders

Marine acrochaetioid algae; cells with one to several parietal plastids of varying shape (lobed to spiral to irregular), with or without pyrenoids; plants reproducing primarily by monosporangia; where known, sexual life histories triphasic.

TYPE SPECIES AND BASIONYM: C. bonnemaisoniae Batters (1896: 8).

SYNONYMS: *Chantransia bonnemaisoniae* (Batters) Levring (1937: 94) and others as listed in Garbary (1987).

Colaconema amphiroae (Drew) J. T. Harper *et* G. W. Saunders, *comb. nov*.

BASIONYM: *Rhodochorton amphiroae* Drew (1928: 179).

SYNONYMS: *Acrochaetium amphiroae* (Drew) Papenfuss (1945: 312); *Audouinella amphiroae* (Drew) Garbary in Hansen *et al.* (1981: 117).

Colaconema asparagopsis Chemin (1926: 902)

Colaconema caespitosa (J. Agardh) J. T. Harper *et* G. W. Saunders, *comb. nov*.

BASIONYM: *Callithamnion caespitosum* J. Agardh (1851: 18).

SYNONYMS: Acrochaetium caespitosum (J. Agardh) Nägeli (1861: 405); Audouinella caespitosa (J. Agardh) Dixon in Parke *et* Dixon (1976: 590); *Chantransia caespitosa* (J. Agardh) Batters (1896: 9); *Rhodothamniella caespitosa* (J. Agardh) Feldmann (1954: 68).

Colaconema dasyae (Collins) J. T. Harper *et* G. W. Saunders, *comb. nov*.

BASIONYM: *Acrochaetium dasyae* Collins (1906: 191).

SYNONYMS: *Audouinella dasyae* (Collins) Woelkerling (1973*b*: 545); *Chantransia dasyae* (Collins) Collins (1911: 186).

Colaconema daviesii (Dillwyn) Stegenga (1985: 317)

Colaconema endophytica (Batters) J. T. Harper *et* G. W. Saunders, *comb. nov*.

BASIONYM: *Acrochaetium endophyticum* Batters (1896: 386).

SYNONYMS: Audouinella endophytica (Batters) Dixon in Parke et Dixon (1976: 590); Chantransia endophytica (Batters) De Toni (1924: 63).

Colaconema pectinata (Kylin) J. T. Harper *et* G. W. Saunders, *comb. nov*.

BASIONYM: *Chantransia pectinatum* Kylin (1906: 120).

SYNONYMS: Acrochaetium pectinatum (Kylin) Hamel (1927: 103); Audouinella pectinata (Kylin) Papenfuss (1945: 326); Rhodochorton pectinatum (Kylin) Rosenvinge (1935: 7).

Colaconema proskaueri (West) J. T. Harper *et* G. W. Saunders, *comb. nov*.

BASIONYM: *Acrochaetium proskaueri* West (1972: 383).

SYNONYMS: *Audouinella proskaueri* (West) Garbary in Garbary, Hansen *et* Scagel (1982: 45).

Colaconema rhizoidea (Drew) J. T. Harper *et* G. W. Saunders, *comb. nov*.

BASIONYM: *Rhodochorton rhizoideum* Drew (1928: 182).

SYNONYMS: Acrochaetium rhizoideum (Drew) Jao (1937: 102); Audouinella rhizoidea (Drew) Garbary (1979: 490).

Colaconema tetraspora (Garbary *et* Rueness) J. T. Harper *et* G. W. Saunders, *comb. nov*.

BASIONYM: *Audouinella tetraspora* Garbary *et* Rueness (1980: 17).

Having *Colaconema* serve as the sole genus within the Colaconemataceae is not the final solution to the problem of generic delineation within the Colaconematales, but it is a foundation for further research to build a revised and comprehensive classification for these organisms. The molecular phylogenies produced herein provide a template for assessing characteristics within each of these lineages. Extensive morphological and anatomical observations combined with the sequencing of key taxa are most certainly required to elucidate exactly how many genera and possibly families should ultimately be recognized within the Colaconematales.

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