AN ABSTRACT OF THE DISSERTATION OF

John A. Wheeler for the degree of Doctor of Philosophy in Botany and Plant Pathology presented on January 30, 1998. Title: Molecular Phylogenetic Analyses of *Riccia* and Marchantiales.

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Abstract approved: _____

Aaron Liston

This dissertation consists of three main subproject manuscripts. In **manuscript 1**, preliminary molecular phylogenies of the Marchantiales are presented. The marchantioid sample includes 10 carpocephalate taxa and 24 acarpocephalate taxa (emphasizing *Riccia*), *Monoclea*, *Sphaerocarpos*, and *Riella*. Three Metzgeriales (*Fossombronia*, *Pellia* and *Blasia*), the hornwort *Anthoceros*, four mosses and *Coleochaete* are also sampled. Cladistic analyses are based on three culled nucleotide sequence alignments: 1) partial nuclear-encoded Large Subunit rDNA 2) the plastid-encoded *trn*L- region and 3) combined data. Relative rate tests reveal significant heterogeneity in the nuclear LSU rDNA data. *Lunularia* positions as the most basal of sampled Marchantiopsida; Sphaerocarpales, *Marchantia* and *Corsinia* represent early diverging lines. Monophyletic Aytoniaceae, Cleveaceae and *Riccia* are indicated. Topologies imply that extant acarpocephalate taxa are derived from carpocephalate forms. *Monoclea* positions well within Marchantiales *sensu stricto*. A well-supported long branch unites all sampled Marchantiopsida and isolates this clade from other liverworts and bryophytes. An unresolved marchantioid polytomy follows the well-supported basal nodes. This polytomy may correspond to an explosive radiation of taxa coincident with extreme conditions and ecological reorganizations of the Permo-Triassic. In **manuscript 2**, focused analyses of *Riccia* are presented. Nuclear, plastid and combined data strict consensus topologies based on 17-18 species of *Riccia* (representing 5/8 of subgenera) are largely congruent with respect to terminal groups; basal resolution is poor, the possible signature of an explosive initial species radiation

during the Permo-Triassic. Unexpected placement of several taxa is well-supported suggesting a propensity in *Riccia* for volatile morphology not reflected in the underlying genetic history. In **manuscript 3**, an alternative hypothesis is articulated to explain the origin of a marchantialean complex thallus from a *Sphaerocarpos*- or *Geothallus*-like model. The complex thallus is envisioned to have originated from a transitional form with a highly regularized, bilaterally-symmetrical reticulum of fused dorsal lappets. This lappet-modular hypothesis is largely derived from the concepts of Burgeff (1943, Verlag von Gustav Fischer, Jena) and Doyle (1962, *University of California Publications in Botany* 33: 185-268) and attempts to reconcile the novel observations of both workers.

Molecular Phylogenetic Analyses of *Riccia* and Marchantiales

by

John A. Wheeler

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented January 30, 1998
Commencement June 1998

Doctor of Philosophy dissertation of John A. Wheeler presented on January 30, 1998

APPROVED:

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Major Professor, Representing Botany and Plant Pathology

Redacted for Privacy

Chair of Department of Botany and Plant Pathology

Redacted for Privacy

Dean of Graduate School

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John A. Wheeler, Author

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1.1. Introduction

What biological innovations occurred on planet earth during the origin of land plants? What was the chain of events in that original mysterious arena of embryophyte radiation across the Paleozoic terrestrial landscape? Of extant relictual taxa, which most closely resemble any of those original morphological experiments? And can a glimpse of any of those original experiments be inferred despite the cumulative haze of morphological and molecular autapomorphy?

Liverworts (Table 1.1) derive from some of the earliest land plant experiments and almost certainly trace back to the initial radiation of terrestrial eoembryophytes (reviewed by Kenrick and Crane 1997). Microfossils assigned to the "bryophyte grade" first appear about 480 MYA in the mid-Ordovician (Gray et al. 1982, Graham 1993). Microfossil evidence [spores, cuticule-like sheets and tube-like fragments] suggests that some of the first land plant morphologies "may have outwardly resembled modern prostrate, thalloid liverworts" (Gray and Shear 1992; Graham 1993).

Extant marchantioid liverworts (Marchantiopsida: consisting of Monocleales, Sphaerocarpales and Marchantiales) are the heterogeneous terminal taxa of an extremely old lineage. Long phylogenetic isolation from other extant bryophyte stem groups (other liverworts, mosses and hornworts) is supported by several recent molecular phylogenetic analyses (Waters et al. 1992; Capesius 1995; Bopp and Capesius 1996; Capesius and Bopp 1997; Lewis et al. 1997; Wheeler, in prep. [Chapter 2]). Taken together, these studies affirm that extant marchantioids are monophyletic and suggest that this clade may well trace back to an ancestor that appeared near the dawn of land plant evolution. The concept of a basal or near-basal Marchantiopsida

Table 1.1. Current higher level classification of liverworts (Hepaticae), combining features from Bartholomew-Begin (1990), Schuster (1992b) and Crandall-Stotler (1997).

Subdivision	**Class**	**Order**
Hepaticae (liverworts)	Jungermanniopsida	Jungermanniales
		Calobryales
		Treubiales
		Metzgeriales
	Marchantiopsida	Marchantiales
		Monocleales
		Sphaerocarpales

Table 1.2. Intraordinal classification of traditional Marchantiales *sensu stricto*. After Bischler (1988) following Schuster (1979). Genera sampled in this study are indicated in bold face.

Order	**Suborder**	**Family**	***Genus***	**No. of species**
Marchantiales	**Corsiniineae**	Corsiniaceae	***Corsinia***	1
			Cronisia	1
	Carrpineae	Monocarpaceae	*Monocarpus*	1
	Targioniineae	Aitchisoniellaceae	*Aitchisoniella*	1
		Targioniaceae	***Targionia***	3
			Cyathodium	10
	Marchantiineae	Lunulariaceae	***Lunularia***	1
		Wiesnerellaceae	*Wiesnerella*	1
		Conocephalaceae	*Conocephalum*	2
		Aytoniaceae	***Reboulia***	1
			Mannia	10
			Asterella	20
			Cryptomitrium	1
			Plagiochasma	16
		Cleveaceae	***Athalamia***	6
			Sauteria	2
			Peltolepis	1
		Exormothecaceae	*Exormotheca*	7
			Stephensoniella	1
		Marchantiaceae	***Marchantia***	45
			Preissia	1
			Bucegia	1
			Neohodgsonia	1
			Dumortiera	1
		Monosoleniaceae	*Monosolenium*	1
	Ricciineae	Oxymitraceae	***Oxymitra***	2
	(riccioids)	Ricciaceae	***Ricciocarpus***	1
			Riccia	~ 200

within embryophytes was suggested earlier based on a wide variety of morphological and biochemical characters (Mishler and Churchill 1984; Mishler and Churchill 1985; Mishler 1986; Bremer et al. 1987).

Macrofossils similar to modern Metzgeriales (e.g. *Pallaviciniites* and *Blasiites*) begin to appear by the mid-Paleozoic (Devonian and lower-Carboniferous, respectively). No definitive Marchantialean macrofossils (i.e. those that exhibit preserved air pores) are documented until the Triassic (reviewed in Krassilov and Schuster 1984), a discrepancy of over 150 million years. However, new micro- and macrofossil evidence suggests that marchantioids did indeed originate in the Paleozoic. Transmission electron microscopic analysis of spore wall ultrastructure led Taylor (1995) to assign putative sphaerocarpalean-hepatic affinity to the Lower Silurian microfossil *Dyadospora*. A recently-discovered coalified Lower Devonian macrofossil containing spore tetrads "similar to those first recorded in the Ordovician" exhibit a suite of "individual cellular features [that] match those in extant hepatics" (Edwards et al 1995); these authors go on to discuss gametophytic features "reminiscent of" Marchantiales.

Based on ecology and modern distributions of putatively relictual taxa, Schuster (1981, 1984, 1992b) argues that the jungermannioids (Jungermanniopsida) and marchantioids (Marchantiopsida) followed distinct evolutionary paths from the beginning and diversified into very different sorts of terrestrial habitats. According to Schuster, extant putatively-relictual Jungermanniopsida are concentrated in relatively equitable, shady habitats with cool, moist oceanic climates while extant Marchantiopsida are concentrated in seasonally-warm, seasonally-dry, strongly-illuminated habitats with continental climates (Schuster 1984). He speculates that jungermannioids might have evolved from ancestors that penetrated inland via river and stream drainages by exploiting water-saturated terrestrial microhabitats such as rills, cascades and splash zones; in contrast, marchantioids may trace back to "amphibious ancestors that invaded the fluctuating margins of shallow lakes and ponds - environments subject to desiccation" (Schuster 1981; 1992c: p. 25). Fluctuating desiccation-prone marginal microhabitats are also proposed by Graham (1993) to explain how a charophycean *Coleochaete*-like alga might have invaded terrestrial

surfaces. A very similar scenario is presented (or implied) by Mishler and Churchill (1985: figure 5) and followed by Niklas (1997: figure 4.7).

Marchantiales *sensu stricto* currently consists of five suborders, 14 families and 28 genera (Bischler 1988). Of these 28 genera, 16 are monotypic and three are ditypic (Table 1.2). Gametophytes are morphologically simple to relatively complex. Tissue organization is typically very complex relative to other liverwort groups, with structurally-intricate photosynthetic and non-photosynthetic (storage) layers; many taxa exhibit elaborated air chambers. Sporophytes are associated with an extensive variety of auxiliary gametophytic structures; these various units are then submerged/ sessile on the vegetative thallus or elevated on specialized branch-like organs called carpocephala. "Structural reorganizations [of reproductive and/or vegetative structures] are frequent" (Bischler 1988). Long phylogenetic isolation of extant forms coupled with apparent widespread extinction of linking morphologies, frustrates the assessment of homology among modern terminal taxa (Schuster 1992b). The pattern of past evolution is obscure even among relatively character-rich, carpocephalate groups (Perold 1994). Marchantiales is characterized by its morphologically distinct monotypes; however, the order does contain a few speciose radiations, e.g. *Marchantia* (with about 45 species; Bischler 1988) and *Riccia* (perhaps 200 species; Perold 1991).

In *Riccia*, individual plants are mostly small (thalli generally 0.5-4 mm wide) and often occur as flat rosette-forming gametophytes. In *Riccia* we see the simplest sporophyte of any extant land plant. There is no carpocephalum; the sporophyte is submerged and virtually hidden in the tissues of the vegetative thallus. There is apparently no foot or seta (Schuster 1992b). At maturity, the spherical sporophyte consists merely of spores enclosed in a delicate capsule. Spores can be among the largest exhibited by any liverwort; these are typically very thick-walled, durable and long-lived; spores are passively released upon decay of the capsule wall and surrounding thallus.

Numerically speaking, *Riccia* basically occupies its own suborder within Marchantiales (i.e. Ricciineae, which it shares with just two other genera: monotypic *Ricciocarpus* and ditypic *Oxymitra*). The large cosmopolitan genus *Riccia* is unparalleled among marchantioid liverworts (Marchantiopsida), and perhaps all

bryophytes, with respect to **intrageneric** variation in a wide variety of characters and behaviors. Within this single genus, species vary widely in ecology, habitat, life history strategy, sexuality and cytology. Morphological variation occurs in growth form, size, color, thallus shape, thallus ornamentation, thallus ramification pattern, epidermal structure, tissue organization, ventral scale morphology, spore shape, spore ornamentation and spore size. The genus contains a spectrum from delicate ephemeral taxa to perennial xeromorphic clones (even free-floating aquatics). Some taxa are bisexual but others are weakly or strongly heterothallic-unisexual. Meiospores are usually detached but in certain taxa they are permanently united as tetrads. Spores can be trilete to apolar; spore ornamentation is smooth, verruculate, foveolate, areolate, reticulate, vermiculate or papillate. Cytological variation is "astonishing" compared to other hepatics (Schuster1992b); extensive cytological study by Bornefeld (1984; 1987; 1989) demonstrates that taxa are haploid, polyploid, aneuploid or "nothopolyploid" (n= 8, 9, 10, 12, 15, 16, 17, 18, 20, 24, or 48). The range of narrow, regional and continental endemic taxa are known. Many extant species occur as widespread intercontinental disjunct populations.

So what do we know at this point? 1) Liverworts may be among the very earliest diverging land plant lineages. 2) Marchantioid and jungermannioid liverworts are strongly isolated morphologically and genetically and apparently followed different evolutionary paths from the very beginning. 3) Extant marchantioids are a heterogeneous mix of evolutionarily stenotypic (relictual) and evolutionarily active (speciose) groups; the polarity of many characters (e.g. presence/ absence of carpocephala and air chambers) is unknown. 4) Acarpocephalate riccioids are morphologically isolated within Marchantiales and therefore may represent one of the first branching events in the marchantialean radiation.

What was the morphology and ecology of the proto- and/or eomarchantioid? What was the evolutionary trajectory (polarity) of important characters such as carpocephala and air chambers? Is the acarpocephalate genus *Riccia* relatively derived or basal? Might the collective array of putative plesiomorphies seen in extant *Riccia* represent a conceptual portal back in time to an original transmigration to land? Or do extant ephemeral colonizers of modern freshwater/dry-land transitional surfaces

represent a secondary evolution from perennial xeromorphs? Illuminating these and other tantalizing mysteries about marchantioid and riccioid phylogeny and character evolution depends on a clear comprehension of phylogenetic relationships in the Marchantiopsida.

1.2. Previous phylogenetic analyses involving marchantioid liverworts

Issues of monophyly and the phylogenetic position of Marchantiopsida have been controversial. The phylogenetic analyses of Garbary et al. (1993), based on male gametogenesis characters, place marchantioid exemplars (*Sphaerocarpos* and *Marchantia*) as paraphyletic relative to the metzgerialean liverwort *Blasia* and derived within a monophyletic bryophyte clade. Other morphological cladistic analyses of land plants position an unresolved Marchantiopsida at the base of liverworts (Hepaticae) which is, in turn, basal to a paraphyletic Bryophyta (Mishler and Churchill 1985). Most earlier molecular-based reconstructions (Mishler et al. 1992, 1994; Waters et al. 1992; Manhart 1994; Hiesel et al. 1994; Bopp and Capesius 1995; Kranz et al. 1995) are collectively characterized by a general lack of consensus. The position of Marchantiopsida remains controversial (contrast Hedderson et al. 1996 with Bopp and Capesius 1996).

Sampling within Marchantiopsida was greatly improved in two recent comparable phylogenetic projects: nuclear 18S rDNA (Bopp and Capesius 1996; Capesius and Bopp 1997) and chloroplast *rbc*L (Lewis, Mishler and Vilgalys 1997) analyses. The trees of Bopp and Capesius show a striking basal dichotomy between Marchantiopsida and another clade that includes all other bryophyte exemplars (mosses, hornworts and jungermannioids). Phylogenetic isolation and monophyly of Marchantiopsida are well supported (100% bootstrap). In their trees, Sphaerocarpales is basal to Marchantiales; Monocleales is not sampled.

In the chloroplast *rbc*L-based analyses of Lewis at al. (1997), Marchantiopsida is highly isolated (by a long branch), strongly monophyletic (high bootstrap and decay values), and near basal within liverworts; only *Haplomitrium* (Calobryales) is an earlier

branch in some topologies. Sphaerocarpales is basal to Marchantiales but shares a branch with *Lunularia* on some trees. *Monoclea* positions within Marchantiales, implying that separate ordinal status of Monocleales is unwarranted.

1.3. The position of *Riccia* among land plants

Historically, the phylogenetic position of *Riccia* has been a volatile, contentious issue. A persistent traditional view (following antithetic theory) positions *Riccia* near the base of land plants by virtue of its small gametophyte and extremely simple embedded sporophyte. For example, Ricciaceae are the first land plant morphologies presented in the popular modern textbook by Bold et al. (1987). In 1910, Cavers (following Lotsy 1909) introduced a new "phylogenetic" classification of the bryophytes based on a fundamental "*Sphaero-Riccia*" ancestral type. In their view the larger more elaborate sporophytes of other liverworts, mosses and hornworts (and tracheophytes) were derived from this *Sphaero-Riccia* ancestor (Schuster 1966).

Goebel (1910) was the first to suggest that the *Riccia*-type morphology was in fact derived, the product of extreme morphological reduction and streamlining. Schuster (1981, 1992) completely rejects the idea of an archetypal *Riccia*; the concept of an interpolated (antithetic) sporophyte is an irritation to him, an unfortunate "phoenix" of an idea that will not die. Schuster (1966) writes, " the modern systems all have one feature in common: they attempt to derive the gametophytes of the Hepaticae [indeed all plants] from erect rather than prostrate or thallose progenitors." In such modern schemes, thalloid taxa are derived.

But now a wildcard has been thrown into the debate by recent extensive study of putative algal ancestors. Comprehensive research by Graham and others (Graham 1984; 1993, Mishler and Churchill 1984, Graham, Delwiche and Mishler 1991) increasingly supports a haplobiontic (zygotic) charophycean algal ancestor of land plants. Based on a morphological cladistic analysis, Mishler and Churchill (1984) propose ("disinter" in Schuster's opinion) the idea of delayed meiosis in the transitional ancestor resulting in a quantum shift from zygotic to sporic meiosis and a resultant

hepatic archetype with extremely simple sporophytes. This sort of "interpolation scenario" has been fleshed out by Hemsley (1994) who evaluates the fossil thalloid *Parka* as a possible intermediary model between a thalloid *Coleochaete*-like form and true embryophytes.

The fossil record sheds little light on the position of *Riccia*. Schuster cites the late appearance of marchantioid fossils (relative to metzgerioids) as evidence of a later Mesozoic radiation. But putative ricciaceous fossils from the Permo-Triassic (Lundblad 1954) seem derived and xeromorphic by Schuster's own standards; delicate mesomorphic-ephemeral *Riccia* morphologies (plesiomorphic in Schuster's own estimation) might never yield recognizable fossils.

1.4. The genus *Riccia*: previous phylogenetic concepts and taxonomic history

Because of its ultimate sporophytic simplicity, *Riccia* is usually prominent in discussions of the marchantioid carpocephalum. Early attempts to model carpocephalum evolution among extant marchantioids invariably position *Riccia* at the base; progressively elaborate carpocephala evolved in progressively derived taxa (Schiffner 1895; Howe 1923; Evans 1923). Goebel (1910) suggested that *Riccia* was derived, morphologically simplified by reduction from a *Marchantia*-like (carpocephalate) ancestor. Schuster (1992c) suggests that neither linear series is useful; he argues that both *Riccia* and *Marchantia* are derived. He would derive both morphologies from a quasi-carpocephalate *Cronisia* / *Corsinia*-type ancestor, forms that exhibit a sessile (but not embedded) sporophyte and involucre.

The only previous attempt (based on isozymes) to reconstruct relationships within *Riccia* using explicit methods detected only autapomorphic variation (Dewey 1988); however, this study suggests that interspecific divergence is relatively high. With just two enzyme systems, each of 16 exemplar species (all from Subgenus *Riccia*) was resolved with a diagnostic phenotype. In a detailed isozyme study of *Riccia dictyospora* in the southeastern United States, Dewey (1989) detected a

complex of three cryptic "sibling species" with mean genetic identities of $I = 0.211 - 0.454$, values lower than found among most angiosperm congeners.

A review of taxonomic history of Ricciaceae by Duthie and Garside (1939) begins in 1696 with the works of John Ray. In 1729, Micheli presented names and illustrations for *Riccia*, *Lunularia*, *Blasia*, *Marchantia* and *Anthoceros* (Schofield 1985). Lamy (1976) summarizes the history of classification in Marchantiales; even early systems invariably included a category for *Riccia*-like taxa (those with a submerged or sessile sporophyte that, in turn, exhibited a reduced seta and foot, i.e. *Riccia*, *Corsinia*, *Oxymitra*, and even *Sphaerocarpos*).

Perold (1995) summarizes the volatile taxonomic history of Ricciaceae during the interval: 1937-1995. She notes that the preceding 240 years was similarly marked by various "attempts to subdivide and rearrange the taxa in this large and puzzling family..." Her post-1937 taxonomic history recounts the completion of 31 regional treatments including India (Pandé and Udar 1958), New Zealand (Campbell 1975, 1979), Europe (Grolle 1976, 1983), Australia (Na-Thalang 1980), Mediterranean countries (Jovet-Ast 1986), Fennoscandia (Damsholt and Hallingbäch 1986), southern Africa (Volk and Perold; Perold 1984-1991), Latin America (Jovet-Ast 1993), North America (Schuster 1992) and sub-Saharan Africa (Perold 1995).

To date, eight subgenera have been formally designated: *Riccia* (Micheli) L. [1753]; *Ricciella* (A. Braun) Bisch. [1898]; *Thallocarpus* (Lind.) Jovet-Ast [1976]; *Leptoriccia* Schust. [1984]; *Viridisquamata* Jovet-Ast [1984]; *Chartacea* Perold [1986]; *Pannosae* Perold [1991] and *Triseriata* Jovet-Ast [1996]. Prior to Schuster (1992a), few formal taxonomic categories were designated below the subgenus and the few sections that were named typically described divergent monotypic elements within subgenera; regional workers preferred to arrange most species into informal groups or subgroups.

One especially problematic group has been subgenus *Riccia*. This group includes about 65% of the entire genus (about 120 species). In 1992, Schuster published a novel classification of subgenus *Riccia* that included 10 new sections. As justification he writes, "...the still appalling taxonomy of subg. *Riccia* reflects the fact that recent workers have not attempted its subdivision into natural subunits." Schuster

also notes the wide range of chromosome numbers in the group (n = 8, 9, 10, 12, 15, 24, 36, 48) as an indication of the need for subdivision. But Perold (1995) notes that six of Schuster's new sections are monotypic and wonders if this sort of higher-taxon name "proliferation" is really progressive. She advocates the use of informal groups in anticipation of a worldwide synthesis of regional treatments; until then, she worries that rash sectional designations will only complicate an already ponderous and tangled nomenclature.

1.5. Phylogenetic data used in this study

The nuclear-encoded ribosomal DNA (rDNA) cistron has proven to be a rich source of information for phylogeny reconstruction. Numerous studies attest to its utility for resolving recent, intermediate and ancient divergence events. The nuclear Large Subunit (LSU) rDNA gene consists of highly conserved "core" regions interspersed among "variable domains" or "expansion segments." Core region sequences exhibit the deepest phylogenetic signal; variable domain sequences ostensibly resolve divergence events in the 50-300 MYA range (Larson 1991b). Selected core and/or expansion segment sequences have been used to examine relatively deep cladogenesis in diverse organisms such as amphibians (Larson 1991a), Chlorophyta (Chapman & Buchheim 1991), metazoans (Christen et al. 1991), volvocine flagellates (Larson et al. 1992), ciliates (Baroin-Tourancheau et al. 1992), *Drosophila* (Pelandakis & Solignac 1993), basidiomycetes (Hibbett & Vilgalys 1993), oysters (Littlewood 1994), unicellular/ colonial green flagellates (Buchheim et al. 1994), frogs (Kjer 1995), dinoflagellates (Zardoya et al.1995), omphalinoid mushrooms (Lutzoni 1997), ascomycetes (Spatafora 1998) and seed plants (Kuzoff 1997; Ro et al. 1997).

A set of chloroplast primers designed to amplify across a contiguous suite of tRNA, spacer and intron sequences was introduced by Taberlet *et al.* in 1991. Like the nuclear LSU rDNA sequence, this entire sequence consists of conserved regions (various tRNA exons) interspersed by more variable regions (two intergenic spacers and a single type I intron- the *trn*L intron). Phylogenetic antiquity of the *trn*L intron is

noteworthy; this immobilized intron was apparently present prior to the divergence of the plastid from its cyanobacterial ancestor (endosymbiont) about one billion years ago (Kuhsel et al. 1990). Conserved domains and secondary structure across a broad phylogenetic range of organisms (Kuhsel et al. 1990) led Taberlet et al. (1991) to recommend this intron for "evolutionary studies at higher taxonomic levels." Sequences from the *trn*L intron and/or more conserved adjacent regions have been used recently in concert with other gene sequences to examine phylogeny in diverse plant groups such as Rhamnaceae (Richardson et al. 1997), palms (Baker et al. 1997), Cyperaceae (Yen and Olmstead 1997), leptosporangiate ferns (Ranker et al. 1997) and arthrodontous mosses (Cox and Hedderson 1997).

1.6. Research plan and organization

The initial goal of this phylogenetics project was to examine monophyly, position and deeper (higher-level) relationships within *Riccia* using nucleotide sequences from the nuclear LSU rDNA and the plastid trnL-region. Prevailing uncertainty about relationships within the Marchantiales, however, required such wide outgroup sampling that the riccioid analysis soon became nested within and simultaneous with a greater marchantioid analysis. Detailed results of the riccioid study appear in Chapter 3 of this dissertation. Relationships within and across the Marchantiopsida are presented in Chapter 2. Chapter 4 presents the argument for an alternative theory to explain the origin of a complex marchantioid thallus.

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Chapter 2

Preliminary Phylogenetic Reconstructions of the Ancient Marchantioid Liverwort Radiation

John Wheeler
Department of Botany and Plant Pathology
Oregon State University, Corvallis OR, 97331

2.1 Abstract

Preliminary molecular phylogenies of the complex-thalloid liverworts (Marchantiales) were reconstructed using independent nuclear and plastid data sets to explore relative age, relationships and character evolution in this ancient group. The marchantioid sample includes 10 carpocephalate taxa and 24 acarpocephalate taxa (emphasizing *Riccia*). *Monoclea*, *Sphaerocarpos*, *Riella*, three Metzgeriales (*Fossombronia*, *Pellia* and *Blasia*), the hornwort *Anthoceros*, four mosses and outgroup *Coleochaete* are also sampled. Cladistic analyses are based on three nucleotide sequence alignments: 1) partial nuclear-encoded Large Subunit rDNA (LSU rDNA), 2) the plastid-encoded *trn*L- region and 3) a combined data set consisting of concatenated nuclear and plastid alignments. Alignment ambiguous regions of each alignment were culled. Selected pairwise comparisons reveal significant rate heterogeneity in the nuclear LSU rDNA data; metzgerioid liverworts, hornworts and primitive mosses evolve significantly slower than other taxa relative to the outgroup *Coleochaete*. The LSU rDNA genes of some marchantioid taxa and derived mosses are apparently evolving relatively fast. Rate heterogeneity is documented within Marchantiales *sensu stricto*. *Lunularia* positions as the most basal of sampled Marchantiopsida; Sphaerocarpales, *Marchantia* and *Corsinia* represent early diverging lines. A monophyletic Aytoniaceae, Cleveaceae and *Riccia* are indicated. Topologies imply that extant acarpocephalate taxa are derived from carpocephalate forms. *Monoclea* positions well within Marchantiales *sensu stricto*. A well-supported long branch unites

all sampled Marchantiopsida and isolates this clade from other liverworts and bryophytes. This long branch may suggest extensive extinction of proto- and eomarchantioid forms that led to modern taxa. A major theme of topologies presented here is the unresolved marchantioid polytomy that follows the well-supported basal nodes. This polytomy may correspond to an explosive radiation of marchantioid forms (e.g. Aytoniaceae, Cleveaceae, *Targionia*, *Monoclea* and riccioids) coincident with extreme conditions and ecological reorganizations of the Permo-Triassic. The origin of Marchantiopsida probably occurred long before; amidst, perhaps, a series of long-extinct *Blasia*-like ancestors that colonized and innovated on any of various xeric surfaces (either cool or warm) that were available throughout embryophyte history in the Paleozoic.

2.2 Introduction

Within extant liverworts, Schuster (1958, 1984, 1992b) emphasizes the "profound" differences between Jungermannidae (Jungermanniopsida) and Marchantiidae (Marchantiopsida) and invokes these two groups as the earliest phylogenetic divergence in liverwort evolutionary history: his "initial dichotomy" (Schuster 1984: p. 913). Recent morphology- and molecular-based analyses basically agree with Schuster's concept and support the recognition of two fundamental liverwort stem groups: 1) Jungermanniopsida: Haplomitriales, Metzgeriales, Treubiales, and Jungermanniales and 2) Marchantiopsida: Sphaerocarpales, Marchantiales, and Monocleales (Bartholomew-Began 1990; Bopp and Capesius 1996; Crandall-Stotler 1997; Lewis et al. 1997). Throughout the remainder of this paper, I will refer to taxa from these two stem groups as the 'jungermannioids' and 'marchantioids' respectively.

Monophyly and the phylogenetic position of Marchantiopsida have been controversial. The phylogenetic analyses of Garbary et al. (1993), based on male gametogenesis characters, place marchantioid exemplars (*Sphaerocarpos* and *Marchantia*) as paraphyletic relative to the metzgerialean liverwort *Blasia* and derived within a monophyletic bryophyte clade. Other morphological cladistic analyses of land

plants position an unresolved Marchantiopsida at the base of liverworts (Hepaticae) which is, in turn, basal to a paraphyletic Bryophyta (Mishler and Churchill 1985). Due to limited and inconsistent taxon sampling, most earlier molecular-based reconstructions (Mishler et al. 1992, 1994; Waters et al. 1992; Manhart 1994; Hiesel et al. 1994; Bopp and Capesius 1995b; Kranz *et al.* 1995) are collectively characterized by a general lack of consensus. The position of Marchantiopsida remains controversial (contrast Hedderson et al. 1996 with Capesius and Bopp 1997).

Sampling within Marchantiopsida was greatly improved in two recent comparable phylogenetic projects: nuclear 18S rDNA (Bopp and Capesius 1996; Capesius and Bopp 1997) and chloroplast *rbc*L (Lewis, Mishler and Vilgalys 1997) analyses. The trees of Bopp and Capesius show a striking basal dichotomy between Marchantiopsida and another clade that includes all other bryophyte exemplars (mosses, hornwort and jungermannioids). Phylogenetic isolation and monophyly of Marchantiopsida is well supported (100% bootstrap). In their trees, Sphaerocarpales is basal to Marchantiales; Monocleales is not sampled.

In the chloroplast *rbc*L-based analyses of Lewis at al. (1997), Marchantiopsida is highly isolated (by a long branch), strongly monophyletic (high bootstrap and decay values), and near basal within liverworts; only *Haplomitrium* (Calobryales) is an earlier branch in some topologies. Sphaerocarpales is basal to Marchantiales but shares a branch with *Lunularia* on some trees. *Monoclea* positions within Marchantiales. In addition to topological isolation, marchantioids are distinct from other embryophyte lineages by a significantly slower relative rate of sequence divergence in the *rbc*L gene. Using the charophycean alga *Coleochaete* as reference, Lewis at al. show that other sampled embryophytes (including *Haplomitrium* + other jungermannioids) typically accumulate twice as many nucleotide transitions per unit time. Slower relative substitution rate is apparently not limited to the plastid; in 11 of 12 mitochondrial genes surveyed by Laroche et al. (1995), *Marchantia* was significantly slower ($P > 0.01$) than angiosperms (rooted on the chlorophyte alga *Prototheca*).

Blepharoplast features indicate that among extant jungermannioids examined to date, only *Blasia* resembles sampled Marchantiopsida (Rushing et al. 1995; Brown et al. 1995; Pass and Renzaglia 1995). Based on spermatid morphology and the occurrence

of archaic monoplastidic meiosis, Pass and Renzaglia (1995) recommend elevating *Blasia* (and *Cavicularia*) to the Order Blasiales; moreover, these authors also recommend realigning Blasiales into the marchantioid stem. Neither Lewis at al. (1997) nor Bopp and Capesius (1996) sample *Blasia*; to my knowledge no previous study has sequenced this important taxon.

The nuclear-encoded ribosomal DNA (rDNA) cistron has proven to be a rich source of information for phylogeny reconstruction. Numerous studies attest to its utility for resolving recent, intermediate and ancient divergence events. The nuclear Large Subunit (LSU) rDNA gene consists of highly conserved "core" regions interspersed among "variable domains" or "expansion segments." Core region sequences exhibit the deepest phylogenetic signal; variable domain sequences ostensibly resolve divergence events in the 50-300 MYA range (Larson 1991b). Selected core and/or expansion segment sequences have been used to examine relatively deep cladogenesis in diverse organisms such as amphibians (Larson 1991a), Chlorophyta (Chapman & Buchheim 1991), metazoans (Christen et al. 1991), volvocine flagellates (Larson et al. 1992), ciliates (Baroin-Tourancheau et al. 1992), *Drosophila* (Pelandakis & Solignac 1993), basidiomycetes (Hibbett & Vilgalys 1993), oysters (Littlewood 1994), unicellular/ colonial green flagellates (Buchheim et al. 1994), frogs (Kjer 1995), dinoflagellates (Zardoya et al.1995), omphalinoid mushrooms (Lutzoni 1997), ascomycetes (Spatafora 1998) and seed plants (Kuzoff 1997; Ro et al. 1997).

A set of chloroplast primers designed to amplify across a contiguous suite of tRNA, spacer and intron sequences was introduced by Taberlet et al. in 1991. Like the nuclear LSU rDNA sequence, this entire sequence consists of conserved regions (various tRNA exons) interspersed by more variable regions (two intergenic spacers and a single type I intron- the *trn*L intron). Phylogenetic antiquity of the *trn*L intron is noteworthy; this immobilized intron was apparently present prior to the divergence of the plastid from its cyanobacterial ancestor (endosymbiont) about one billion years ago (Kuhsel et al. 1990). Conserved domains and secondary structure across a broad phylogenetic range of organisms (Kuhsel et al. 1990) led Taberlet et al. (1991) to recommend this intron for "evolutionary studies at higher taxonomic levels." Sequences from the *trn*L intron and/or more conserved adjacent regions have been used recently in

concert with other gene sequences to examine phylogeny in diverse plant groups such as Rhamnaceae (Richardson et al. 1997), palms (Baker et al. 1997), Cyperaceae (Yen and Olmstead 1997), leptosporangiate ferns (Ranker et al. 1997) and arthrodontous mosses (Cox and Hedderson 1997).

The genus *Riccia* is unparalleled in the Marchantiales (and perhaps all Hepaticae) with respect to intrageneric variation in diverse features such as morphology, cytology, life history and ecology. This worldwide genus is a large (± 200 species) and taxonomically puzzling group. Taxonomic history and concepts have been somewhat confusing and idiosyncratic (Perold 1995) and a higher-level comprehension of the entire group has been largely intractable based on morphological characters alone. The initial goal of this study was to examine monophyly, phylogenetic position and deeper (higher-level) relationships within *Riccia* using nucleotide sequences from the nuclear LSU rDNA and the plastid trnL-region. Prevailing uncertainty about relationships within the Marchantiales, however, required such wide outgroup sampling that the 'riccioid' analysis soon became essentially simultaneous with a greater 'marchantioid' analysis. Detailed results of the 'riccioid' study will appear elsewhere (Wheeler, in prep. [Chapter 3]). This paper presents an examination of relationships within and across the Marchantiopsida. The topologies presented here are considered preliminary; more conclusive results await dense sampling of the complete range of extant marchantioid diversity.

2.3. Materials and Methods

Tissues were field-collected or acquired as gifts of duplicate herbarium material (Table 2.1). Single clones were sampled whenever this was possible to ascertain. A sample of young (apical meristematic) tissue was placed into a plastic tube with water and vigorously shaken in a vortexer to free attached soil particles and other contaminants. This process was repeated until water changes contained no apparent debris. These washed tissues were then carefully examined under a dissecting scope to detect any attached foreign tissues (i.e. moss protonemata, minute plant rootlets, etc.).

Table 2.1. Sample taxa used in this study with voucher details. **NN** = Nalini Nadkarni; **SMP** = S. M. Perold; **WM** = Wes Messinger. **OSC** = Oregon State University, USA; **PRE** = Pretoria, RSA; **UC** = University of California, Berkeley, CA, USA.

Taxon	Voucher details
ALGAL OUTGROUP	
Coleochaete scutata	OSC; Wheeler 265; Carolina Bio. Supply Co.; Lot # 15-2128; 19 Feb 1996
MOSSES	
Dendroalsia abietina	OSC; Wheeler 254; Avery Park, Benton Co.; Oregon, USA; 19 Oct 1995
Metaneckera menziesii	OSC; Wheeler 253; Avery Park, Benton Co.; Oregon, USA; 19 Oct 1995
Sphagnum recurvum	OSC; Wheeler 263; Mercer Lake, Lane Co.; Oregon, USA; 30 Dec 1995
Tetraphis pellucida	OSC; Wheeler 258; Tenmile Creek, Lane Co.; Oregon, USA; 30 Dec 1995
HORNWORTS	
Anthoceros punctatus 1	OSC; Wheeler 124; Quartz Cr., Josephine Co.; Oregon, USA; 24 Apr 1994
Anthoceros punctatus 2	OSC; Wheeler 256; Adair Village, Benton Co.; Oregon, USA; 26 Dec 1995
LIVERWORTS	
Asterella bolanderi	UC; Norris 80866; southern Sierra Nevada Mtns., California, USA; Apr 1993
Asterella californica	UC; Norris 80914; southern Sierra Nevada Mtns., California, USA; Apr 1993
Asterella gracilis	OSC; Wheeler 221; Eagle Cr., Hood River Co.; Oregon, USA; 15 Apr 1995
Athalamia hyalina	OSC; Wheeler 219; Columbia R.,Multnomah Co.; Oregon, USA; 15 Apr 1995
Blasia pusilus	OSC; Wheeler 233; Santiam River, Linn Co.; Oregon, USA; 8 Jul 1995
Corsinia coriandrina	OSC; Wheeler 166; near Bastrop, Bastrop Co.; Texas, USA; 31 Mar 1995
Cryptomitrium tenerum	UC; Norris 80911; southern Sierra Nevada Mtns., California, USA; Apr 1993
Fossombronia foveolata	OSC; Wheeler 257; Yakina Head, Lincoln Co.; Oregon, USA; 29 Dec 1995
Lunularia cruciata	OSC; Wheeler 201; OSU campus, Benton Co.; Oregon, USA; 12 Apr 95
Marchantia polymorpha	OSC; Wheeler 236; Deschutes R., Deschutes Co.; Oregon, USA; 8 Jul 1995
Monoclea gottschei	OSC; Wheeler 247 (from NN); Monte Verde, Costa Rica; 3 Aug 1995
Oxymitra cristata	PRE; Koekemoer 1024 (from SMP); Olifantshoek, Cape, Africa; Dec 1992
Oxymitra incrassata	OSC; Wheeler 180; near Willow City, Gillespie Co.;Texas, USA; 3 Apr 1995
Pellia epiphylla	OSC; Wheeler 098; Issaquah, King Co.; Washington, USA; 21 Apr 1994
Peltolepis quadrata	OSC; Wagner 8198; Elkhorn Mtns., Baker Co.; Oregon, USA; 19 Aug 1996
Plagiochasma rupestre	OSC; Wheeler 005 (from WM); Brewster Co., Texas, USA; Dec 1991
Reboulia hemisphaerica	OSC; Wheeler 229; Skamania, Skamania Co.; Washingon, USA; 16 apr 1995
Riccia albida	OSC; Wheeler 454; near Sonora, Sutton Co.; Texas, USA; 9 Jan 97
Riccia albolimbata	OSC; Wheeler 455; near Sonora, Sutton Co.; Texas, USA; 9 Jan97
Riccia atromarginata	OSC; Wheeler 450; Squaw Pk., Phoenix, Pima Co.; Arizona, USA; 5 Jan 97
Riccia beyrichiana	OSC; Wheeler 172; near Utley, Bastrop Co.; Texas, USA; 1 Apr 1995
Riccia cavernosa	OSC; Wheeler 252; near Monroe, Benton Co.; Oregon, USA; 8 Jul 1995
Riccia frostii	OSC; Wheeler 234; Smith Rocks, Deschutes Co.; Oregon, USA; 8 Jul 1995
Riccia gougetiana	OSC; Wheeler 169; near Paige, Bastrop Co.; Texas, USA; 31 Mar 1995
Riccia huebeneriana	OSC; Wheeler 249; White R., Washington Co.; Arkansas, USA; 17 Oct 1995
Riccia lamellosa	OSC; Wheeler 493; Murrieta, Riverside Co.; California, USA; 15 Jan 1997
Riccia macrocarpa	OSC; Wheeler 204; Tehama Co.; California, USA; 13 Apr 1995
Riccia membranacea	OSC; Wheeler 248; White R., Washington Co.; Arkansas, USA; 17 Oct 1995
Riccia nigrella	OSC; Wheeler 086; Murrieta, Riverside Co.; California, USA; 30 Dec 1993
Riccia papulosa	OSC; Camacho 1283; Frankland River; Western Australia; 20 Jun 1995
Riccia schelpei	PRE: Oliver 9873 (from SMP); Namaqualand, NW Cape, Africa; 29 Jun 1991
Riccia sorocarpa	OSC; Wheeler 567; OSU campus, Benton Co.; Oregon, USA; 30 May 1997
Riccia tomentosa	PRE; Perold 2157 (from SMP); Namaqualand, Cape, Africa; 29 Aug 1988
Riccia trichocarpa	OSC; Wheeler 509; Griffin Park, Josephine Co.; Oregon, USA; 5 Apr 1997
Riccia villosa	PRE; Oliver 8039 (from SMP); Khamiesberg, Cape, Africa; 01 Sep 1983
Ricciocarpus natans (1)	OSC; Wheeler 251; near Monroe, Benton Co.; Oregon, USA; 19 Oct 95
Ricciocarpus natans (2)	OSC; Wheeler 218; Willamette Park, Benton Co.; Oregon, USA; 15 Apr 95
Riella americana	OSC; Wheeler 453; Davis Mtns., Jeff Davis Co.; Texas, USA; 8 Jan 1997
Sphaerocarpos texanus (1)	OSC; Wheeler 231; Corvallis, Benton Co.; Oregon, USA; 17 Apr 1995
Sphaerocarpos texanus (2)	OSC; Wheeler 053; Willamette Park, Benton Co.; Oregon, USA; 5 Apr 1993
Targionia hypophylla	OSC; Wheeler 446; Squaw Peak, Maricopa Co.; Arizona, USA; 4 Jan 97

Live contaminant tissues are an ever-present danger in field-collected marchantioid specimens because in nature these often occur in intimate association with mosses, hornworts and even cryptic terrestrial jungermannioids (e.g. virtually filamentous *Cephaloziella* sp.).

In early stages of this study, total genomic DNA was extracted according to the CTAB micro-prep method of Doyle and Doyle (1987) with minor modifications (see Liston and Wheeler 1994). In later stages, DNA was extracted using DNeasy Plant Mini Kits (Qiagen, Chatsworth, CA) following the manufacturer's protocol.

Nuclear-encoded partial LSU rDNA amplicons (PCR-derived gene segments) and plastid-encoded *trn*L-region amplicons (Figures 2.1 and 2.2, respectively) were produced by polymerase chain reaction (PCR). Forward primer ITS3 (White et al. 1990) and reverse primer LR1010 (designed for this study) were used to amplify the nuclear amplicon. Forward primer C and reverse primer F (Taberlet 1991) were used to amplify the plastid amplicon (Table 2.2). These same external primers and other internal primers (Table 2.2) were then used in subsequent sequencing reactions. Each PCR reaction mixture (100 µl) contained: 10 mM Tris-HCl, pH8.3; 50 mM KCl; 1.5-2.0 mM MgCl2; 0.005% Tween 20 ; 0.005% NP-40; 0.001% gelatin; 0.1 mM each dATP, dTTP, dCTP and dGTP; 50 pmol of each primer; and 2.5 units of Replitherm polymerase (Epicentre Technologies, Madison, WI).

Reaction mixtures were covered with mineral oil and heated to 72 C (Erlich et al. 1991) prior to the addition of genomic DNA. Each of 35 PCR cycles (MJ Research thermocycler) was programmed as follows: 94 C for 1 min, 57 C for 45 s and 72 C for 2 min with a 6 min additional final extension step. Reactions were then held at 10 C on the thermocycler block until removed. The shorter *trn*L-region amplicons were usually produced in 50 µl reactions. Experimentation with alternative DNA polymerases i.e. Amplitherm (Epicentre Technologies, Madison, WI) or Taq (Promega: Madison, WI), was sometimes necessary when using total DNA isolations derived from older dried material. Products were visualized with ethidium bromide on 1% agarose gel. Satisfactory amplicons were gel-purified (Qiagen, Chatsworth, CA) and then processed by cycle sequencing and dye-terminator chemistry on an ABI model 373A or 377 automated fluorescent sequencer at the Oregon State Univ. Central Services Laboratory.

Figure 2.1. Map of the nuclear-encoded LSU rDNA region and PCR amplicon used in this study.

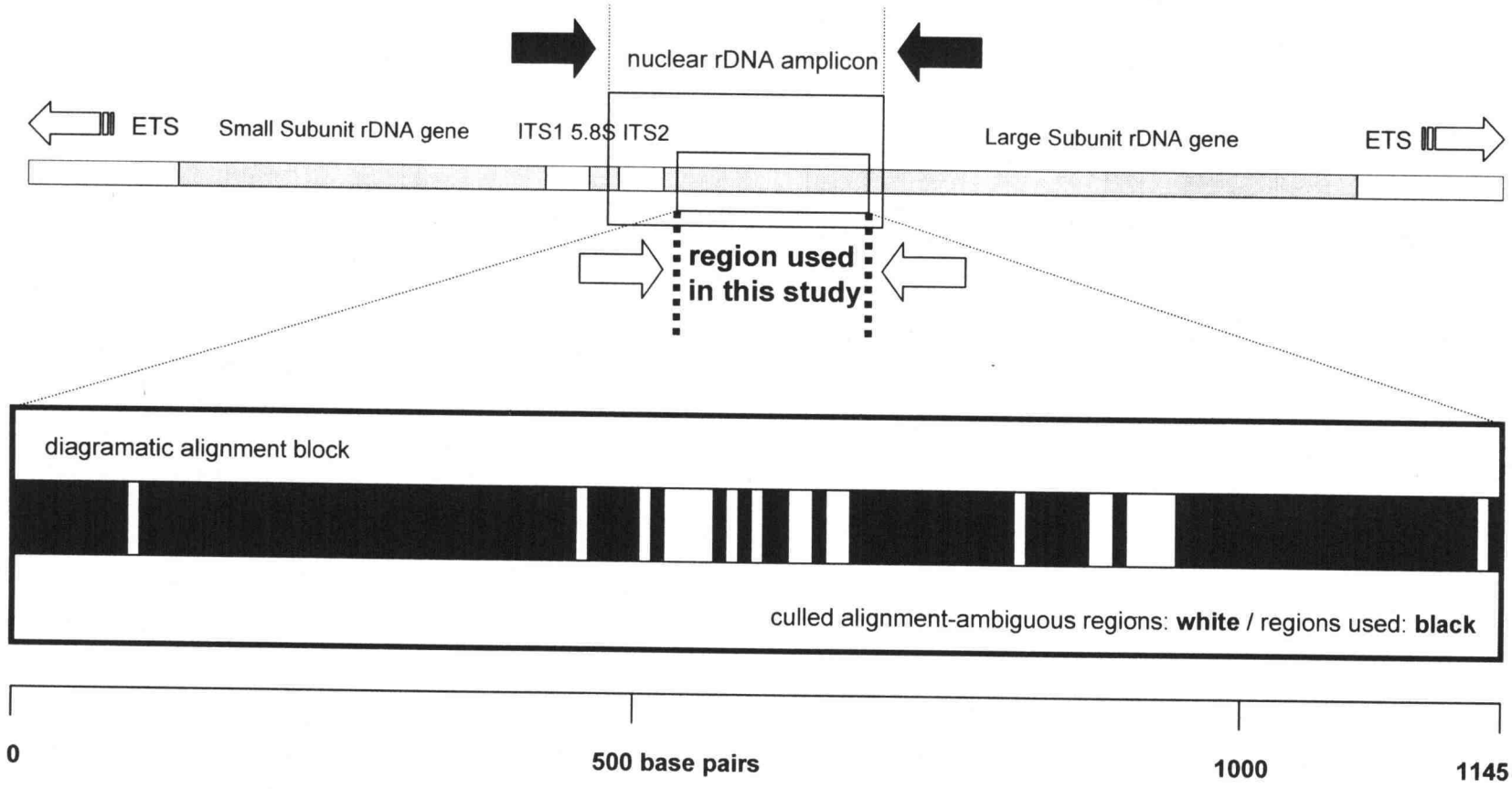
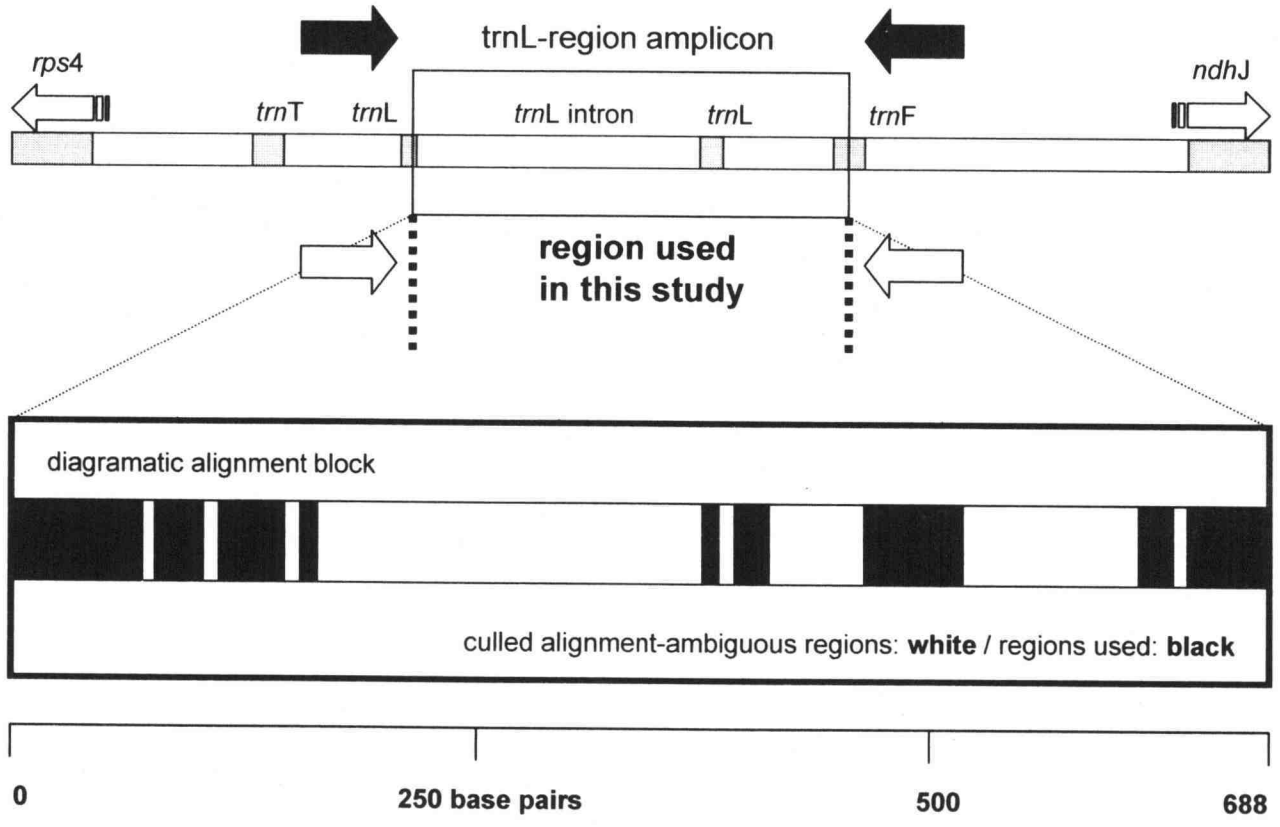

nuclear rDNA amplicon
ETS
Small Subunit rDNA gene
ITS1 5.8S ITS2
Large Subunit rDNA gene
ETS
region used
in this study
diagramatic alignment block
culled alignment-ambiguous regions: white / regions used: black
0
500 base pairs
1000
1145

Figure 2.2. Map of the plastid-encoded *trn*L-region and PCR amplicon used in this study.


trnL-region amplicon
rps4
trnT
trnL
trnL intron
trnL
trnF
ndhJ
region used
in this study
diagramatic alignment block
culled alignment-ambiguous regions: white / regions used: black
0
250 base pairs
500
688

Wild-collected liverwort thallus tissues generally contain endophytic fungi; higher (more stringent) annealing temperatures were used when the standard reaction conditions produced unwanted (putative fungal) bands. The initial sequencing read from each amplicon was compared to GenBank and EMBL databases with a BLASTN similarity search (Altschul et al. 1990) for early detection of mistakenly amplified sequences (for a discussion of this problem see Camacho et al. 1997).

Table 2.2. Primer sequences used for PCR amplification and sequencing in this study. Arrows designate direction of primer. Tm is the calculated melting (annealing) temperature. Primers designed specifically for this project are so indicated; the 3' position of these primers in the LSU rDNA gene (relative to *Lycopersicon*) are indicated by the numbers incorporated into each primer name.

Name		Sequences 5′-3′	Tm	Source:
NUCLEAR				
ITS3	⇨	GCAACGATGAAGAACGCAGC	64.3	White et al. 1990
LR1010	⇦	GCCTCTAATCATTGGCTTTACC	59.1	this study
LF47	⇨	ACCCGCTGAGTTTAAGCATATC	58.1	this study
LR654	⇦	TTGGTCCGTGTTTCAAGACG	62.1	this study
PLASTID				
Universal C	⇨	CGAAATCGGTAGACGCTACG	60.8	Taberlet et al. 1991
Universal F	⇦	ATTTGAACTGGTGACACGAG	56.1	Taberlet et al. 1991

The nuclear-encoded LSU rDNA subproject involved sequencing 36 marchantioid exemplars, three metzgerioid liverworts, two hornworts, four mosses and the alga *Coleochaete* (Table 2.1). The LSU rDNA taxon sample includes duplicate *Ricciocarpus natans*, *Sphaerocarpos texanus* and *Anthoceros punctatus* accessions as internal controls. Sampling for the plastid *trn*L-region subproject was limited to marchantioids only (Marchantiopsida: Marchantiales, Sphaerocarpales and Monocleales) and the outgroup *Blasia*. Marchantioid sampling was equivalent across

the two data sets (nuclear vs. plastid) except that *Riccia papulosa* is missing in the *trn*L-region data set.

Sequence files were manipulated using GCG8 (Genetics Computer Group 1994) or GCG9 (Genetics Computer Group 1996). An initial automated alignment generated with the Pileup program in GCG (gap creation penalty = 2.0; gap length penalty = 0.2) was imported into GDE (Genetic Data Environment: Smith et al. 1994) for manual adjustment and the convenient creation of NEXUS files. Alignment-ambiguous blocks of positions were excluded from both the LSU rDNA and *trn*L-region alignments. In this way, one preferred "culled" alignment (Gatesy et al. 1994) was obtained for each of the two data sets. A copy of the full LSU rDNA culled alignment (48 taxa) was trimmed down to a marchantioids-only culled alignment (37 taxa). This derivative LSU rDNA marchantioids-only alignment and the plastid *trn*L-region culled alignment were analyzed separately and then combined in a "total evidence" analysis.

The UNIX test version 4.0d59 of PAUP* (David L. Swofford) on a SUN 670 MP computer was used for unweighted parsimony analyses. Alignment gaps were treated as missing data. Heuristic search options were set as follows: 100 replicate searches (nreps=100) with random addition sequences (addseq=rand), no maxtrees limit and tree bisection and reconnection (TBR) branch swapping. In PAUP* these settings automatically report any occurrence of islands of equally most-parsimonious trees (Maddison 1991). Bootstrap support (Felsenstein 1985) for each topology was determined using the "simple addition sequence" option, mulpars = on and maxtrees = 500 in PAUP*. Tree files generated with PAUP* were examined and manipulated using the program TREEVIEW (Page 1996). Decay values were calculated using the clade constraint method (Eernisse and Kluge 1993) as described by Morgan (1997). The full LSU rDNA (48 taxa) analysis was rooted on *Coleochaete*. Separate LSU rDNA and *trn*L-region "marchantioids only" analyses were each rooted on *Blasia*. The combined (nuclear+plastid) analysis was rooted on *Blasia*.

Selected pairwise and groupwise relative rate tests were performed on nuclear LSU rDNA sequences using version 2.0 of PHYLTEST (Kumar 1995). This program calculates relative rate using the two-cluster test of Takezaki, Rzhetsky and Nei (1995)

and enables the user to contrast individual sequences (pairwise) or multiple sequences (groups or clades).

2.4. Results

2.4.1 *Sequences and alignments*

The individual PCR-amplified LSU rDNA sequences vary in length from 941 bp (*Coleochaete*) to 1015 bp (*Athalamia*). After manual adjustment and masking of ambiguous sites, the final full (48 taxa) LSU rDNA culled alignment (Appendix 1) is 905 bp in length. Pairwise sequence divergence (uncorrected *p* distance), calculated from this culled alignment, ranges from 0.003 (*Riccia sorocarpa* / *R. trichocarpa*) to 0.155 (*Athalamia* / *Dendroalsia*). Compared to the outgroup *Blasia*, marchantioid sequence divergence ranges from 0.061 (*Sphaerocarpos*) to 0.103 (*Athalamia*). Homogeneity of base frequencies across taxa was confirmed (P = 1.000) with the Chi-square test in PAUP*. Observed means and ranges of base frequencies are **A**: 0.243 (0.237-0.255); **C**: 0.242 (0.231-0.255); **G**: 0.337 (0.317-0.348); **T**: 0.177 (0.165-0.197).

The *trn*L-region amplicon sequences vary in length from 458 bp (*Riella americana*) to 577 bp (*Reboulia hemisphaerica*). Following adjustments and masking of ambiguous sites, the final *trn*L-region (36 taxa) culled alignment (Appendix 2) is 348 bp in length. Based on this culled alignment, pairwise sequence divergence (uncorrected *p* distance: ranges from 0.003 (*Riccia frostii* / *R. cavernosa*) to 0.127 (*Riccia albolimbata* / *Blasia*). Relative to the outgroup *Blasia*, sequence divergence among other sample taxa ranges from 0.086 (*Peltolepis*) to 0.127 (*Riccia albolimbata*). Base frequencies are homogeneous across taxa (P = 1.000: Chi-square test); means and ranges are **A**: 0.361 (0.347-0.375); **C**: 0.167 (0.157-0.179); **G**: 0.198 (0.184-0.208); **T**: 0.274 (0.259-0.288).

2.4.2. *Relative rate tests*

Selected pairwise comparisons (Table 2.3) reveal significant rate heterogeneity in the nuclear LSU rDNA data set; metzgerioid liverworts (*Fossombronia* and *Blasia*),

Table 2.3. Selected pairwise relative rate tests performed on nuclear LSU rDNA sequences using version 2.0 of PHYLTEST (Kumar 1995). Pairwise uncorrected *p* distance values are above the diagonal; relative rate test Z-scores are below the diagonal. Bold-face Z-scores are significant at the 5% level; bold-underlined values are significant at the 1% level. Arrows point to the taxon with a faster rate of sequence evolution. Pairwise distance to *Coleochaete* is indicated at the top of the table.

Coleochaete	0.098	0.102	0.102	0.104	0.106	0.110	0.124	0.126	0.128	0.130	0.136	0.137	0.143	0.151
sequence divergence (uncorrected "p") / relative rate scores (pairwise)	Fossombronia	Blasia	Anthoceros	Sphagnum	Tetraphis	Pellia	Lunularia	Riella	Sphaerocarpos	Marchantia	Metaneckera	Dendroalsia	Monoclea	Corsinia
Fossombronia		0.025	0.044	0.036	0.041	0.041	0.079	0.081	0.075	0.095	0.079	0.088	0.103	0.109
Blasia	0.793		0.051	0.041	0.043	0.045	0.067	0.067	0.062	0.080	0.081	0.091	0.093	0.096
Anthoceros	0.585	0.068		0.059	0.047	0.061	0.097	0.097	0.093	0.109	0.087	0.096	0.124	0.123
Sphagnum	0.769	0.329	0.254		0.049	0.073	0.094	0.091	0.091	0.105	0.087	0.096	0.114	0.114
Tetraphis	1.134	0.606	0.485	0.193		0.060	0.096	0.091	0.089	0.107	0.076	0.080	0.123	0.121
Pellia	**1.985**	1.315	0.971	0.678	0.561		0.093	0.095	0.087	0.103	0.106	0.115	0.115	0.113
Lunularia	**2.899**	**2.677**	**2.230**	**2.032**	1.875	1.485		0.055	0.051	0.056	0.119	0.127	0.080	0.071
Riella	**3.141**	**2.932**	**2.372**	**2.238**	**2.171**	1.663	0.241		0.035	0.057	0.114	0.123	0.074	0.073
Sphaerocarpos	**3.594**	**3.421**	**2.677**	**2.550**	**2.496**	**2.022**	0.576	0.412		0.054	0.116	0.125	0.072	0.066
Marchantia	**3.239**	**3.095**	**2.633**	**2.447**	**2.325**	1.930	0.189	0.486	0.189		0.124	0.135	0.063	0.064
Metaneckera	**4.121**	**3.717**	**3.567**	**3.313**	**3.453**	**2.498**	1.114	1.018	0.745	0.606		0.033	0.139	0.140
Dendroalsia	**3.972**	**3.596**	**3.463**	**3.229**	**3.438**	**2.476**	1.162	0.963	0.812	0.678	0.199		0.146	0.151
Monoclea	**4.379**	**4.239**	**3.632**	**3.618**	**3.354**	**3.147**	**2.139**	**2.048**	1.848	1.745	0.622	0.511		0.073
Corsinia	**5.047**	**5.009**	**4.298**	**4.367**	**4.115**	**3.906**	**3.210**	**3.008**	**2.907**	**2.725**	1.265	1.119	0.911	

the hornwort *Anthoceros* and primitive mosses (*Sphagnum* and *Tetraphis*) evolve significantly slower than other taxa relative to the outgroup *Coleochaete*. The LSU rDNA genes of some marchantioid taxa (e.g. *Corsinia* and *Monoclea*) and derived mosses (*Dendroalsia* and *Metaneckera*) are apparently evolving relatively fast ($P < 0.001$). The marchantioids *Lunularia*, *Sphaerocarpos*, *Riella* and *Marchantia* exhibit an intermediate rate of sequence evolution.

Relative rate tests that compare putative clades or intuitive groups are summarized in Table 2.4. Rate heterogeneity is documented within Marchantiales *sensu stricto*; i.e. the *Oxymitra* clade evolves slower than remaining pooled Marchantiales while sampled Cleveaceae and *Corsinia* are evolving significantly faster than other pooled Marchantiales. Within *Riccia* certain pairwise tests are significant (not shown); however, no rate difference could be detected between xeromorphic (perennial clone-forming) species and a numerically balanced sample of mesophytic (ephemeral) species.

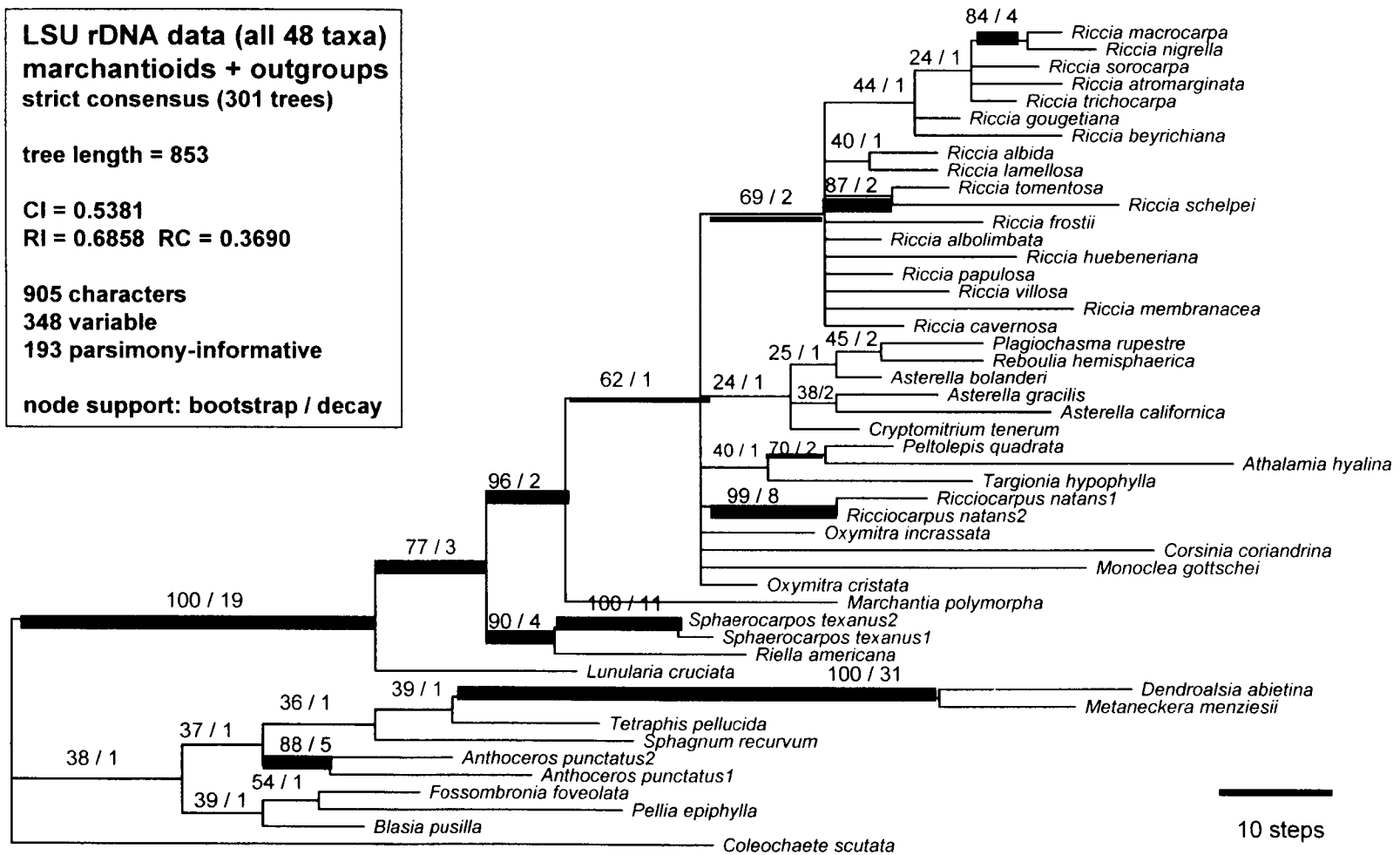
2.4.3. *Phylogenetic analyses*

Analysis 1: culled nuclear LSU rDNA alignment [all 48 taxa]: This alignment exhibits 557 constant sites, 348 variable sites and 193 informative sites. Heuristic searching of the full LSU rDNA culled alignment with unweighted parsimony results in 301 shortest trees distributed among four islands (216, 44, 14 and 27 trees respectively), tree length = 853, CI = 0.5381, RI = 0.6858, RC = 0.3690. The strict consensus of these 301 trees (Figure 2.3) places *Lunularia* at the base of sampled Marchantiopsida. *Riella* and *Sphaerocarpos* (Sphaerocarpales) are monophyletic but intercalated between *Lunularia* and *Marchantia*. The later taxon is basal to remaining marchantioids (including *Monoclea*) which radiate as a polytomy. Sampled Aytoniaceae, Cleveaceae and *Riccia* form monophyletic groups, respectively. *Targionia* positions on a branch with Cleveaceae. The marchantioid clade (all sampled Marchantiopsida) is strongly supported by bootstrap and decay values (100% and 19 steps respectively). A monophyletic *Riccia* is indicated with moderate support (bootstrap 69%; decay = 2). Strict consensus trees obtained for each of the four islands separately (not shown), differ chiefly in the relative positions of acarpocephalate marchantioid taxa. The relative

Table 2.4. Relative rate tests that compare putative clades or intuitive groups. Analyses were performed on nuclear LSU rDNA sequences using version 2.0 of PHYLTEST (Kumar 1995). Relative rate test Z-scores are above the diagonal; arrows below the diagonal point to the taxon or clade with a faster relative rate of sequence evolution. Bold-face Z-scores are significant at the 5% level; bold-underlined values are significant at the 1% level. 1 = pooled Marchantiales; 2 = pooled *Riccia*; 3 = sample of four xeromorphic *Riccia* species i.e. *Riccia nigrella*, *R. atromarginata*, *R. lamellosa*, *R. albolimbata*; 4 = sample of four mesomorphic *Riccia* species i.e. *Riccia frostii*, *R. cavernosa*, *R. membranacea*, *R. huebeneriana*. The number of exemplar taxa included in each clade is shown in parentheses.

Relative rate scores (group/ group) / comparisons showing unequal rate	Metzgeriales (3 sp.)	basal mosses(2 sp.)	hornworts (2 sp.)	Sphaerocarpales (3)	Oxymitra (2 sp.)	Marchantiales (%) [1]	Aytoniaceae (6 sp.)	Riccia (%) [2]	Riccia (4 xero sp.) [3]	Riccia (4 meso sp.) [4]	derived mosses (2)	Monoclea (1)	Cleveaceae (2 sp.)	Corsinia (1 sp.)
Metzgeriales (3)		0.286	0.419	**3.127**	**3.040**	**3.961**	**3.709**	**3.899**	**3.770**	**3.959**	**3.704**	**4.092**	**4.370**	**4.861**
basal mosses (2)			0.738	**2.671**	**2.639**	**3.426**	**3.237**	**3.382**	**3.278**	**3.435**	**3.832**	**3.675**	**3.935**	**4.477**
hornworts (2)				**2.940**	**2.901**	**3.632**	**3.480**	**3.555**	**3.448**	**3.609**	**4.082**	**3.901**	**4.144**	**4.594**
Sphaerocarpales (3)	⇦	⇦	⇦		0.353	1.387	1.323	1.302	1.163	1.476	0.985	**2.123**	**2.441**	**3.200**
Oxymitra (2)	⇦	⇦	⇦			**1.978**	1.144	1.454	1.212	1.595	0.756	**2.426**	**3.060**	**3.424**
Marchantiales (%) [1]	⇦	⇦	⇦		⇦		1.923	1.167	1.166	0.631	0.256	1.336	**2.216**	**2.873**
Aytoniaceae (6)	⇦	⇦	⇦					0.027	0.169	0.242	0.210	1.459	**1.992**	**2.500**
Riccia (%) [2]	⇦	⇦	⇦						0.497	0.660	0.222	1.446	1.784	**2.523**
Riccia (4 xero.) [3]	⇦	⇦	⇦							0.682	0.284	1.552	1.874	**2.545**
Riccia (4 meso.) [4]	⇦	⇦	⇦								0.106	1.202	1.454	**2.328**
derived mosses (2)	⇦	⇦	⇦									0.584	0.649	1.225
Monoclea (1)	⇦	⇦	⇦	⇦	⇦								0.036	0.910
Cleveaceae (2)	⇦	⇦	⇦	⇦	⇦	⇦	⇦							1.035
Corsinia (1)	⇦	⇦	⇦	⇦	⇦	⇦	⇦	⇦	⇦	⇦				

Figure 2.3. Full nuclear LSU rDNA data: strict consensus tree (all 48 taxa). Heuristic search of the full LSU rDNA culled alignment with unweighted parsimony. Tree length = 853, CI = 0.5381, RI = 0.6858, RC = 0.3690.


LSU rDNA data (all 48 taxa)
marchantioids + outgroups
strict consensus (301 trees)
tree length = 853
CI = 0.5381
RI = 0.6858 RC = 0.3690
905 characters
348 variable
193 parsimony-informative
node support: bootstrap / decay
84 / 4
Riccia macrocarpa
Riccia nigrella
24 / 1
Riccia sorocarpa
44 / 1
Riccia atromarginata
Riccia trichocarpa
Riccia gougetiana
Riccia beyrichiana
40 / 1
Riccia albida
Riccia lamellosa
87 / 2
Riccia tomentosa
69 / 2
Riccia schelpei
Riccia frostii
Riccia albolimbata
Riccia huebeneriana
Riccia papulosa
Riccia villosa
Riccia membranacea
Riccia cavernosa
45 / 2
Plagiochasma rupestre
25 / 1
Reboulia hemisphaerica
Asterella bolanderi
62 / 1
24 / 1
38/2
Asterella gracilis
Asterella californica
Cryptomitrium tenerum
40 / 1
70 / 2
Peltolepis quadrata
Athalamia hyalina
96 / 2
Targionia hypophylla
99 / 8
Ricciocarpus natans1
Ricciocarpus natans2
Oxymitra incrassata
77 / 3
Corsinia coriandrina
Monoclea gottschei
Oxymitra cristata
100 / 19
100 / 11
Marchantia polymorpha
90 / 4
Sphaerocarpos texanus2
Sphaerocarpos texanus1
Riella americana
Lunularia cruciata
100 / 31
39 / 1
Dendroalsia abietina
Metaneckera menziesii
36 / 1
Tetraphis pellucida
37 / 1
Sphagnum recurvum
88 / 5
38 / 1
Anthoceros punctatus2
Anthoceros punctatus1
54 / 1
Fossombronia foveolata
39 / 1
Pellia epiphylla
Blasia pusilla
Coleochaete scutata
10 steps

positions of non-marchantioid outgroups, *Lunularia*, *Sphaerocarpos*, *Riella* and *Marchantia* are identical across all trees (all islands). Constraining Sphaerocarpales to the base of remaining marchantioids required three additional steps; topologies that constrain *Monoclea* to the base required 17 additional steps.

Analysis 2: culled nuclear LSU rDNA alignment [marchantioids rooted on *Blasia*]: This alignment contains 645 constant sites, 260 variable sites and 114 informative sites. Heuristic searching (with *Coleochaete* and other non-marchantioid outgroups removed), results in 287 shortest trees distributed among four islands (215, 15, 43 and 14 trees respectively), tree length =557, CI = 0.5583, RI = 0.5907, RC = 0.3298. The strict consensus tree of all 287 trees (not shown) results in a marchantioids topology identical to that seen in the strict consensus tree of Analysis 1.

Analysis 3: culled plastid *trn*L-region alignment [marchantioids rooted on *Blasia*]: This alignment contains 238 constant sites, 108 variable sites and 52 informative sites. Heuristic searching results in a single island of 663 shortest trees, tree length = 215, CI = 0.6512, RI = 0.7292, RC = 0.4749. Rooted on *Blasia*, the strict consensus of these trees (Figure 2.4) positions *Sphaerocarpos* and *Marchantia* at the base of sampled Marchantiopsida. Remaining taxa are largely unresolved; however, a derived riccioid clade is suggested consisting of all *Riccia* exemplars, *Ricciocarpus* and both *Oxymitra* exemplars (bootstrap 70%; decay = 1). A monophyletic *Oxymitra* is strongly supported by the *trn*L- region data with bootstrap and decay values of 94% and 4, respectively.

Analysis 4: combined alignment [marchantioids rooted on *Blasia*]: This alignment contains 874 constant sites, 368 variable sites and 164 informative sites. This analysis results in 11 shortest trees distributed in two islands (10 and 1 tree respectively), tree length = 788, CI = 0.5698, RI = 0.5871, RC = 0.3345. Strict consensus of all eleven trees (Figure 2.5) indicates that the relative positions of *Lunularia*, *Riella*, *Sphaerocarpos* and *Marchantia*, based on the nuclear data, are not changed by the addition of the plastid data. A monophyletic Aytoniaceae is upheld. Putative affinity of *Targionia* with Cleveaceae is preserved; *Monoclea* positions at the base of this *Targionia* + Cleveaceae clade. Monophyly of the genus Riccia is upheld; however, monophyly of suborder Ricciineae (*Riccia*, *Ricciocarpus* and *Oxymitra*) is equivocal in the combined analysis. Strict consensus of the ten trees in Island 1 (Figure 2.6)

Figure 2.4. Plastid trnL-region data: strict consensus tree (marchantioids + *Blasia*). Heuristic search with unweighted parsimony results in a single island of 663 shortest trees, tree length = 215, CI = 0.6512, RI = 0.7292, RC = 0.4749.

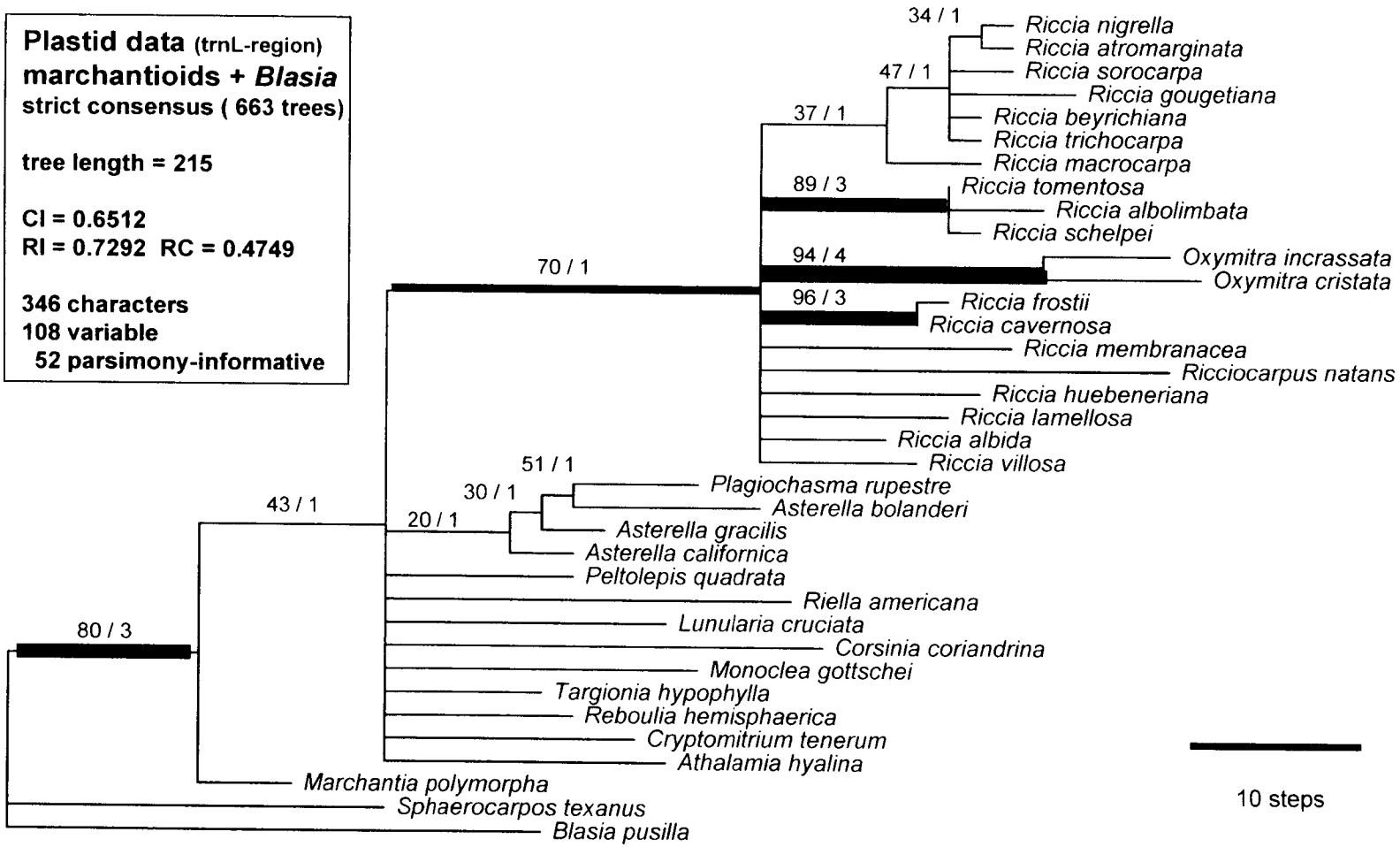

Plastid data (trnL-region)
marchantioids + Blasia
strict consensus (663 trees)
tree length = 215
CI = 0.6512
RI = 0.7292 RC = 0.4749
346 characters
108 variable
52 parsimony-informative
34 / 1
Riccia nigrella
Riccia atromarginata
47 / 1
Riccia sorocarpa
Riccia gougetiana
37 / 1
Riccia beyrichiana
Riccia trichocarpa
Riccia macrocarpa
89 / 3
Riccia tomentosa
Riccia albolimbata
Riccia schelpei
94 / 4
Oxymitra incrassata
Oxymitra cristata
70 / 1
96 / 3
Riccia frostii
Riccia cavernosa
Riccia membranacea
Ricciocarpus natans
Riccia huebeneriana
Riccia lamellosa
Riccia albida
Riccia villosa
51 / 1
Plagiochasma rupestre
30 / 1
Asterella bolanderi
43 / 1
20 / 1
Asterella gracilis
Asterella californica
Peltolepis quadrata
Riella americana
80 / 3
Lunularia cruciata
Corsinia coriandrina
Monoclea gottschei
Targionia hypophylla
Reboulia hemisphaerica
Cryptomitrium tenerum
Athalamia hyalina
Marchantia polymorpha
Sphaerocarpos texanus
Blasia pusilla
10 steps

Figure 2.5. Combined data: strict consensus tree (marchantioids + *Blasia*).). Heuristic search with unweighted parsimony. This analysis results in 11 shortest trees distributed in two islands (10 and 1 tree respectively), tree length = 788, CI = 0.5698, RI = 0.5871, RC = 0.3345.

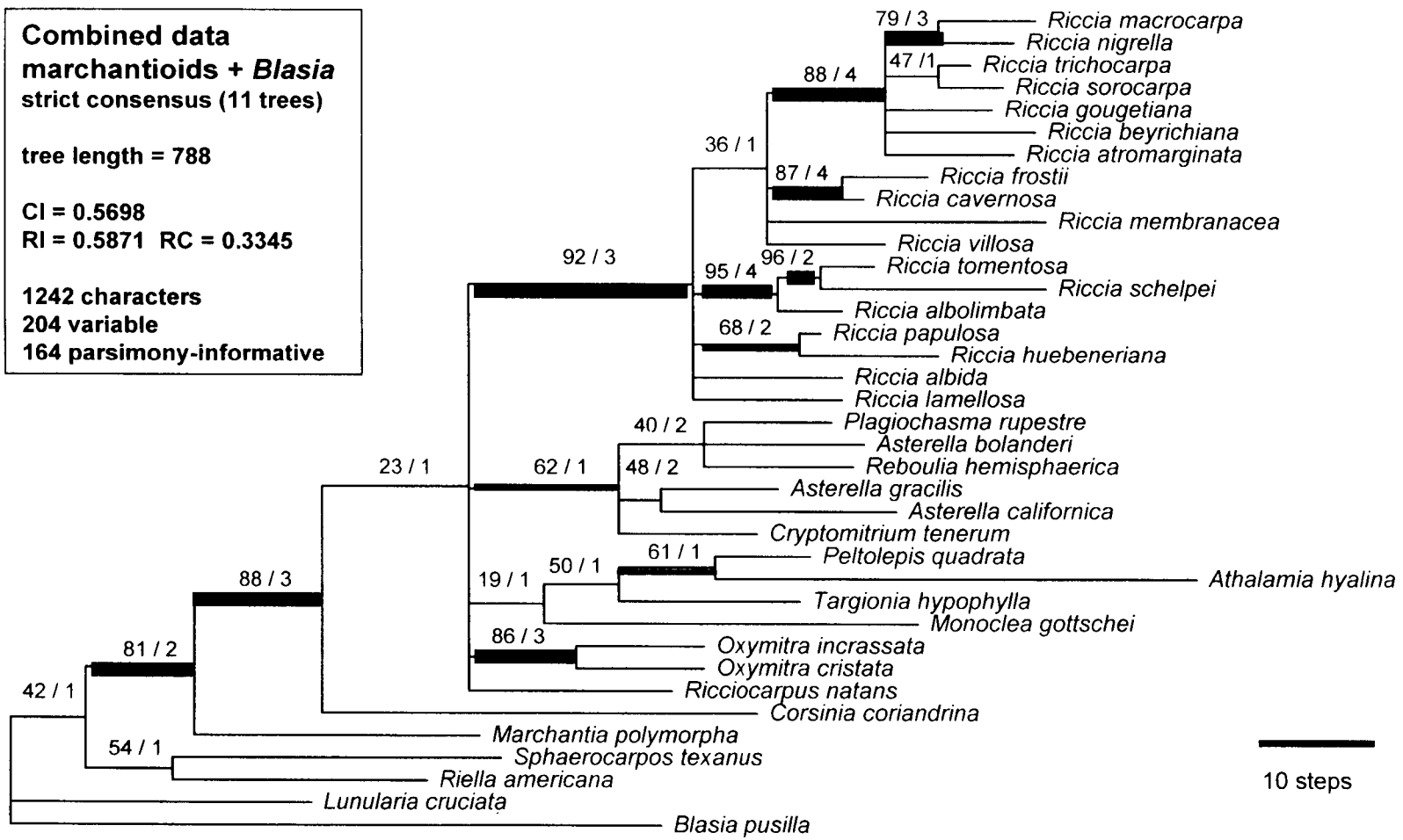

Combined data
marchantioids + Blasia
strict consensus (11 trees)
tree length = 788
CI = 0.5698
RI = 0.5871 RC = 0.3345
1242 characters
204 variable
164 parsimony-informative
79 / 3
Riccia macrocarpa
Riccia nigrella
47 /1
Riccia trichocarpa
Riccia sorocarpa
88 / 4
Riccia gougetiana
Riccia beyrichiana
Riccia atromarginata
36 / 1
87 / 4
Riccia frostii
Riccia cavernosa
Riccia membranacea
Riccia villosa
92 / 3
96 / 2
Riccia tomentosa
95 / 4
Riccia schelpei
Riccia albolimbata
68 / 2
Riccia papulosa
Riccia huebeneriana
Riccia albida
Riccia lamellosa
40 / 2
Plagiochasma rupestre
Asterella bolanderi
23 / 1
62 / 1
48 / 2
Reboulia hemisphaerica
Asterella gracilis
Asterella californica
Cryptomitrium tenerum
61 / 1
Peltolepis quadrata
88 / 3
50 / 1
19 / 1
Athalamia hyalina
Targionia hypophylla
Monoclea gottschei
86 / 3
81 / 2
Oxymitra incrassata
Oxymitra cristata
42 / 1
Ricciocarpus natans
Corsinia coriandrina
Marchantia polymorpha
54 / 1
Sphaerocarpos texanus
Riella americana
Lunularia cruciata
Blasia pusilla
10 steps

Figure 2.6. Combined data: Island 1, strict consensus of 10 trees (marchantioids + *Blasia*). Heuristic search with unweighted parsimony. Tree length = 788, CI = 0.5698, RI = 0.5871, RC = 0.3345.

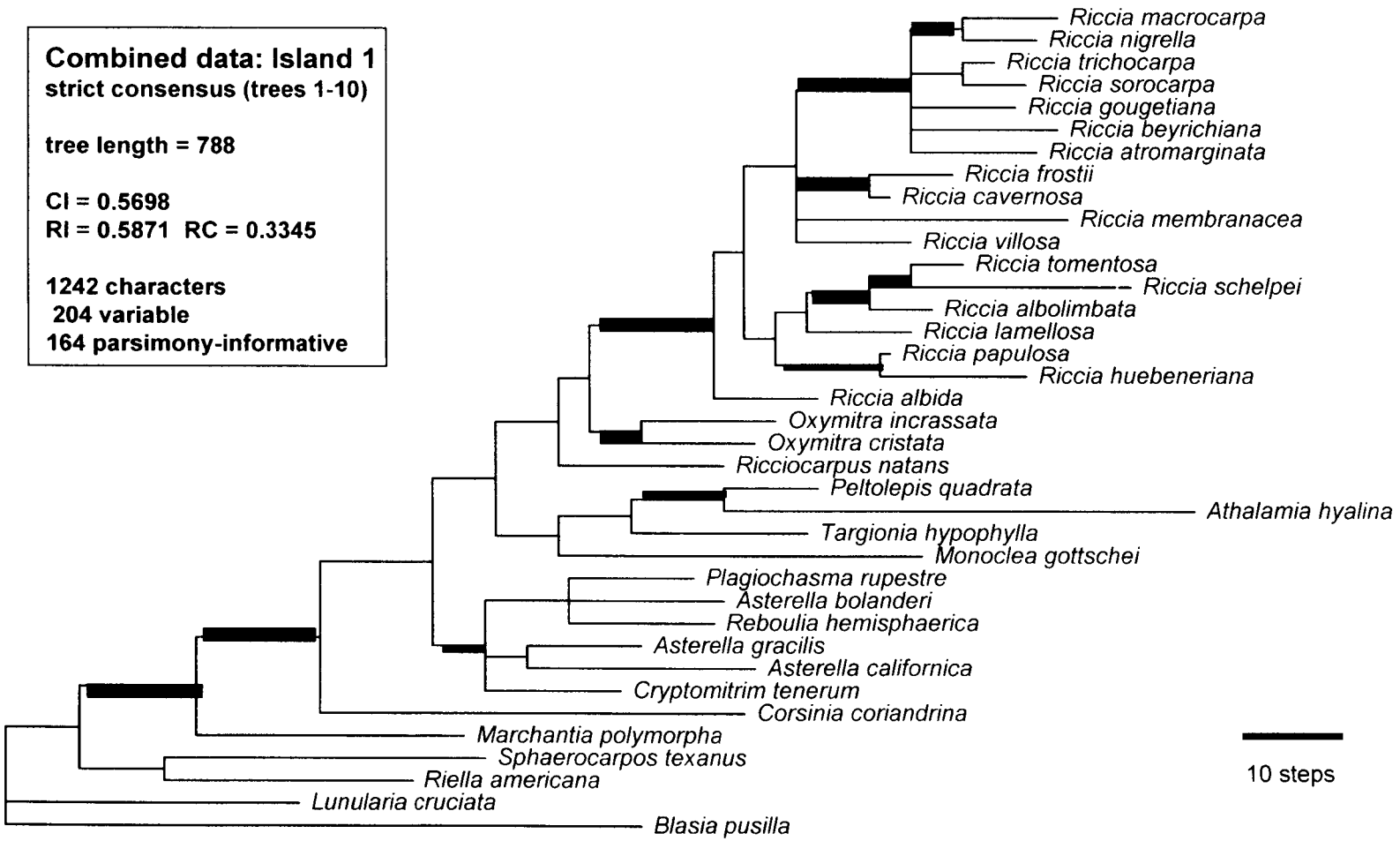
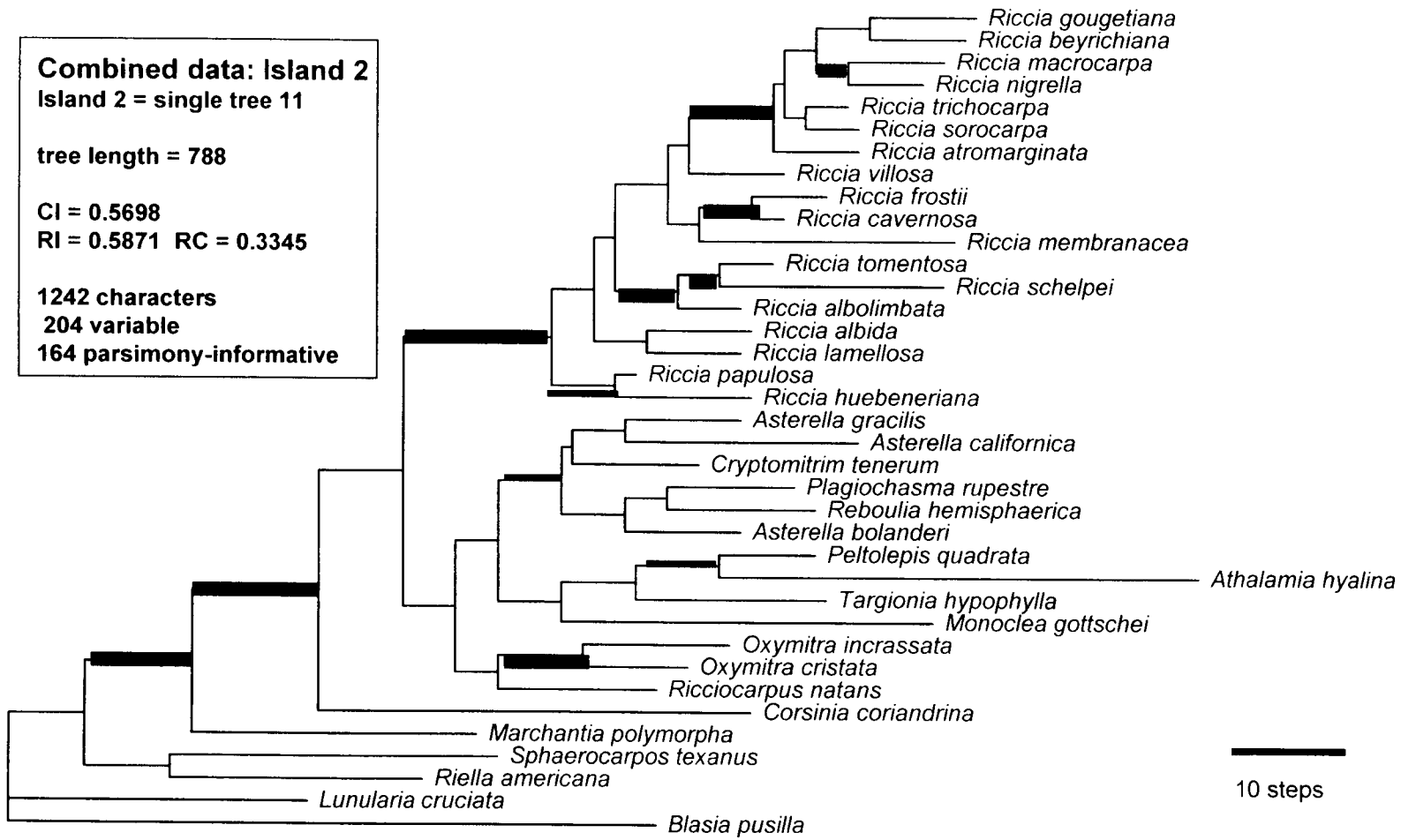

Combined data: Island 1
strict consensus (trees 1-10)
tree length = 788
CI = 0.5698
RI = 0.5871 RC = 0.3345
1242 characters
204 variable
164 parsimony-informative
Riccia macrocarpa
Riccia nigrella
Riccia trichocarpa
Riccia sorocarpa
Riccia gougetiana
Riccia beyrichiana
Riccia atromarginata
Riccia frostii
Riccia cavernosa
Riccia membranacea
Riccia villosa
Riccia tomentosa
Riccia schelpei
Riccia albolimbata
Riccia lamellosa
Riccia papulosa
Riccia huebeneriana
Riccia albida
Oxymitra incrassata
Oxymitra cristata
Ricciocarpus natans
Peltolepis quadrata
Athalamia hyalina
Targionia hypophylla
Monoclea gottschei
Plagiochasma rupestre
Asterella bolanderi
Reboulia hemisphaerica
Asterella gracilis
Asterella californica
Cryptomitrim tenerum
Corsinia coriandrina
Marchantia polymorpha
Sphaerocarpos texanus
Riella americana
Lunularia cruciata
Blasia pusilla
10 steps

Figure 2.7. Combined data: Island 2, single tree (marchantioids + *Blasia*). Heuristic search with unweighted parsimony. Tree length = 788, CI = 0.5698, RI = 0.5871, RC = 0.3345.


Combined data: Island 2
Island 2 = single tree 11
tree length = 788
CI = 0.5698
RI = 0.5871 RC = 0.3345
1242 characters
204 variable
164 parsimony-informative
Riccia gougetiana
Riccia beyrichiana
Riccia macrocarpa
Riccia nigrella
Riccia trichocarpa
Riccia sorocarpa
Riccia atromarginata
Riccia villosa
Riccia frostii
Riccia cavernosa
Riccia membranacea
Riccia tomentosa
Riccia schelpei
Riccia albolimbata
Riccia albida
Riccia lamellosa
Riccia papulosa
Riccia huebeneriana
Asterella gracilis
Asterella californica
Cryptomitrim tenerum
Plagiochasma rupestre
Reboulia hemisphaerica
Asterella bolanderi
Peltolepis quadrata
Athalamia hyalina
Targionia hypophylla
Monoclea gottschei
Oxymitra incrassata
Oxymitra cristata
Ricciocarpus natans
Corsinia coriandrina
Marchantia polymorpha
Sphaerocarpos texanus
Riella americana
Lunularia cruciata
Blasia pusilla
10 steps

supports this concept; however, Island 2 (single tree 11) suggests paraphyly of *Oxymitra* and *Ricciocarpus* relative to other marchantioids (Figure 2.7).

A survey of the three main strict-consensus topologies (nuclear, plastid and combined) shows that for several branches, support is increased in the combined tree. For example, a monophyletic *Riccia* is indicated by bootstraps of 69% and 70% in the nuclear and plastid trees, respectively; combining the data results in a bootstrap of 92%; decay values are also increased. Aytoniaceae is supported at the 24% and 0% levels, respectively; combining the data resulted in a bootstrap of 62%.

2.5. Discussion

2.5.1. *Methodological considerations and cautions*

The potential for paralogous copies of the nuclear LSU rDNA (Telford and Holland 1997) and cryptic sequence simplicity in this same gene (Bult et al. 1995) dictates the need for methodological vigilance. Cryptic sequence simplicity amounts to simple repeated motifs in ribosomal gene sequences that diverge from one another and thereby confound the determination of positional homology. I did not test for this phenomenon; however, I reason that this problem would be concentrated in variable regions of the alignment, regions that were largely culled because of alignment ambiguity. Confidence in the utility of LSU rDNA for phylogeny reconstruction is upheld in two recent studies: tree topology of the angiosperm family Ranunculaceae (Ro et al. 1997), based on ca. 1100 base pairs (both core and interspersed expansion segments), was deemed "highly congruent" with two other independently published topologies (based on *rbc*L, *atp*B and 18S rDNA). Recent analyses of complete nuclear LSU rDNA sequences from diverse angiosperms and two gnetalean outgroups "yielded topologies highly concordant with those based on analysis of 18S rDNA and *rbc*L" (Kuzoff et al. 1997).

The presence of cryptic pseudogene sequences in the data set can also confound phylogeny reconstruction (Buckler et al. 1997). While certain sequences used in this study are significantly divergent (see discussion of relative rate tests), nucleotide

composition was homogeneous across taxa. This homogeneity would not be expected if the data set were contaminated by a pseudogene sequence(s); bias toward "deamination-driven substitutions at methylation sites" is a red flag indicating the presence of a psuedogene or multiple pseudogenes (Buckler et al. 1997). An examination for this deamination phenomenon in rDNA sequences used in this study did not reveal any obvious outliers.

Sampling choices and bias can affect phylogenetic tree topology. Lewis et al. (1997) eliminated one taxon (*Jubula*) from their analyses due to a severe topological effect. Previous studies that emphasize marchantioid liverworts (e.g. Bopp and Capesius 1996; Lewis et al. 1997) have been biased toward carpocephalate taxa with ratios of 6:2 and 7:2 respectively; in contrast, this study is biased toward acarpocephalate Marchantiales (ratio 10:24).

Relatively high autapomorphic load in some sequences used in this analysis can also potentially confound their placement on the phylogeny (Felstenstein 1978). However, long branch attraction tends to pull affected taxa to the base (Aguinaldo et al. 1997; Buckler et al. 1997); if the topological positions of sequence-divergent taxa such as *Monoclea* and *Corsinia* are obscured in this study (due to long-branch attraction) then I predict that these taxa have been collapsed from even more resolved terminal positions within Marchantiales.

The issue of combining independent data sets is still widely debated (Huelsenbeck et al. 1996; Cunningham 1997a; 1997b); the relative efficacy of various data- combinability tests is also currently debated. While I did not perform combinability tests, adding the plastid data to the nuclear data (i.e. combining) did result in improved bootstrap support for several nodes without changing the fundamental topology (see Results).

2.5.2. *Phylogenetic analyses and possible implications*

The riccioid sample: Combined-Data Island 1 (Figure 2.6) depicts a monophyletic Ricciineae (*Riccia* + *Ricciocarpus* + *Oxymitra*) but Island 2 (Figure 2.7) does not. Despite equivocal support, the topology derived from Island 1 (Figure 2.6) is preferred for the following reason: operationally, both *Oxymitra* sequences and the *Ricciocarpus*

sequence readily align to the *Riccia* block of sequences in more variable (culled) portions of the *trn*L-region; based on this fact, tentative confidence in suborder Ricciineae seems reasonable. These culled regions will be added in focused analyses of the Ricciineae (Wheeler, in prep., Chapter 3).

The genus *Riccia* is a remarkably variable group with impressive **intrageneric** variation in ecology, habitat, life history strategy, gross morphology, spore morphology and cytology. Cytological variation is "astonishing" compared to other hepatics (Schuster 1992); extensive cytological study by Bornefeld (1984; 1987; 1989) demonstrates that taxa are haploid, polyploid, aneuploid or "nothopolyploid" (n=8, 9, 10, 12, 15, 16, 17, 18, 20, 24, or 48). The range of narrow, regional and continental endemic taxa are known; several species occur as intercontinental disjuncts that may have dispersed prior to the fission of Pangea (Frey and Kürschner 1988).

A wide range of morphologically and ecologically divergent *Riccia* exemplars are sampled in this study. The possibility that *Riccia* might be a polyphyletic catch-all, derived from independently reduced marchantioid lineages is not supported. All analyses point to a monophyletic *Riccia*. All topologies imply that this genus was derived, ultimately, from a carpocephalate ancestor (assuming a single origin of the carpocephalum) because both *Lunularia* and *Marchantia* are basal to *Riccia* with good support on the pertinent branches.

The position of Sphaerocarpales: The embedding of sampled Sphaerocarpales (*Riella* and *Sphaerocarpos*) between *Lunularia* and *Marchantia* is unexpected. This position may be a spurious result of homoplasious nucleotide substitutions that are attracting these sphaerocarpalean exemplars to more derived taxa in Marchantiales. However, this interpretation is contradicted by reasonably high bootstrap and decay support for these nodes in the full 48-taxon LSU rDNA topology. In the *rbc*L-based trees of Lewis et al. (1997), in fact, several trees exhibit a shared branch between *Lunularia* and Sphaerocarpales. Sphaerocarpales is basal to all Marchantiales in the trees of Bopp and Capesius (1996) but unfortunately *Lunularia* was not sampled. The sporophyte of *Lunularia* has been invoked as the most primitive type observed in extant Marchantiales (Schuster 1992b).

An admittedly unorthodox hypothesis would be that Sphaerocarpales is, in fact, derived from within basal Marchantiales *sensu stricto*. The implication would be that Sphaerocarpales is the result of reduction and specialization from carpocephalate marchantialean stock. In support of this hypothesis, consider the continuing confusion that surrounds the unique monotype *Monocarpus*. This minute ephemeral is apparently very reduced and specialized. Superficially it does resemble *Sphaerocarpos* (Scott 1985). Originally aligned with Sphaerocarpales by its discoverer (Carr 1956), the species was later transferred to Marchantiales (Proskauer 1961). Grolle (1983), citing the biochemical data of Markham (1980), returned *Monocarpus* to the Sphaerocarpales; however, Schuster (1992b) disagrees. In his original descriptions, Carr (1956) suggests that this plant might represent an intermediate morphology between Sphaerocarpales and Marchantiales. Proskauer was convinced that the main plant body consists of a highly reduced carpocephalum. Resolution of this mystery awaits future sampling of this tiny enigmatic plant.

The position of *Monoclea*: The phylogenetic position and affinities of this unusual mesophyte have been controversial. *Monoclea* exhibits a mixture of jungermannioid and marchantioid features (Schuster 1984; p.1040). On the weight of evidence, Schuster established the Order Monocleales to account for only two species, *Monoclea fosteri* and *Monoclea gottschei*; however, there is no lack of precedent in the historical literature for the concept of placing *Monoclea* within Marchantiales (e.g. Campbell 1898; Müller 1939; Burgeff 1943; Proskauer 1951; Hässel de Menendez 1962).

How might one account for the odd morphology of *Monoclea*? Rather loose morphogenetic control has been documented in some extant marchantialean taxa; atypical organization of gametangia on the thallus (e.g. bisexual receptacles) has been documented on otherwise normal thalli in *Preissia*, *Marchantia*, *Dumortiera*, *Monoselenium* and *Reboulia* (reviewed by Haupt 1926). Gross-morphologically aberrant (yet fertile) carpocephala and thalli occur in *Preissia* (Györffy 1946; Denizot 1963a; Schuster 1992b), *Marchantia* (Burgeff 1943; Denizot 1963b), *Asterella* (Pande et al. 1953) and *Reboulia* (Burgeff 1943). The exhaustive research of Burgeff (1943) meticulously documents a remarkable propensity in the genus *Marchantia* for bizarre morphologies, both in the vegetative thallus and carpocephalum.

Following a careful morphological examination of semi-aquatic *Monoclea* specimens collected in Jamaica, Johnson (1904) concluded that the "absence of air chambers and ventral scales is probably due to the nearly aquatic habit of the plant." Extreme simplification and/or reduction of both air pores and ventral scales is seen in certain extant marchantialean taxa such as *Dumortiera* (Schuster 1992b) and *Cyathodium* (Srivastava and Dixit 1996). Perhaps extant populations of *Monoclea* (growing in the modern spectrum of semi-aquatic to terrestrial habitats) trace back to a marchantialean ancestor that was permanently modified during a semi-amphibious phase of morphological evolution. Given the apparent tolerance for imprecise morphogenesis in some marchantioids and reductive morphological specializations seen in other extant taxa, a super-specialized *Monoclea* derived from within Marchantiales *sensu stricto* seems plausible.

Affinity of *Monoclea* with the carpocephalate marchantialean genus *Dumortiera* is strongly supported by *rbc*L data (Lewis et al.1997), consistent with the hypotheses of some earlier authors. Independent placement of *Monoclea* **within** extant Marchantiales with chloroplast *rbc*L (Lewis et al. paper) and with nuclear LSU rDNA and chloroplast trnL-region data (this study) suggests that ordinal status is unwarranted and that a model for the proto-marchantioid should be sought elsewhere.

The position of *Blasia*: The monotype *Blasia* is a "unique" and "extraordinary organism", "a cool-temperate to low-arctic circumboreal species, widespread in temporary or 'difficult' environments" (Schuster 1992b). Renzaglia (1982) states that this species is "one of the most interesting and complex of the Metzgeriales." *Blasia* (and sister genus *Cavicularia*) differ from all other metzgerioids by the presence of two-ranked ventral scales, a feature seen in many marchantioid taxa. *Blasia* can develop leaf-like lobes on elongate thalli but typically occurs as a thalloid plant with a discrete-rosette habit (Renzaglia 1982; personal observation). Though weakly supported, *Blasia* is basal to other metzgerioid samples (*Pellia* and *Fossombronia*) in the LSU rDNA phylogeny presented here; moreover, a separate "liverworts only" analysis (not shown) positions *Blasia* on a branch that leads to all sampled Marchantiopsida (when rooted on *Fossombronia*).

2.5.3. *Origin and evolution of marchantioid liverworts*

At the Paleozoic-Mesozoic boundary (Permo-Triassic), terranes corresponding to modern continents were organized into the Pangean supercontinent (Figure 2.8). Frey and Kürschner (1988) discuss what they term the "Xerothermic Pangaean" bryophyte flora. Based on modern distribution and ecology, they propose a "Permo-Triassic continental Pangaean range" for *Targionia hypophylla*, *Plagiochasma ruprestre*, *Oxymitra paleacea* (=*incrassata*), *Riccia lamellosa* and others. These four species were sampled in this study. Despite the apparent antiquity of these stenotypic species, the phylogenies presented here imply that these taxa are relatively derived within Marchantiales. In the LSU rDNA and combined data topologies (Figures 2.3 and 2.5), each of these four taxa traces back to an apparent star radiation (polytomy) that may represent an explosive phase of evolution in marchantioid forms during and immediately after the Permo-Triassic global crisis. Schuster (1992b) also suggests a Pangean origin for *Corsinia*; like *Oxymitra incrassata* and others mentioned above, this species may have dispersed across the landscape prior to the fission of Pangea (e.g. Figures 2.9 and 2.10).

Based on the fossil record and morphological trends seen in many extant taxa, Schuster (1981, 1984, 1992b) argues that xeromorphic marchantioids (Marchantiales and Sphaerocarpales) originated and radiated much later than the main jungermannioid radiation. Macrofossils similar to modern Metzgeriales (e.g. *Pallaviciniites* and *Blasiites*) begin to appear by the mid-Paleozoic (Devonian and lower-Carboniferous, respectively). The early-Mesozoic appearance of definitive marchantioid fossils seems correlated with global climate changes that occurred in concert with the late-Paleozoic development of the Pangean supercontinent. Schuster (1992b) asserts that marchantioids evolved and rapidly radiated into resultant new "immense barren areas with only seasonal moisture".

Warm, exposed desiccating environments undoubtedly did extensively expand in the Permo-Triassic. The end-Paleozoic (Permo-Triassic) crisis resulted in unprecedented global extinctions and desolation; however, rapid biological and ecological "reorganization" saw explosive radiations in many groups of organisms (Erwin 1993; Morris *et al.* 1995; Anderson *et al.* 1996). The ensuing environmental

Pangean supercontinent: Permo-Triassic Ca. 250 Ma

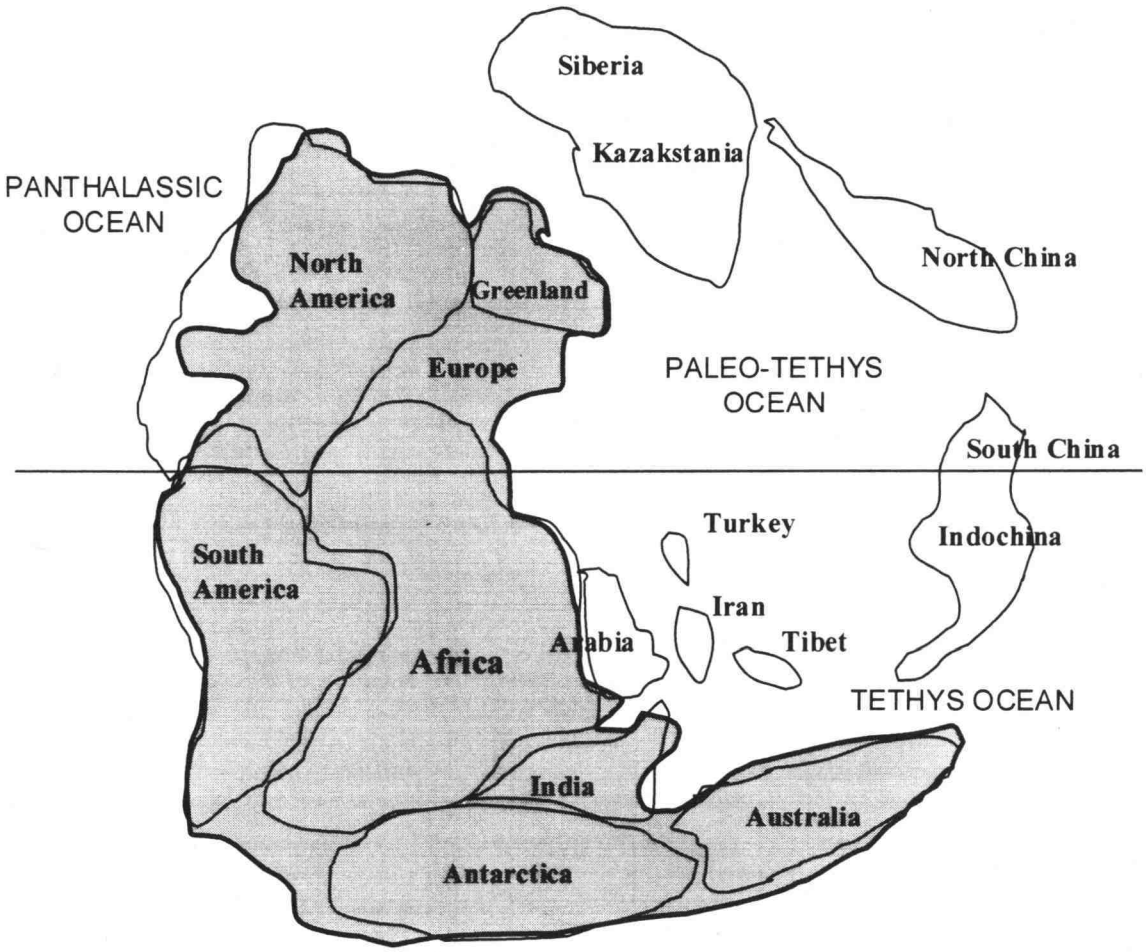


Figure 2.8. Reconstruction of paleocontinental configuration at the Permo-Triassic boundary ca. 250 million years ago. The Pangean supercontinent is shaded; terranes corresponding to modern continents and subcontinents are indicated. Redrawn and simplified from a Late Permian (255 Ma) map created by C. R. Scotese (1997: the Paleomap Project). A vast interior desert apparently occupied most of adjoining South American and African terranes; other regional deserts occupied large continental areas on the northern and southern complexes. The Pangean supercontinent remained intact until about 180 Ma. The Permo-Triassic global crisis reached its zentith at about 245 Ma (Raup 1994), a time of unprecedented global extinction and rapid subsequent ecological reorganization.

and ecological vacuum probably did favor a radiation of xeromorphic marchantioids. However, past environments that would have selected for xeromorphy need not have been seasonally **warm**. Aridity is not dependent on temperature *per se*; many modern desiccating environments are, in fact, routinely **cool** (if not cold). Nor are cool environments new; Rogers (1993) points out that "at least some glaciation was continuous from the Ordovician through the Permian." Glaciation in the late-Ordovician/ early- Silurian was centered at the edge of Gondwana on the modern Sahara region of North Africa (Frakes et al. 1992). Average global temperatures were apparently quite cool at this time with pulses of glaciation comparable to the more recent Pleistocene (Rogers 1993). Glaciations and associated katabatic winds result in large shifting expanses of cool, barren windswept surfaces (Pielou 1991). If eoembryophytes originated in the Ordovician, then it follows that surfaces in cool marginal (xeric) environments might have been available for colonization by some of the very first land plants.

Did marchantioid xeromorphy originate in response to increasing seasonal heat and aridity of the Permo-Triassic arena or were pre-adapted (xeromorphic) marchantioids queued for radiation, having already evolved much earlier in the cold, arid habitats associated with Paleozoic glaciations? Whether any of the earliest liverworts colonized cold Paleozoic surfaces is unknown. Modern *Blasia* is reported from arctic habitats in Greenland; here this species occurs on unstable frost-heaving soils and overwinters in the vegetative state, intact thalli surviving months of burial by snow (Schuster 1992a: p. 538). Modern carpocephalate marchantioids are well represented in modern seasonally cold habitats (Bischler 1988). Of modern liverworts (both jungermannioids and marchantioids) that occur in the Western Himalayas, marchantioids reach the highest elevations. In this region, most liverwort species occur between ca. 5000 and 10000 feet (ca. 1500 to 3200 meters) above sea level. But Kashyap (1972) notes that three marchantioid taxa: *Preissia*, *Marchantia* (Marchantiaceae) and *Sauteria* (Cleveaceae), reach elevations of ca. 15000 ft. (ca. 4600 meters) in the mountain passes.

Alternatively, selection pressure for xeromorphic characters might have also occurred on edaphic islands such as rock outcrops, table mountains or unstable inimical soil types. Modern marchantioid diversity often reaches its highest local development

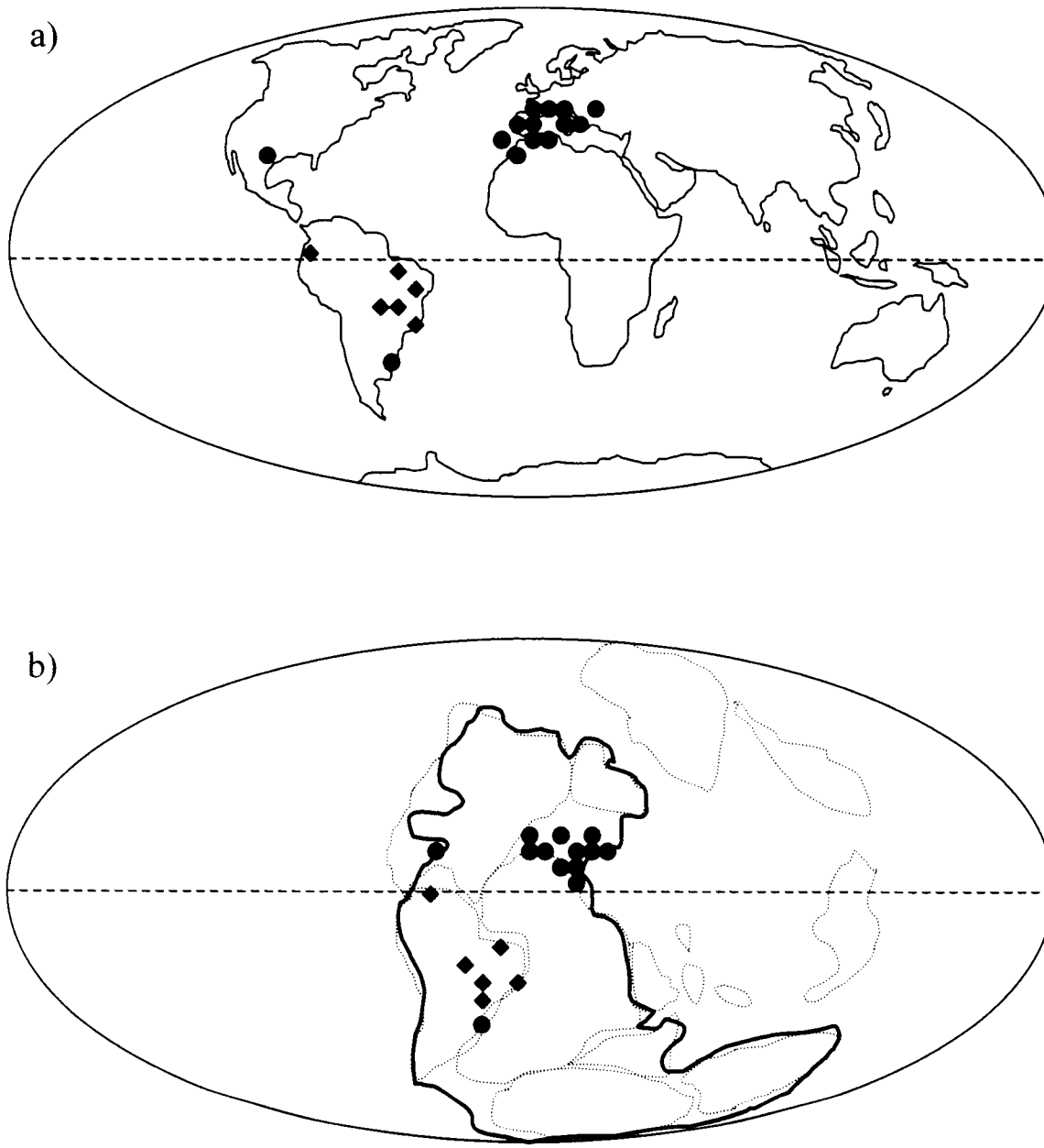


Figure 2.9. Distribution map of Corsiniaceae. **–a.** Known localities are indicated on a map of the modern world. Solid circles indicate known distribution of the widespread *Corsinia coriandrina*; diamonds indicate the known distribution of the south American endemic *Cronisia paradoxa*. **-b.** the same modern localities transferred to approximately corresponding positions on a map of the Permo-Triassic world (ca. 250 Ma). The Pangean supercontinent is drawn with a bold border; constituent modern continents are indicated with dashed borders. Redrawn from maps created by C. R. Scotese 1997 (Paleomap Preoject). Distribution data from Schuster (1992b) and Vital (1974). Transfer of Mediterranean locations on the Pangean supercontinent are necessarily somewhat approximate.

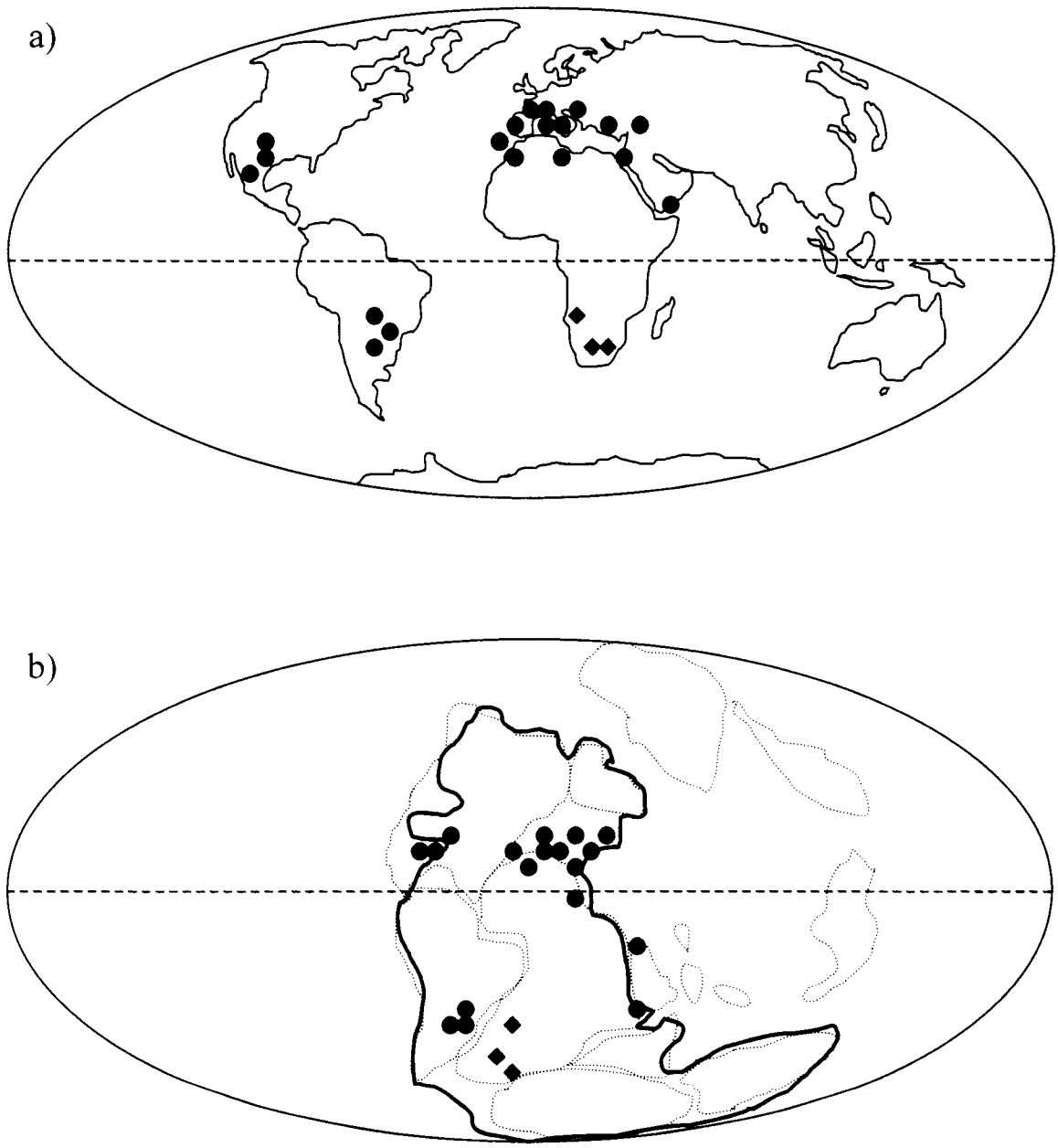


Figure 2.10. Distribution map of *Oxymitra*. **–a.** Known localities are indicated on a map of the modern world. Solid circles indicate known distribution of the widespread *Oxymitra incrassata*; diamonds indicate the known distribution of the south African endemic (polyploid) *Oxymitra cristata*. **-b.** the same modern localities transferred to approximately corresponding positions on a map of the Permo-Triassic world (ca. 250 Ma). The Pangean supercontinent is drawn with a bold border; terranes of constituent modern continents are indicated with dashed borders. Redrawn from maps created by C. R. Scotese 1997 (Paleomap Project). Distribution data from Schuster 1992b; Frey and Kürschner 1988. Transfer of Mediterranean locations on the Pangean supercontinent are necessarily somewhat approximate.

in such places. Optimal habitats include granitic outcrops and domelands, exposed bedrocks, the surface of table basalts and raw freshly-exposed erosional surfaces (personal observations). Analogous edaphically-controlled habitats have presumably existed throughout the ages during both cool (e.g. Ordovician-Silurian boundary) and warm (e.g. Devonian) climate modes.

The results of this study affirm the long phylogenetic isolation of extant Marchantiopsida previously demonstrated by other studies (Waters et al. 1992; Capesius 1995; Bopp and Capesius 1996; Capesius and Bopp 1997; Lewis et al. 1997). A long history (well-supported branch) unites all extant Marchantiopsida sampled to date and isolates this clade from other liverworts. This long branch may suggest extensive extinction of proto- and eomarchantioid forms that led to modern taxa. A major theme of topologies presented here is the unresolved marchantioid polytomy that follows the well-supported basal nodes. I speculate that this polytomy corresponds to an explosive radiation of marchantioid forms coincident with extreme conditions of the Permo-Triassic as Schuster (1981; 1992c) suggests. However, the origin of Marchantiopsida probably occurred long before; amidst, perhaps, a series of long-extinct *Blasia*-like ancestors that colonized and innovated on any of various xeric surfaces (either cool or warm) that were available throughout embryophyte history in the Paleozoic.

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Chapter 3

A Phylogenetic Analysis of the Genus *Riccia* L. (Hepaticae)

John Wheeler
Department of Botany and Plant Pathology
Oregon State University, Corvallis OR, 97331

2.1. Abstract

Molecular phylogenies of the marchantioid liverwort genus *Riccia* were reconstructed using independent nuclear and plastid data sets in the hope of better understanding relative age, relationships and character evolution in this taxonomically puzzling and ancient radiation. Cladistic analyses are based on three nucleotide sequence alignments: 1) partial nuclear-encoded Large Subunit rDNA (LSU rDNA), 2) the plastid-encoded *trn*L- region and 3) a combined data set consisting of concatenated nuclear and plastid alignments. Alignment ambiguous regions of each alignment were culled. Independently-derived strict consensus topologies based on 17-18 species of *Riccia* representing 5/8 of subgenera and 13/19 of sections (*sensu* Schuster 1992a) are largely congruent; combining the data results in a strict consensus tree with even higher overall bootstrap and decay support. A comparison of the nuclear and plastid trees reveals that five specific clades are common to both; moreover, in the combined strict consensus topology, seven clades are indicated with bootstrap support greater than 65%. All analyses (nuclear, plastid and combined) affirm the biological reality of a monophyletic *Riccia* (rooted on outgroups *Ricciocarpus* and *Oxymitra*). Resolution and/or support for basal relationships in *Riccia* is weak in all three topologies, the possible signature of an explosive initial species radiation during the Permo-Triassic. Striking morphological divergence within well-supported terminal clades, suggests a propensity in *Riccia* for volatile morphology that is not reflected in the underlying genetic history; characters emphasized in prior systematic treatments are apparently

unstable and therefore largely unreliable for the purpose of discriminating phylogenetically meaningful higher-level intrageneric taxa.

2.2 Introduction

Extant marchantioid liverworts (Marchantiopsida: consisting of Monocleales, Sphaerocarpales and Marchantiales) are the heterogeneous terminal taxa of an ancient lineage. Their monophyly and phylogenetic distance from other extant bryophyte stem groups (jungermannioid liverworts, mosses and hornworts) is supported by several recent molecular phylogenetic analyses (Waters et al. 1992; Capesius 1995; Bopp and Capesius 1996; Lewis et al. 1997; Capesius and Bopp 1997; Wheeler (in prep., Chapter 2). Marchantiales *sensu stricto* consists of five suborders, 14 families and 28 genera (Bischler 1988). Of these 28 genera, 16 are monotypic and three are ditypic. Within Marchantiales, "structural reorganizations are frequent" (Bischler 1988). Sporophytes are associated with an extensive variety of auxiliary gametophytic structures; these various units are then usually sessile on the horizontal vegetative thallus or elevated on specialized vertical branches called carpocephala. Long phylogenetic isolation of extant forms coupled with apparent widespread extinction of linking morphologies, frustrates the assessment of homology in and between modern terminal taxa (Schuster 1992b). The pattern of past evolution is obscure even among relatively character-rich, carpocephalate groups (Perold 1994). Marchantiales is characterized by its morphologically distinct monotypes; however, the order does contain a few speciose radiations, e.g. the carpocephalate genus *Marchantia* (with about 45 species; Bischler 1988) and acarpocephalate *Riccia* (perhaps 200 species worldwide; Perold 1991).

The large cosmopolitan genus *Riccia* is unparalleled among marchantioid liverworts (Marchantiopsida), and perhaps all bryophytes, with respect to intrageneric variation in a wide variety of characters and behaviors. Within this single genus, species vary widely in ecology, habitat, life history strategy, sexuality and cytology. Morphological variation occurs in growth form, size, color, thallus shape, thallus ornamentation, thallus ramification pattern, epidermal structure, tissue organization,

ventral scale morphology, spore shape, spore ornamentation and spore size. The genus contains delicate ephemeral taxa that can complete an entire life cycle (spore to spore) in 5-6 weeks. Other species occur as perennial xeromorphic clones, part of the cryptobiotic soil crust communities of warm deserts. Taxa are known from subarctic and alpine sites; others from the banks of lowland tropical rivers. Certain species are free-floating aquatics. Some taxa are bisexual but others are weakly or strongly heterothallic-unisexual. Meiospores are usually detached but in certain taxa they are permanently united as tetrads. Spores can be trilete to apolar; spore ornamentation is smooth, verruculate, foveolate, areolate, reticulate, vermiculate or papillate. Cytological variation is "astonishing" compared to other hepatics (Schuster1992b); extensive cytological study by Bornefeld (1984; 1987; 1989) demonstrates that taxa are haploid, polyploid, aneuploid or "nothopolyploid" (n= 8, 9, 10, 12, 15, 16, 17, 18, 20, 24, or 48). Narrow, regional and continental endemic taxa are known. Several species occur as intercontinental disjunct populations.

Suborder Ricciineae consists of *Riccia* and two other genera: monotypic *Ricciocarpus* and ditypic *Oxymitra*. The recent description of a new monotypic Australian *Riccia* subgenus (*Triseriata*; Jovet-Ast 1996) brings the total number of subgenera to eight; of these, five are monotypic (Table 3.1). Schuster (1992b) has recently proposed a controversial classification of *Riccia* consisting of 19 sections; of these, 10 are monotypic. Although not accepted by all workers, this new classification does indicate that while the genus is large and certainly contains some relatively recent ongoing radiations (e.g. the African section *Pilifer*: Perold 1991), morphologically-isolated monotypes are a common feature of the group.

In *Riccia*, individual plants are mostly small (thalli 0.5-4 mm wide) and often occur as flat rosette-forming gametophytes. Plants typically grow on strongly-illuminated litter-free surfaces of mineral soil where cover of vascular plants is low due to thin soils or periodic disturbance; thalli are closely and firmly attached to the substrate by numerous rhizoids. In *Riccia* we see the simplest sporophyte of any extant land plant. There is no carpocephalum; the sporophyte is submerged and virtually hidden in the tissues of the vegetative thallus. There is apparently no foot or seta (Schuster 1992b). At maturity, the spherical sporophyte consists merely of spores

Table 3.1. *Riccia* exemplars used in this study. Monotypic subgenera are indicated by an asterisk. Sampled species are indicated in bold face.

Genus	**Subgenus**	**EXEMPLAR**
Riccia	*Leptoriccia**	***membranacea***
	Thallocarpus	*curtisii* (complex)
	*Pannosae**	***tomentosa***
	Ricciella	***cavernosa***
		frostii
		huebeneriana
		papulosa
	*Chartacea**	***schelpei***
	*Viridisquamata**	*caroliniana*
	*Triseriata**	*singularis*
	Riccia	***albida***
		albolimbata
		atromarginata
		beyrichiana
		gougetiana
		lamellosa
		macrocarpa
		nigrella
		sorocarpa
		trichocarpa
		villosa

Table 3.2. Sampling of putative Sections within Subgenus *Riccia* (*sensu* Schuster 1992). Sampled sections are indicated in bold face.

Subgenus	**Section**	**EXEMPLAR**
Riccia	***Albidae***	*albida*
	Atromarginatae	*atromarginata*
	Albosquamatae	*albosquamata*
	Bicarinatae	*bicarinata*
	Ciliatae	*trichocarpa*
	Ciliiferae	*gougetiana*
	Lamellosae	*albolimbata*
	Pilifer	*villosa*
	Riccia	*beyrichiana*
		lamellosa
		macrocarpa
		nigrella
	Sommieri	*sommieri*
	Sorocarpae	*sorocarpa*

enclosed in a delicate capsule; the unistratose capsule wall is continuous with surrounding tissues and seems ± reabsorbed at sporophyte maturity. Spores can be among the largest of any liverwort; these are typically very thick-walled, durable and long-lived. Spores are passively released upon decay of the capsule wall and surrounding thallus.

Ephemeral taxa are of particular phylogenetic interest. Collectively, they often exhibit several features considered plesiomorphic by most workers (Jovet-Ast 1987, Perold 1991, Schuster 1992b). Putative plesiomorphies include: mesomorphy, unisexual-heterothallism, and uncomplicated cytology (i.e. absence of polyploidy or aneuploidy). Thallus ontogeny has also been invoked as an indicator of phylogenetic position within *Riccia*; air pores on a 'spongy' thallus are considered plesiomorphic while simple schizogenous air canals (penetrating a denser 'solid' thallus) may represent a derived (neotenic?) xeromorphic specialization (Schuster 1992b). Mesomorphic ephemeral taxa exhibit the widest range of known spore shape and spore ornamentation. Permanently united spore tetrads are found only in one small group of heterothallic-unisexual species (subgenus *Thallocarpus*).

The fossil record of *Riccia* is equivocal. The relatively late appearance of definitive marchantioid fossils (those with convincing preserved air-pores) has been considered as evidence that the entire order mostly traces to a later Mesozoic radiation (Schuster 1992b). But in *Riccia*, air pores are usually somewhat amorphous if present at all; moreover, putative ricciaceous fossils from near the Permo-Triassic boundary (Lundblad 1954) seem derived and xeromorphic by Schuster's own standards. However, ricciaceous affinity of these same fossils (*Ricciopsis scanica* Lundblad and *R. florinii* Lundblad) is rejected by Grolle (1983). The fossilization potential of *Riccia* is probably very low because species that occur in sedimenting habitats tend to be mesomorphic and delicate. Thalli with durable (xeromorphic) characteristics tend to inhabit upland sites where sedimentary processes are more unlikely. Possible late-Paleozoic or early-Mesozoic age for the genus *Riccia* is suggested by the modern biogeography of several extant species; these species occur as intercontinental disjunct populations that may have dispersed across the landscape prior to the fission of the

Pangean supercontinent (Jovet-Ast 1973; Jovet-Ast 1986; Frey and Kürschner 1988; Perold 1991; Schuster 1992a).

The nuclear-encoded ribosomal DNA (rDNA) cistron has proven to be a rich source of information for phylogeny reconstruction. Numerous studies attest to its utility for resolving recent, intermediate and ancient divergence events. The nuclear large subunit (LSU) rDNA gene consists of highly conserved "core" regions interspersed among "variable domains" or "expansion segments." Core region sequences exhibit the deepest phylogenetic signal; variable domain sequences reportedly resolve divergence events in the 50-300 MYA range (Larson 1991b). Selected core and/or expansion segment sequences have been used to examine relatively deep cladogenesis in diverse organisms such as amphibians (Larson 1991a), Chlorophyta (Chapman & Buchheim 1991), metazoans (Christen et al. 1991), volvocine flagellates (Larson et al. 1992), ciliates (Baroin-Tourancheau et al. 1992), *Drosophila* (Pelandakis & Solignac 1993), basidiomycetes (Hibbett & Vilgalys 1993), oysters (Littlewood 1994), unicellular/ colonial green flagellates (Buchheim et al. 1994), frogs (Kjer 1995), dinoflagellates (Zardoya et al.1995), omphalinoid mushrooms (Lutzoni 1997), ascomycetes (Spatafora 1998) and seed plants (Kuzoff 1997; Ro et al. 1997).

A set of chloroplast primers designed to amplify across a contiguous suite of tRNA, spacer and intron sequences was introduced by Taberlet et al. in 1991. Like the nuclear LSU rDNA sequence, this entire sequence consists of conserved regions (various tRNA exons) interspersed by more variable regions (two intergenic spacers and a single type I intron- the *trn*L intron). Phylogenetic antiquity of the *trn*L intron is noteworthy; this immobilized intron was apparently present prior to the divergence of the plastid from its cyanobacterial ancestor (endosymbiont) about one billion years ago (Kuhsel et al. 1990). Conserved domains and secondary structure across a broad phylogenetic range of organisms (Kuhsel et al. 1990) led Taberlet et al. (1991) to recommend this intron for "evolutionary studies at higher taxonomic levels." Sequences from the *trn*L intron and/or more conserved adjacent regions have been used recently in concert with other gene sequences to examine phylogeny in diverse plant groups such as Rhamnaceae (Richardson et al. 1997), palms (Baker et al. 1997), Cyperaceae (Yen

and Olmstead 1997), leptosporangiate ferns (Ranker et al. 1997) and arthrodontous mosses (Cox and Hedderson 1997).

The genus *Riccia* is a large and taxonomically puzzling group. Taxonomic history and concepts have been somewhat confusing and idiosyncratic (Perold 1995) and a higher-level comprehension of the entire group has been largely intractable based on morphological characters alone. The main goal of this study was to examine monophyly of *Riccia* and relationships within the genus using nucleotide sequences from the nuclear LSU rDNA and the plastid *trn*L-region. Prevailing uncertainty about relationships within the Marchantiales, however, required such wide outgroup sampling that the riccioid analysis soon became essentially simultaneous with a greater marchantioid analysis. Detailed results of the marchantioid study will appear elsewhere (Wheeler, in prep., Chapter 2).

This paper presents an examination of relationships within *Riccia* based on a taxonomically broad sample (Tables 3.1 and 3.2). The topologies presented here are considered preliminary; more conclusive results await dense sampling of the complete range of extant riccioid diversity. However, the independent conformation of several topological features (across independent nuclear and plastid data sets) in the trees presented here, suggests that some current concepts of classification are artificial. Several robust (independently confirmed) clades are surprising and imply that a propensity for volatile morphology is confounding our attempts to understand relationships in this taxonomically difficult group.

3.3. Materials and Methods

Sampling was guided by the recent classification of Schuster (1992) in an attempt to include the widest possible range of morphological diversity in the group (Tables 3.1 and 3.2). Tissues were field-collected or acquired as gifts of duplicate herbarium material (Table 3.3).

Single clones were sampled whenever this was possible to ascertain. Tissues were first carefully cleaned and examined for externally attached contaminants; live

Table 3.3. Sample taxa used in this study with voucher details. **SMP** = S. M. Perold; **OSC** = Oregon State University, USA; **PRE** = Pretoria, RSA.

Taxon	Voucher details
Oxymitra cristata	PRE; Koekemoer 1024 (from SMP); Olifantshoek, Cape, Africa; Dec 1992
Oxymitra incrassata	OSC; Wheeler 180; near Willow City, Gillespie Co.;Texas, USA; 3 Apr 1995
Riccia albida	OSC; Wheeler 454; near Sonora, Sutton Co.; Texas, USA; 9 Jan 97
Riccia albolimbata	OSC; Wheeler 455; near Sonora, Sutton Co.; Texas, USA; 9 Jan97
Riccia atromarginata	OSC; Wheeler 450; Squaw Pk., Phoenix, Pima Co.; Arizona, USA; 5 Jan 97
Riccia beyrichiana	OSC; Wheeler 172; near Utley, Bastrop Co.; Texas, USA; 1 Apr 1995
Riccia cavernosa	OSC; Wheeler 252; near Monroe, Benton Co.; Oregon, USA; 8 Jul 1995
Riccia frostii	OSC; Wheeler 234; Smith Rocks, Deschutes Co.; Oregon, USA; 8 Jul 1995
Riccia gougetiana	OSC; Wheeler 169; near Paige, Bastrop Co.; Texas, USA; 31 Mar 1995
Riccia huebeneriana	OSC; Wheeler 249; White R., Washington Co.; Arkansas, USA; 17 Oct 1995
Riccia lamellosa	OSC; Wheeler 493; Murrieta, Riverside Co.; California, USA; 15 Jan 1997
Riccia macrocarpa	OSC; Wheeler 204; Tehama Co.; California, USA; 13 Apr 1995
Riccia membranacea	OSC; Wheeler 248; White R., Washington Co.; Arkansas, USA; 17 Oct 1995
Riccia nigrella	OSC; Wheeler 086; Murrieta, Riverside Co.; California, USA; 30 Dec 1993
Riccia papulosa	OSC; Camacho 1283; Frankland River; Western Australia; 20 Jun 1995
Riccia schelpei	PRE: Oliver 9873 (from SMP); Namaqualand, NW Cape, Africa; 29 Jun 1991
Riccia sorocarpa	OSC; Wheeler 567; OSU campus, Benton Co.; Oregon, USA; 30 May 1997
Riccia tomentosa	PRE; Perold 2157 (from SMP); Namaqualand, Cape, Africa; 29 Aug 1988
Riccia trichocarpa	OSC; Wheeler 509; Griffin Park, Josephine Co.; Oregon, USA; 5 Apr 1997
Riccia villosa	PRE; Oliver 8039 (from SMP); Khamiesberg, Cape, Africa; 01 Sep 1983
Ricciocarpus natans	OSC; Wheeler 251; near Monroe, Benton Co.; Oregon, USA; 19 Oct 95

contaminant tissues are an ever-present danger in field-collected marchantioid specimens because in nature these often occur in intimate association with mosses, hornworts and even cryptic terrestrial jungermannioids (e.g. virtually filamentous *Cephaloziella* sp.). All subsequent tissue handling, DNA isolation, polymerase chain reaction (PCR) and sequencing methods are detailed in Wheeler (in prep., Chapter 2); the following section is a brief summary of main points.

Nuclear-encoded partial LSU rDNA amplicons (PCR-derived gene segments) and plastid-encoded *trn*L-region amplicons (Figures 3.1 and 3.2, respectively) were generated by PCR. Forward primer ITS3 (White et al. 1990) and reverse primer LR1010 (designed for this study) were used to amplify the nuclear amplicon (Table 3.4). Forward primer "C" and reverse primer "F" (Taberlet 1991) were used to amplify the plastid amplicon. These same external primers and other internal primers (Table 3.4) were then used in subsequent sequencing reactions. Satisfactory amplicons were gel-purified (Qiagen, Chatsworth, CA) and then processed by cycle sequencing and dye-terminator chemistry on an ABI model 373A or 377 automated fluorescent sequencer at the Oregon State University Central Services Laboratory.

Table 3.4. Primer sequences used for PCR amplification and sequencing in this study. Arrows designate direction of primer. Tm is the calculated melting (annealing) temperature. Primers designed specifically for this project are so indicated; the 3' position of these primers in the LSU rDNA gene (relative to *Lycopersicon*) are indicated by the numbers incorporated into each primer name.

Name		Sequences 5′-3′	Tm	Source:
NUCLEAR				
ITS3	⇨	GCAACGATGAAGAACGCAGC	64.3	White et al. 1990
LR1010	⇦	GCCTCTAATCATTGGCTTTACC	59.1	this study
LF47	⇨	ACCCGCTGAGTTTAAGCATATC	58.1	this study
LR654	⇦	TTGGTCCGTGTTTCAAGACG	62.1	this study
PLASTID				
Universal C	⇨	CGAAATCGGTAGACGCTACG	60.8	Taberlet et al. 1991
Universal F	⇦	ATTTGAACTGGTGACACGAG	56.1	Taberlet et al. 1991

Figure 3.1. Map of the nuclear-encoded LSU rDNA region and PCR amplicon used in this study.

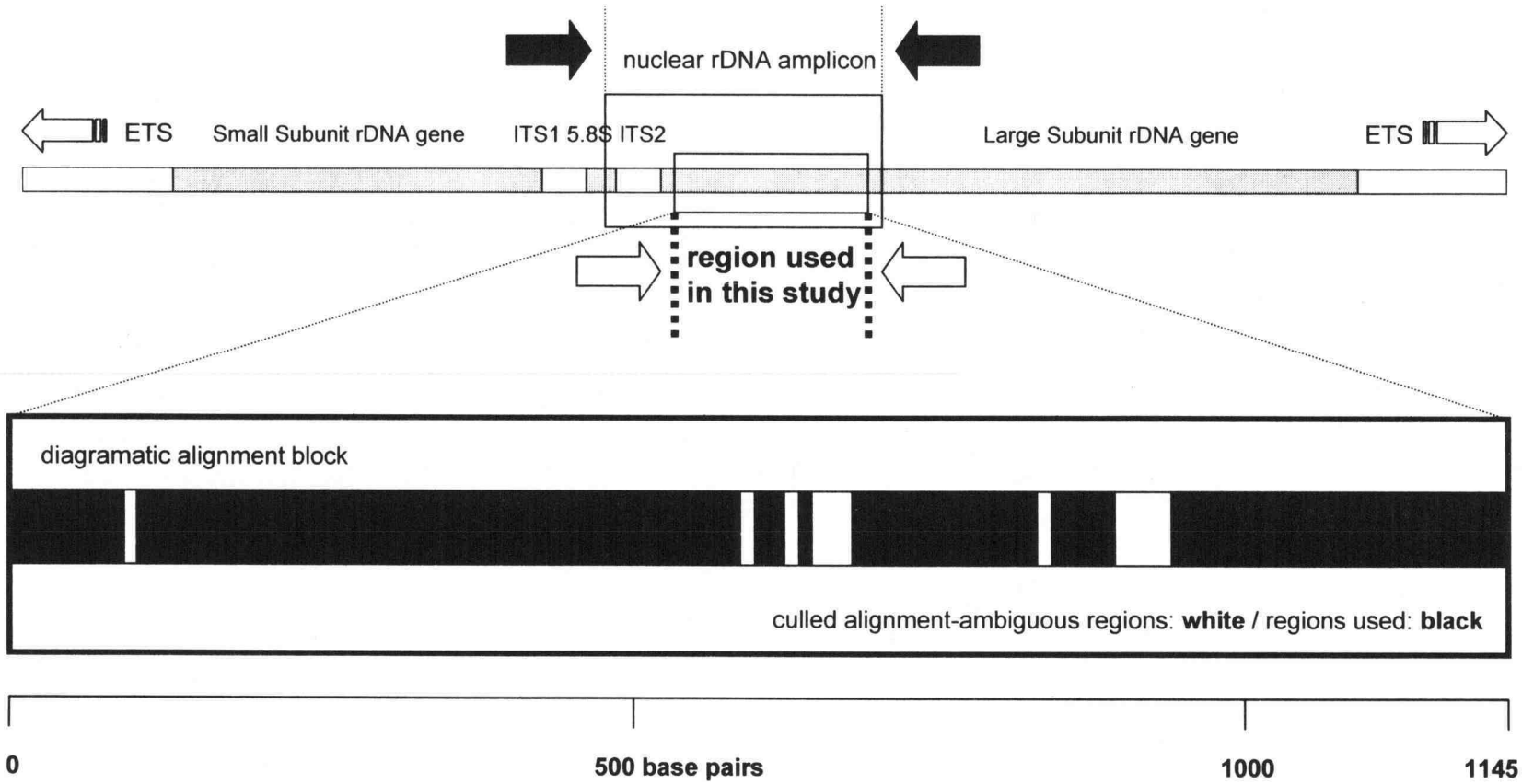
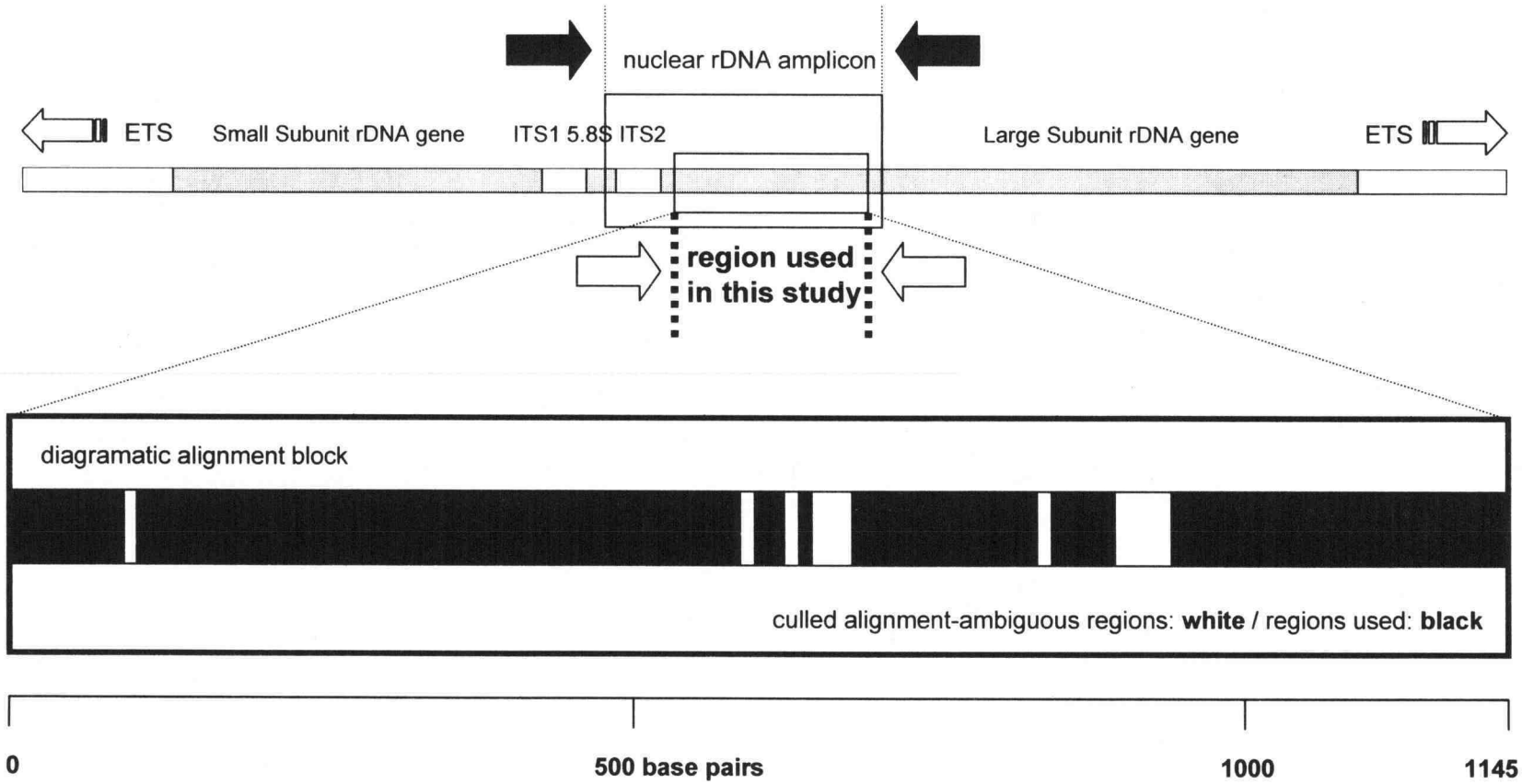

nuclear rDNA amplicon
ETS
Small Subunit rDNA gene
ITS1 5.8S ITS2
Large Subunit rDNA gene
ETS
region used
in this study
diagramatic alignment block
culled alignment-ambiguous regions: white / regions used: black
0
500 base pairs
1000
1145

Figure 3.2. Map of the plastid-encoded *trn*L-region and PCR amplicon used in this study.


nuclear rDNA amplicon
ETS
Small Subunit rDNA gene
ITS1 5.8S ITS2
Large Subunit rDNA gene
ETS
region used
in this study
diagramatic alignment block
culled alignment-ambiguous regions: white / regions used: black
0
500 base pairs
1000
1145

The initial sequencing read from each amplicon was compared to GenBank and EMBL databases with a BLASTN similarity search (Altschul et al. 1990) for early detection of mistakenly amplified sequences. For a discussion of this problem see Camacho et al. (1997). Higher (more stringent) annealing temperatures were used when the standard reaction conditions produced unwanted (putative fungal) bands.

The nuclear-encoded LSU rDNA subproject involved sequencing 18 *Riccia* exemplars, both extant species of *Oxymitra* and the monotypic *Ricciocarpus* (Table 3.3); these three genera comprise the suborder Ricciineae. Sampling was equivalent across the two data sets (nuclear vs. plastid) except for *Riccia papulosa* which is missing in the *trn*L-region data set.

Sequence files were manipulated using GCG8 (Genetics Computer Group 1994) or GCG9 (Genetics Computer Group 1996). An initial automated alignment generated with the Pileup program in GCG (gap creation penalty = 2.0; gap length penalty = 0.2) was imported into GDE (Genetic Data Environment: Smith et al. 1994) for manual adjustment and the convenient creation of NEXUS files. Alignment-ambiguous blocks of positions (Figures 3.1 and 3.2) were excluded from both the LSU rDNA and *trn*L-region alignments. In this way, one preferred "culled" alignment (Gatesy et al. 1994) was obtained for each of the two data sets. This LSU rDNA alignment and the plastid *trn*L-region culled alignment were analyzed separately and then combined in a total evidence analysis.

The UNIX test version 4.0.0d59 of PAUP* (David L. Swofford) on a SUN 670 MP computer was used for unweighted parsimony analyses. Alignment gaps were treated as missing data. Heuristic search options were set as follows: 100 replicate searches (nreps=100) with random addition sequences (addseq=rand), no maxtrees limit and tree bisection and reconnection (TBR) branch swapping. In PAUP* these settings automatically report any occurrence of islands of equally most-parsimonious trees (Maddison 1991). Bootstrap support (Felsenstein 1985) for each topology was determined using the "simple addition sequence" option, mulpars = on and maxtrees = 500 in PAUP*. Tree files generated with PAUP* were examined and manipulated using the program TREEVIEW (Page 1996). Decay values were calculated using the clade constraint method (Eernisse and Kluge 1993) as described by Morgan (1997).

Separate LSU rDNA and *trn*L-region analyses and the final combined (nuclear+plastid) analysis were each rooted on *Ricciocarpus*.

3.4. Results

3.4.1. *Sequences and alignments.*

Individual PCR-amplified LSU rDNA sequences vary in length from 972 bp (*Riccia macrocarpa* and *R. atromarginata*) to 1005 bp (*Riccia membranacea*). After manual adjustment and masking of ambiguous sites, the LSU rDNA culled alignment (Appendix 3) is 949 bp in length. Pairwise sequence divergence (uncorrected *p* distance), calculated from this culled alignment, ranges from 0.012 (*Riccia nigrella* / *R. macrocarpa*) to 0.060 (*R. albolimbata* / *Oxymitra incrassata*). Compared to the outgroup *Ricciocarpus*, sequence divergence ranges from 0.030 (*Riccia gougetiana*) to 0.058 (*Riccia albolimbata*). Homogeneity of base frequencies across taxa was confirmed (P = 1.000) with the Chi-square test in PAUP* Observed means and ranges of base frequencies are **A**: 0.235 (0.231-0.239); **C**: 0.254 (0.247-0.264); **G**: 0.341 (0.330-0.345); **T**: 0.172 (0.165-0.178).

The *trn*L-region amplicon sequences vary in length from 517 bp (*Riccia gougetiana*) to 569 bp (*Riccia macrocarpa*). Following adjustments and masking of ambiguous sites, the final *trn*L-region culled alignment (Appendix 4) is 479 bp in length. Based on this culled alignment, pairwise sequence divergence (uncorrected "P" distance: ranges from 0.004 (*Riccia frostii* / *R. cavernosa*) to 0.094 (*Riccia huebeneriana* / *Oxymitra incrassata*). Relative to the outgroup *Ricciocarpus*, sequence divergence among other sample taxa ranges from 0.055 (*Riccia membranacea*) to 0.083 (*Riccia huebeneriana*). Base frequencies are homogeneous across taxa (P = 1.000: Chi-square test); means and ranges are **A**: 0.395 (0.380-0.404); **C**: 0.142 (0.130-0.151); **G**: 0.166 (0.154-0.176); **T**: 0.297 (0.287-0.305).

3.4.2. *Phylogenetic analyses*

Analysis 1: culled nuclear LSU rDNA alignment: This alignment has 791 constant sites, 158 variable sites and 63 informative sites. Heuristic searching with unweighted

Phylogeny of *Riccia*: nuclear data CI = 0.6523, RI = 0.5659, RC = 0.3691

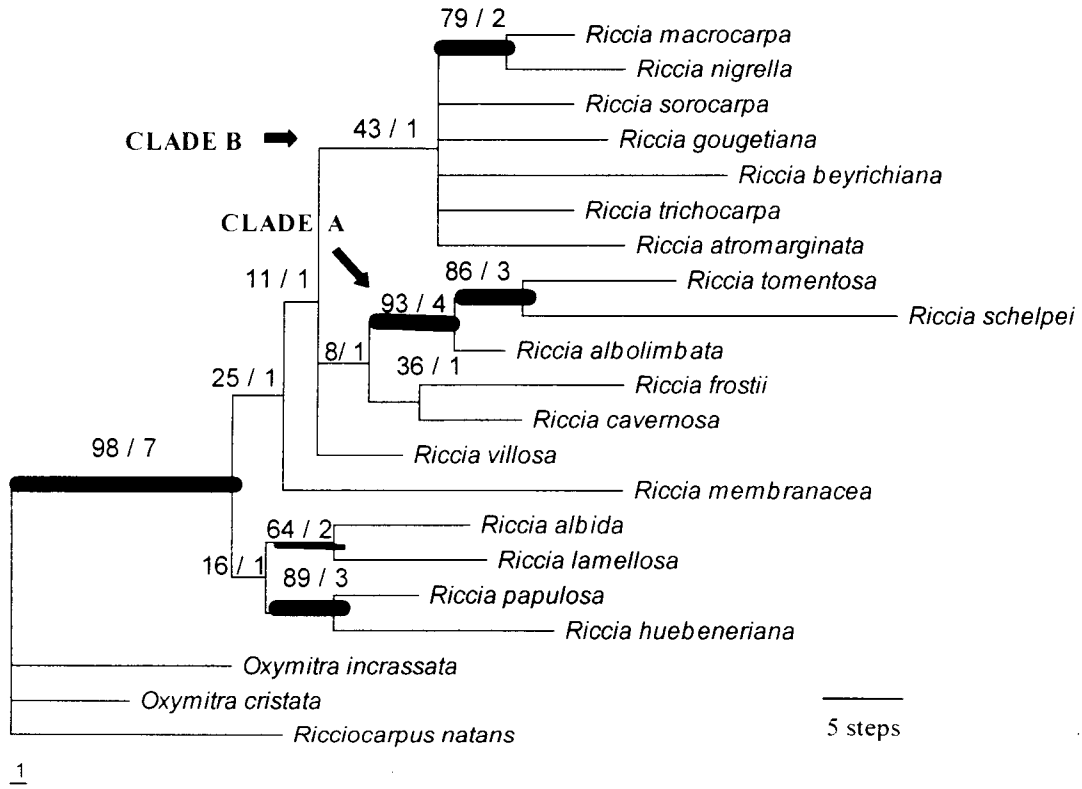


Figure 3.3. Strict consensus phylogeny of *Riccia* based on nuclear data: 949 characters, 791 constant sites, 158 variable sites and 63 informative sites. Heuristic searching with unweighted parsimony results in a single island of 26 shortest trees (tree length = 256). Branch support: [bootstrap %/ decay value].

parsimony results in a single island of 26 shortest trees, tree length = 256, CI = 0.6523, RI = 0.5659, RC = 0.3691. Rooted on *Ricciocarpus*, the strict consensus of these 26 trees (Figure 3.3) supports a monophyletic *Riccia* (bootstrap = 98%; decay = 7). Monophyly of the two *Oxymitra* exemplars is not resolved. Within *Riccia*, the following species pairs or clades are supported by > 50% of bootstrap replicates; each pair/clade is listed with associated bootstrap percentage and decay support, respectively: *Riccia nigrella / macrocarpa* (bootstrap = 79%; decay = 2); *Riccia tomentosa / schelpei* (86%; 3); CLADE A: *Riccia albolimbata / R. tomentosa+schelpei* (93%; 4); *Riccia albida / lamellosa* (64%; 2); *Riccia papulosa / huebeneriana* (89%; 3). The following pairs/clades are resolved with poor (< 50%) bootstrap support: *Riccia frostii / cavernosa* (36%; 1) and a clade of seven morphologically heterogeneous *Riccia* species termed CLADE B [*Riccia gougetiana + beyrichiana + atromarginata + trichocarpa + sorocarpa + macrocarpa + nigrella*] (43%; 1). Basal branches are largely resolved but collapse with just one additional step; bootstrap support for basal branches is also low (< 50%).

Analysis 2: culled plastid trnL-region alignment: This alignment has 365 constant sites, 114 variable sites and 61 informative sites. Heuristic searching results in a single island of 72 shortest trees, tree length = 197, CI = 0.7310, RI = 0.6864, RC = 0.5017. Rooted on *Ricciocarpus*, the strict consensus of these trees (Figure 3.4) supports a monophyletic *Riccia* (90%; 5) and a monophyletic *Oxymitra* (100%; 6). Within *Riccia*, the following species pairs or clades are supported in > 50% of bootstrap replicates; each pair/clade is listed with associated bootstrap percentage and decay support, respectively: *Riccia tomentosa / schelpei* (97%; 3); CLADE A (82%; 2); *Riccia frostii / cavernosa* (100%; 12); CLADE B (77%; 3)]. The *Riccia albida / lamellosa* pair has low support (41%; 1). Most basal branches are poorly resolved and decay with just one additional step; bootstrap support for basal branches is also low (< 50%).

Analysis 3: combined alignment: This alignment has 1156 constant sites, 272 variable sites and 124 informative sites. This total-evidence analysis (culled nuclear LSU rDNA combined with culled plastid trnL-region data), results in a single most-parsimonious tree (Figure 3.5), length = 458, CI = 0.6790, RI = 0.6070, RC = 0.4121. *Riccia* is monophyletic in 98% of bootstrap replicates; eleven additional steps (decay =

Phylogeny of Riccia: plastid data CI = 0.7310, RI = 0.6864, RC = 0.5017

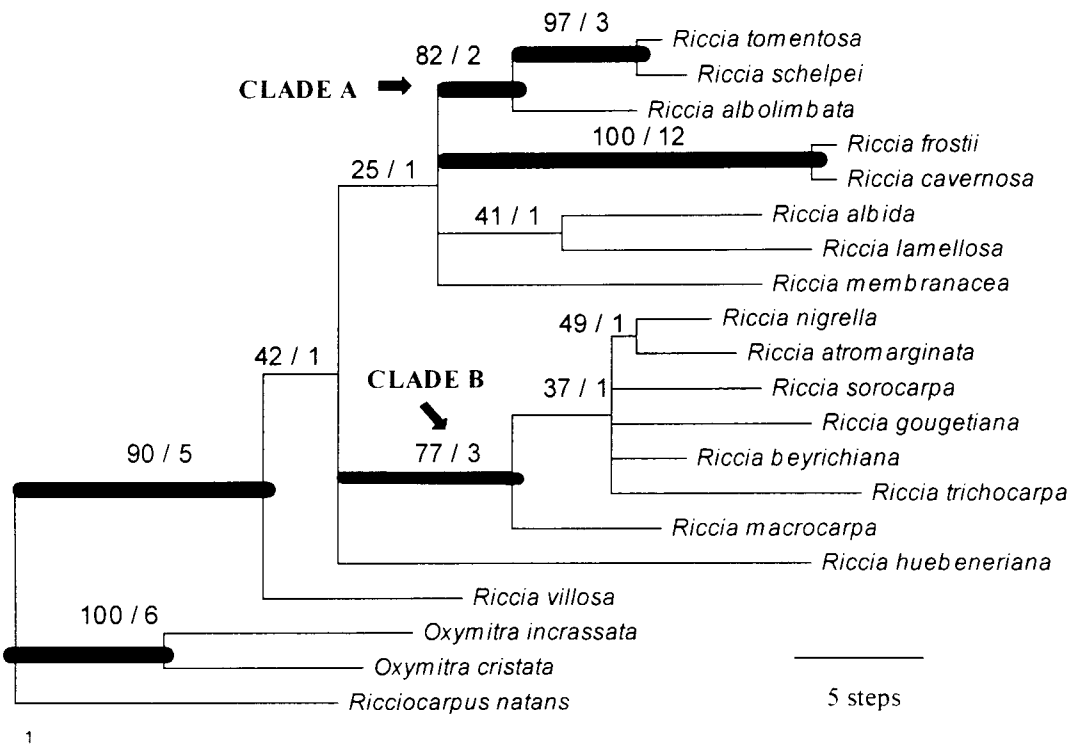


Figure 3.4. Strict consensus phylogeny of *Riccia* based on plastid data: 479 characters, 365 constant sites, 114 variable sites and 61 informative sites. Heuristic searching with unweighted parsimony results in a single island of 72 equally shortest trees (tree length =197). Branch support: [bootstrap %/ decay value].

Phylogeny of Riccia: combined data CI = 0.6790, RI = 0.6070, RC = 0.4121

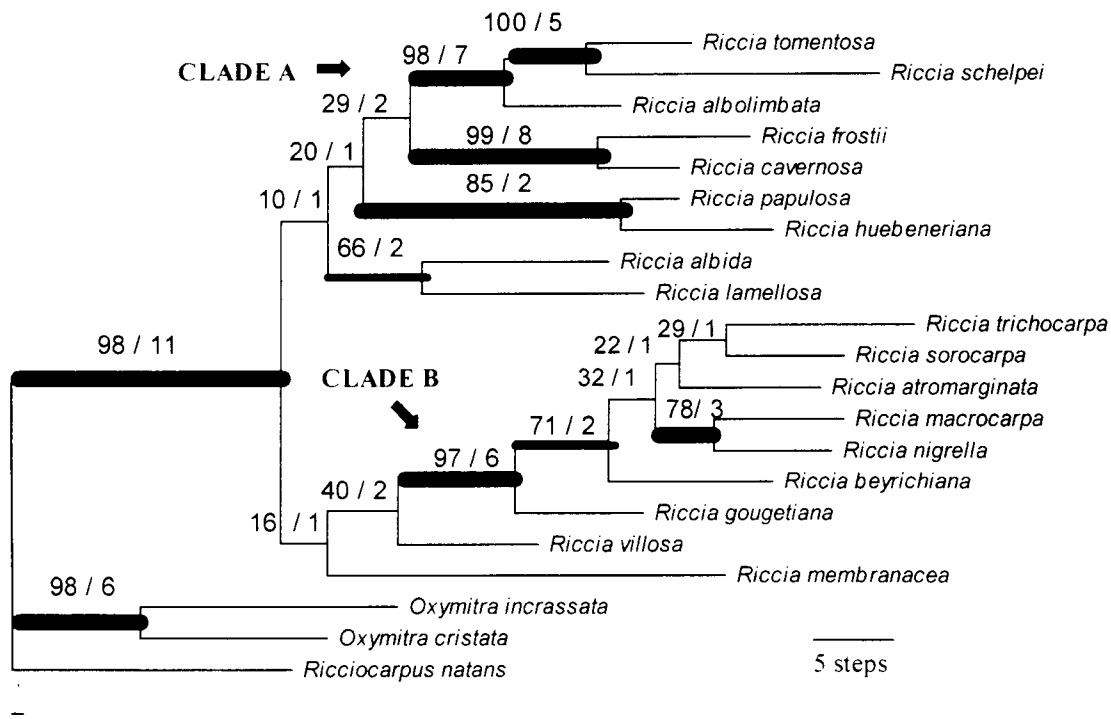


Figure 3.5. Strict consensus phylogeny of *Riccia* based on combined data: 1428 characters, 1156 constant sites, 272 variable sites and 124 informative sites. Heuristic searching with unweighted parsimony results in one single most-parsimonious tree (tree length = 458). Branch support: [bootstrap %/ decay value].

11) are required to collapse *Riccia* with *Oxymitra* or *Ricciocarpus*. Monophyly of the two *Oxymitra* exemplars is well-supported (98%; 6) in the combined data analysis. Within *Riccia*, the following species pairs or clades are supported by > 50% of bootstrap replicates; each pair/clade is listed with associated bootstrap percentage and decay support, respectively: *Riccia tomentosa / schelpei* (100%; 5); CLADE A (98%; 7); *Riccia frostii / cavernosa* (99%; 8); *Riccia papulosa / huebeneriana* (85%; 2); *Riccia albida / lamellosa* (66%; 2); *Riccia nigrella / macrocarpa* (78%; 3) and CLADE B (97%; 6). Basal branches are resolved but bootstrap support is low (<40%); decay values for basal branches are one or two additional steps.

3.5. Discussion

3.5.1. *Phylogenetic relationships*

Outgroups: *Ricciocarpus* and *Oxymitra* (Suborder Ricciineae) were used as outgroups for two reasons: **1)** earlier analyses (Wheeler, in prep., Chapter 2) with a wider sample of marchantioid taxa +*Riccia* had yielded topologies compatible with these choices and **2)** other candidate outgroups (e.g. *Corsinia*, *Targionia* and various carpocephalate taxa) were so divergent in their respective nucleotide sequences that it was impossible to align them with the *Riccia* + *Oxymitra* + *Ricciocarpus* block used here. Further study is needed to clarify the positions of *Ricciocarpus* and *Oxymitra* relative to *Riccia*.

The genus *Oxymitra* contains two extant species (Perold 1993), the widespread subcosmopolitan *Oxymitra incrassata* (n = 9) and the rare south African endemic (autopolyploid?) *Oxymitra cristata* (n = 18). Well-supported monophyly of *Oxymitra* is confirmed in the topology derived from plastid data alone. The addition of the nuclear data did not significantly reduce this conclusion; in the combined data analysis, *Oxymitra* is upheld with 98% bootstrap and good decay support.

Monophyly of the genus *Riccia*: The possibility that *Riccia* might be a polyphyletic catch-all, derived from independently reduced marchantioid lineages is not supported. All analyses (nuclear, plastid and combined) support and affirm the biological reality of a monophyletic *Riccia*. The evidence for monophyly is particularly convincing because

while bootstrap and decay support are strong in each separate analysis (nuclear and plastid, respectively), these same support indices become even higher when the two data sets are combined (Table 3.5). Not only do nuclear and plastid data sets agree in this respect; each corroborates the other in an even stronger total evidence hypothesis.

Table 3.5. Comparison of branch support indices across the three data sets. CLADE A = *Riccia albolimbata* + *R. tomentosa* + *R. schelpei.* CLADE B = *R. gougetiana* + *R. beyrichiana* + *R. atromarginata* +*R. trichocarpa* + *R. sorocarpa* + *R. macrocarpa* + *R. nigrella.*

CLADE SUPPORT	**Nuclear**		**Plastid**		**Combined**	
	% bootstrap	decay	% bootstrap	decay	% bootstrap	decay
R. papulosa / huebeneriana	89	3	NA	NA	85	2
R. frostii / cavernosa	36	1	100	12	99	8
R. albida / lamellosa	64	2	41	1	66	2
R. macrocarpa / nigrella	79	2	NA	NA	78	3
R. tomentosa / schelpei	86	3	97	3	100	5
CLADE A	93	4	82	2	98	7
CLADE B	43	1	77	3	97	6
monophyletic ***Riccia***	98	7	90	5	98	11

Phylogenetic relationships within *Riccia*: Topologies presented here are based on a sample of perhaps only 10% of extant species in the genus *Riccia*. In all, 5/8 of subgenera and 13/19 of sections (*sensu* Schuster 1992) were sampled. In some cases, entire (albeit monotypic) subgenera are sampled; in other cases, large putative complexes or sections are represented by a single exemplar. For these reasons, specific pair-wise affinities implied by the topologies cannot be viewed as precise sister-species relationships because they are derived from so few sample taxa. Precise sister-group resolutions await denser sampling; however, several general observations can be made with relative confidence because of exact congruence between the two independent data sets. A comparison of the nuclear strict consensus and plastid strict consensus trees (Figures 3.3 and 3.4) reveals that five specific clades are common to both; moreover, in

the combined strict consensus topology, seven clades are indicated with bootstrap support greater than 65% (Table 3.5).

The *Riccia papulosa* / *R. huebeneriana* clade: Both are placed in subgenus *Ricciella* (A. Braun) Bisch. Na-Thalong (1980) places the Australian-endemic *Riccia papulosa* in her *'Group Terrestriae'* of Subgenus *Ricciella*. *R. huebeneriana* is not recorded for Australia but would correspond to her 'Group *Aquaticae-Terrestriae'* of the same subgenus since *R. huebeneriana* seems part of the greater *R. fluitans* complex of species (Schuster 1992c). *R. papulosa* is a very large terrestrial species (thallus width to 5 mm) that can occur "even in rather dry habitats"; *R. huebeneriana* is small (thallus width to 1.5 mm) and is restricted as an obligate ephemeral to saturated soils at the edge of streams or standing water. Spores of *R. papulosa* are large (100-140 μm); the spores of *R. huebeneriana* are much smaller at 50-70 μm.

The *Riccia frostii* / *R. cavernosa* clade: While both of these widespread intercontinental species are ephemeral and share a similar life history strategy and ecology (they are often sympatric (personal observation), they are different in several ostensibly profound ways; Schuster (1992) places them in separate sections of Subgenus *Ricciella*. *Riccia frostii* exhibits strongly heterothallic unisexual thalli; *R. cavernosa* is homothallic-bisexual. Because of its relatively compact thallus, *R. frostii* has been invoked as a linking morphology between the xeromorphic 'solid' thallus model and the mesomorphic 'spongy' model (e.g. *R. cavernosa*). *R. cavernosa* was so named because of the exaggerated 'cavernose' nature of its highly chambered mesomorphic thallus. Because of these morphological distinctions, such a long well-supported branch shared by these two species is unexpected and suggests relatively recent divergence from a common ancestor.

The *Riccia lamellosa* / *R. albida* clade: Because of its distinctive calcified thallus, *Riccia albida* was given monotypic sectional status (within Subgenus *Riccia*) by Schuster (1992). Jovet-Ast (1973) has studied collections of *R. albida* (=*crustata*) from North America, Australia and the Mediterranean; she is convinced that the taxon "is a

very ancient and stable species." Frey and Kürschner (1988) argue that *R. lamellosa* is also an ancient stable species; in their estimation, *R. lamellosa* "shows a clear xerothermic Pangaean distribution pattern". Few morphological characters would unite these two species. *R. lamellosa* (Section *Lamellosae;* Subgenus *Riccia*) is larger and has large prominent ventral scales. Ventral scales in *R. albida* are vestigial (Schuster 1992b). One possible synapomorphy for the two species is their respective spores. In both species the spore is subspherical to spherical and lacks the angularity of a typical trilete spore.

The *Riccia macrocarpa* / *R. nigrella* clade: Schuster places both of these species in his Section *Lamellosae* of Subgenus *Riccia.* Both species are xeromorphic with a dense (solid) thallus. Both can occur as long-lived clones that become vegetatively dormant during long dry periods. The apical meristem of *R. macrocarpa* was recently revived after 23 years of storage in an herbarium in Paris (Breuil-See 1993). Both have pigmented ventral scales that probably serve to shield the thallus from UV radiation. Both exhibit distinctive oil-body cells (idioblasts); in *R. nigrella* these occur in the epithelium while in *R. macrocarpa* the idioblasts are scattered within the tissues of the thallus (Perold 1991). *R. macrocarpa* is a relatively large species (thallus width to 2.8 μm); *R. nigrella* is smaller (thallus width to 1.3μm).

The *Riccia tomentosa* / *R. schelpei* clade: A close (well-supported) phylogenetic relationship between these two species across all analyses is surprising. Both of these taxa occur as rare species in a geographically restricted area in southwestern Africa (arid shrublands of Namaqualand) but because of profound morphological distinctions, Perold (1991) has described each as its own monotypic subgenus (*Pannosae* and *Chartacea*, respectively). *R. tomentosa* grows on reddish brown, sandy soil, overlying clay; *R. schelpei* grows on soils derived from decomposed granite (Perold 1986, 1990). The spores of *Riccia tomentosa* are "densely papillate to verruculate and united as permanent tetrads"; the spores of *R. schelpei* are areolate and separate-trilete (Perold 1991). The thallus of *R. tomentosa* is densely ornamented with long hair-like epidermal outgrowths; air pores are crowded and essentially unroofed. The thallus of *R. schelpei*

is naked with well-spaced quasi-stellate pores reminiscent of *Oxymitra* (Perold 1986). The two taxa are strikingly different in overall gestalt. Perold writes that *R. tomentosa* is "dorsally shaggy-haired " and "silvery"' when fresh, white (hairs matted) when dry. In contrast, the dorsal aspect of fresh *R. schelpei* is green and "somewhat greasy", becoming "yellow and parchment-like" when dry.

CLADE A: This clade consists of three sampled species: *Riccia albolimbata* basal to the *R. tomentosa* / *R. schelpei* pair discussed above. The topology of these three taxa is consistent across all analyses (Table 3.5). Support is good in both nuclear and plastid analyses (93% and 82% bootstrap respectively); combining the two data sets results in a topology supported with a 98% bootstrap. *Riccia albolimbata* is probably part of a radiation of white-scaled *Riccias*, a complex that includes *R. lamellosa* (Na-Thalang 1980; Schuster 1992b). The modern distributions of *R. lamellosa* (globally widespread) and *R. albolimbata* (North America and southern Africa: relictual?) are both arguably xerothermic Pangaean (sensu Frey and Kürschner 1988). The outgroup *Oxymitra incrassata* also has prominent whitish-hyaline ventral scales.

CLADE B: This clade is resolved in both the nuclear and plastid analyses; combining the two data sets results in even better support (bootstrap 97%, decay = 6). The clade consists of seven rather heterogeneous species: *Riccia gougetiana, R. beyrichiana, R. atromarginata, R. trichocarpa, R. sorocarpa, R. macrocarpa and R. nigrella* representing 6 of Schuster's sections within Subgenus *Riccia*. Taken together, this clade represents a wide gamut of variation in many characters e.g. ventral scales (prominent to vestigial), ventral scale color (opaque-black to translucent-hyaline), thallus size (width 1.3 – 7mm), ecology (relatively mesomorphic to extremely xeromorphic), sexuality (heterothallic-unisexual to homothallic-bisexual) and thallus ornamentation (smooth to papillate to ciliate).

Outlier species: Two species, *Riccia membranacea* and *R. villosa*, show no strong affinity to any other species in the sample suggesting that each represents a highly isolated element in the genus. Jovet-Ast has argued that *R. membranacea* is an ancient

Riccia; she places this species as a fundamental basal branch in her morphology-based phylogenetic polytomy (Jovet-Ast 1987). *R. membranacea* is unique among all known taxa in the genus with respect to its tiny, apolar papillate spores. Schuster remarks that the thallus reminds him of a fern prothallus, ventral tissues are vestigial; Schuster (1984) has elevated the species to a monotypic subgenus (Subgenus *Leptoriccia*).

Riccia villosa is endemic to southern Africa. The species is notable for its spectacular white overarching ventral scales; because of these large serrate scales (a possible plesiomorphy with *Ricciocarpus* and/or *Oxymitra*), Schuster (1984) was compelled to elevate this species to the monotypic genus *Pteroriccia*. But *Pteroriccia* was soon reduced to synonymy under *Riccia* by Perold (1986) citing numerous linking features between *R. villosa* and other *Riccia* species in Section *Pilifer* (Volk).

3.5.2. *Putative explosive radiation of Riccia*

Possible late-Paleozoic or early-Mesozoic age for the genus *Riccia* is suggested by the modern biogeography of several extant species (xeromorphs: e.g. *R. albida* (=*crustata*), *R. lamellosa*, *R. macrocarpa*, and mesomorphs: e.g. *R. curtisii*, *R. membranacea*, *R. frostii*). These species occur as intercontinental disjunct populations (e.g. Figures 3.6 and 3.7) and may have dispersed across the landscape prior to the fission of Pangea (Jovet-Ast 1973; Jovet-Ast 1986; Frey and Kürschner 1988; Perold 1991; Schuster 1992a).

Note that in all phylogenetic topologies presented here (nuclear, plastid and combined), resolution and/or support for basal relationships within *Riccia* is weak. Weak support at the base of *Riccia* may be the hallmark of an actual explosive initial radiation. If apomorphies accumulate at a relatively consistent rate over geologic time then weak support at the base of a large radiation might be explained as the inevitable consequence of that rapid evolution, i.e. there was simply insufficient time for documentation of the rapid cladogenesis (in the form of synapomorphic signatures).

Frey and Kürschner (1988) discuss what they term the "Xerothermic Pangaean" bryophyte flora. Based on modern distribution and ecology, they propose a "Permo-Triassic continental Pangaean range" for *Targionia hypophylla*, *Plagiochasma ruprestre*, *Oxymitra paleacea* (=*incrassata*), *Riccia lamellosa* and others. If true then

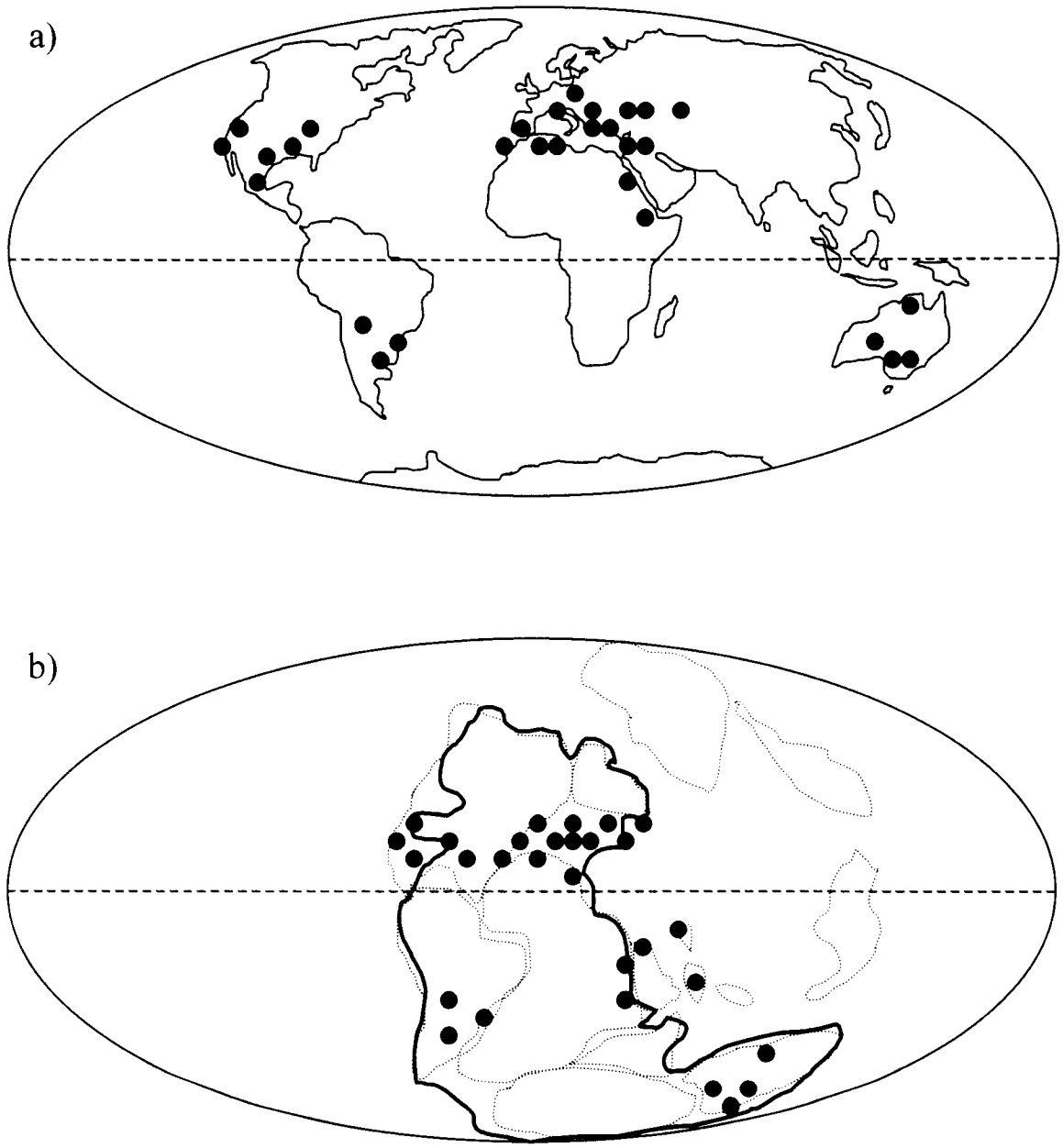


Figure 3.6. Distribution map of *Riccia lamellosa*. **–a.** Representative known localities are indicated by solid circles on a map of the modern world. **-b.** the same modern localities transferred to approximately corresponding positions on a map of the Permo-Triassic world (ca. 250 Ma). The Pangean supercontinent is drawn with a bold border; constituent modern continents are indicated with dashed borders. Redrawn from maps created by C. R. Scotese 1997 (Paleomap Preoject). Distribution data from Jovet-Ast (1986, 1991), Frey and Kürschner (1988) and Schuster (1992b). Transfer of Mediterranean locations on the Pangean supercontinent are necessarily somewhat approximate.

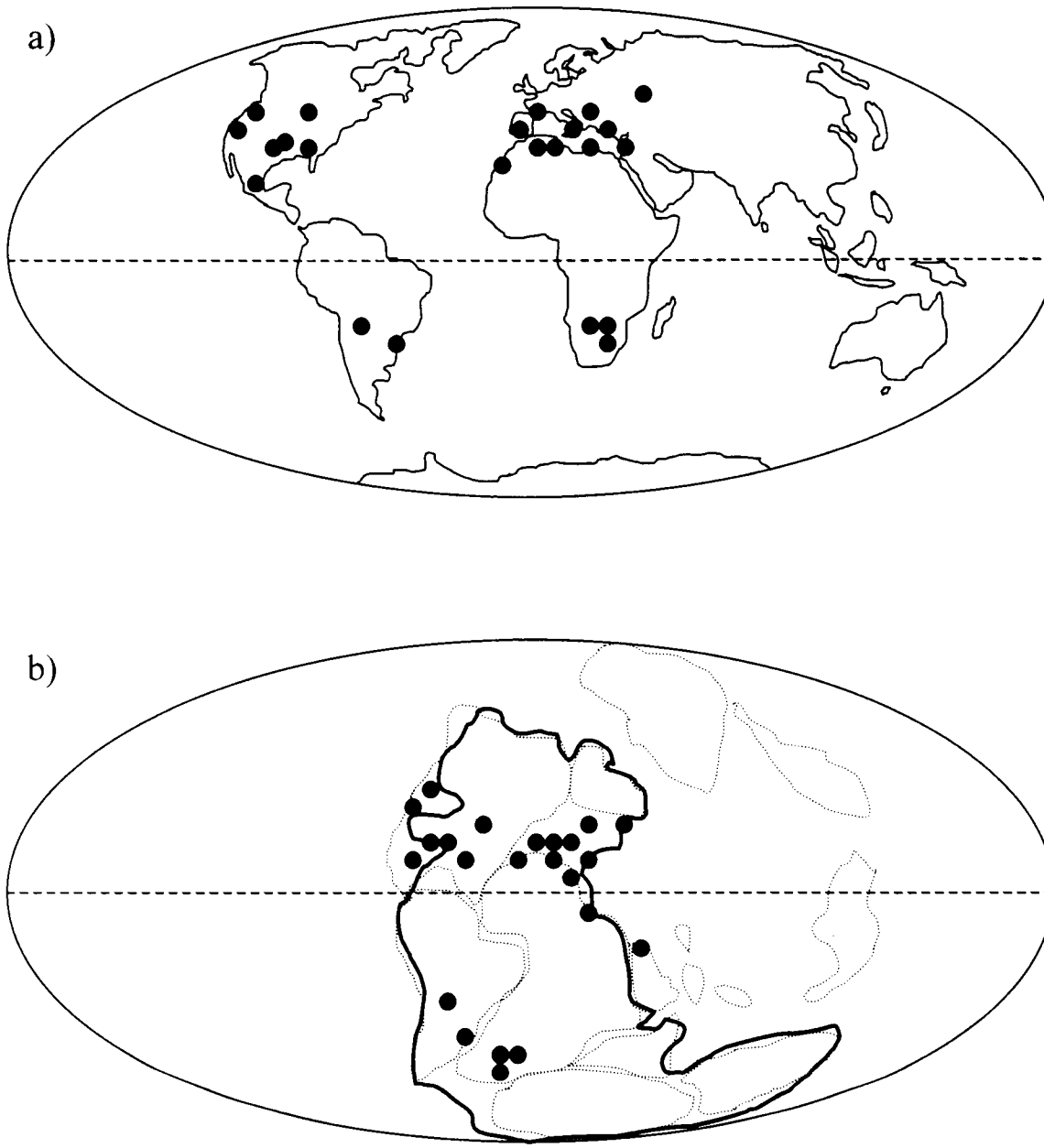


Figure 3.7. Distribution map of *Riccia macrocarpa*. **–a.** Representative known localities are indicated by solid circles on a map of the modern world. **-b.** the same modern localities transferred to approximately corresponding positions on a map of the Permo-Triassic world (ca. 250 Ma). The Pangean supercontinent is drawn with a bold border; constituent modern continents are indicated with dashed borders. Redrawn from maps created by C. R. Scotese 1997 (Paleomap Preoject). Distribution data from Jovet-Ast (1986, 1991), Perold (1991) and Schuster (1992b). Transfer of Mediterranean locations on the Pangean supercontinent are necessarily somewhat approximate.

perhaps the weak basal support for *Riccia* documented in this paper, traces to an explosive phase of evolution in riccioid and other marchantioid forms that was associated with environmental changes of the Permo-Triassic, a time of profound biological and ecological "reorganization" that saw extensive extinctions and then explosive radiations in many groups of organisms (Erwin 1993).

3.5.3. *Volatile morphology in Riccia*

Striking morphological divergence within the well-supported terminal clades discussed above, suggests a propensity in *Riccia* for volatile morphology that is not reflected in the underlying genetic history. Extreme morphological differentiation in closely related taxa is well-documented in many other groups [e.g. island radiations of *Tetramolopium* (Okada et al. 1997) and the Hawaiian silversword alliance (Bruce Baldwin, personal communication)]; however, the topologies presented here suggest that morphology might be positively misleading in *Riccia*. For example, consider Schuster's Section *Lamellosae* of Subgenus *Riccia*: species of this section include *R. lamellosa*, *R. albolimbata*, *R. macrocarpa* and *R. nigrella*. These four taxa were sampled here but do not form a monophyletic clade; in fact, they occur on widely separated branches of the strict consensus trees. The close apparent phylogenetic relationship between African species *R. tomentosa* and *R. schelpei* is remarkable; these two taxa ostensibly represent different monotypic subgenera. The results of this study suggest that characters emphasized in prior systematic treatments are unstable and therefore largely unreliable for the purpose of discriminating phylogenetically meaningful higher-level intrageneric taxa.

3.6. References

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Chapter 4

An Alternative Modular Hypothesis to Explain the Origin and Evolution of a "Complex" Thallus in Marchantioid Liverworts

John Wheeler
Department of Botany and Plant Pathology
Oregon State University, Corvallis OR, 97331

4.1. Abstract

Gametophytes of marchantialean liverworts (Marchantiales *sensu stricto*) are relatively complex compared to those of other embryophytes; air chambers are a unique and prevalent synapomorphy uniting all extant genera. Mehra (1957, *American Journal of Botany* 44: 573-585) argues that the complex chambered thallus seen in extant marchantialean liverworts (e.g. *Marchantia*) can be traced back through a series of logical hypothetical intermediate morphologies to a ± foliose *Petalophyllum*-like ancestor with erect obliquely-oriented unistratose lamellae. Doyle (1962, *University of California Publications in Botany* 33: 185-268) advocates the sphaerocarpalean monotype *Geothallus* as a better model and suggests that some features in *Geothallus* might have some bearing on the origin of a complex (marchantioid) thallus. The developmental observations of *Marchantia* by Burgeff (1943, Verlag von Gustav Fischer. Jena) [i.e. backward-sweeping arcuate lineages of chambers] seem at odds with the basic tenets of the Mehran hypothesis which draws as its evidence, the concept of forward-extending chamber arrays. The lappet-module hypothesis presented here is a synthesis largely derived from the concepts of Burgeff and Doyle and attempts to reconcile the novel observations of both workers. The modern marchantioid complex thallus is here envisioned as a highly regularized reticulum of fused dorsal lappets; backward-sweeping arcuate lineages of air chambers can be envisioned as the product of fused lappet-modules. Tightly controlled, regularized fusion of lappet-modules

would result in a reticulum of chambers that are each bordered by unistratose sidewalls; this senario is consistent with the basic pattern seen in modern chambered thalli.

4.2. Introduction

Gametophytes of marchantialean liverworts (Marchantiales *sensu stricto*) are the most complex of any extant embryophyte (Whittemore 1991). Marchantialean liverworts are often termed the "complex-thalloid" or "chambered" liverworts to reflect this relative morphological complexity. The thallus is generally flat and appressed to the substrate; three distinct tissues layers are typical: a unistratose dorsal epidermis, an chlorophylose aerenchymous layer, and a ventral (often massive) parenchymous storage layer. Air chambers are a unique and prevalent feature in all extant genera, "perhaps the most striking single character in the order" (Proskauer 1961).

About forty years ago, Mehra published the first of three articles in which he proposed and advocated a "new suggestion on the origin of thallus in Marchantiales" (Mehra 1957a; 1957b; 1958). In short, he argued that the complex chambered thallus seen in extant marchantialean liverworts (e.g. *Marchantia*) could be traced back through a series of logical hypothetical intermediate morphologies to a ± foliose *Petalophyllum*-like ancestor (i.e. Metzgeriales) with erect obliquely-oriented unistratose lamellae (Figure 4.1). According to Mehra's theory, secondary strut-like "cross-partitions" evolved between the parallel lamellae to stabilize the increasingly large and three-dimensional thallus; air chambers seen in modern taxa are homologous to the polygonal space or compartment that inevitably formed via cross-linking of lamellae by these hypothetical cross-partitions. In Mehra's model, compartments/ chambers are apparently basically accidental, a consequence of selection for structural strength. Only after thallus compartmentalization had occurred was this incidental invention of the chamber co-opted for other non-structural purposes; elaborations of the chamber unit [chlorophylose-filament carpeting, roofing by epidermis and specialized air pores] were secondary innovations that promoted water use and photosynthetic efficiency.

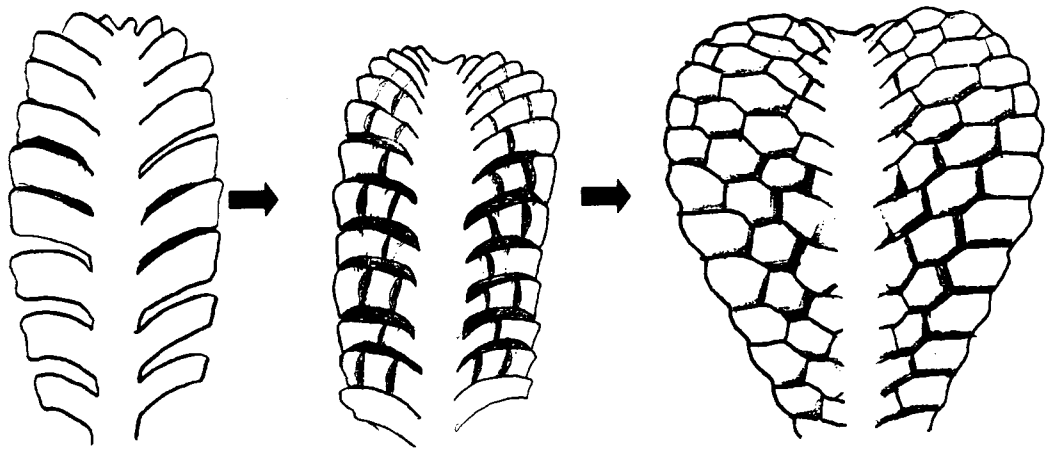

Figure 4.1. Schematic diagram illustrating the the evolution of a complex thallus (right) from a leafy ancestral type (left); the cental image dipicts a putative transitional form with strut-like cross-partitions between lamellae. Modified fromMehra (1957).

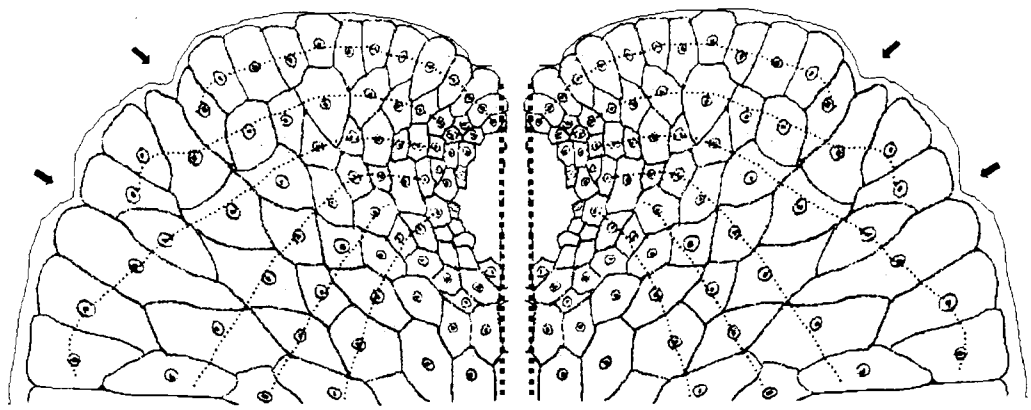

Figure 4.2. Schematic diagram illustrating putative backward-sweeping arcuate chamber lineages. Arrows indicate the position of marginal indentations that Mehra interpreted as the vestiges of ancestral lamella. Modified from Burgeff (1943).

In his careful study of the rare sphaerocarpalean monotype *Geothallus tuberosus*, Doyle (1962) discusses several morphological features he considered germane to the issue of complex-thallus evolution. Though he did not develop a specific morphological model or scenario like the one introduced below, Doyle did apparently see the shadows of a complex-thallus in *Geothallus*:

> ...If, however, Mehra's argument must be used, then surely *Geothallus* might profitable be substituted for *Petalophyllum*. *Geothallus* has all the characters of *Petalophyllum* used by Mehra in developing his argument and in its other characters.... has fewer conflicts with those of the marchantioid group. The development of a reticulum, air pockets, and bottles in *Geothallus*, which arise by direct upgrowth of superficial cells, may be of importance in this consideration (page 225)...

> ...The presence of dorsal lappets, leaf fusion, and lamella and reticulum formation in *Geothallus* may be considered of phylogenetic significance in speculations on the origin of the marchantioid thallus (page 224)...

Schuster (1992a) tacitly accepts a general Mehran model but worries that Mehra's theory is "over ingenious" and concludes that the origin and evolution of the marchantioid complex-thallus remains a largely unsolved mystery. He discusses the possibility that initial divergence of the jungermannioid and marchantioid lineages might have predated the evolution of all leaf-like structures, that metzgerioid and marchantioid thallus morphologies may have evolved separately from a leafless axial common ancestor. He goes on to propose his "unified theory" of thallus evolution: modern complex-thalloid forms trace back to a *Sphaerocarpos*-like progenitor that in turn became superficially *Petalophyllum*-like "by a process of condensation similar to that visualized by [Mehra]."

The only real difference, then, between the Condensation Theory of Mehra and the Unified Theory of Schuster is that Schuster explicitly invokes a sphaerocarpalean ancestor. Schuster is under the impression that Mehra had explicitly invoked the actual

taxon *Petalophyllum* as his model ancestor: "Although Mehra puts much weight on *Petalophyllum* as a starting point in evolution, I am exceedingly skeptical of the validity of any such conceptualization" (Schuster 1992a; page 765). But to be fair, Mehra does not advocate the taxon *Petalophyllum* in the strict sense; he writes, "It is certainly not to be suggested that *Petalophyllum* is an intermediate form in the evolution of the marchantiaceous thallus, but simply that it gives us an insight into the parallel steps which may have worked during the condensation of leafy forms in the remote past, ..." (Mehra 1957b; page 573).

The exhaustive research of Burgeff (1943) meticulously documents the morphology of *Marchantia*. The possible bearing of his observations on the evolution of a complex-thallus is not discussed by either Mehra or Schuster and seems to cast doubt on fundamental tenets implicit in the models of both. The concept of backward-sweeping arcuate lineages of air chambers, as interpreted by Burgeff (see below), is clearly at odds with the concept of forward-extending arrays explicit in Mehra's concept (and then followed by Schuster). Arguments presented here attempt to account for these apparent conflicts with an alternative modular hypothesis to explain the origin of a marchantioid complex-thallus.

4.3. Schizogeny vs. laminar upgrowth

The ontogeny and morphology of marchantialean air chambers and associated pores in has been the subject of much focused study (Leitgeb 1879; Barnes and Land 1907; Hirsh 1910; Evans 1918; Burgeff 1943; Kronestedt 1982; Apostolakos, Galatis and Mitrakos 1982; Apostolakos and Galatis 1984, 1985a, 1985b). Original and persistent questions in these papers have been 1) whether chambers are the result of coordinated epidermal upgrowths (i.e. up from the floor) or, alternatively, whether chambers arise by schizogeny of solid tissues (i.e. down from the roof) and 2) whether chambers originate at the surface or in subsurface tissues as intercellular spaces. The weight of evidence, based on the study of derived modern taxa, supports a schizogenous origin very near the apical initials; incipient chambers (in the form of simple surface

clefts) are usually evident after only 2-3 cell divisions in the median longitudinal plane (Barnes and Land 1907; Evans 1918; Apostolakos, Galatis and Mitrakos 1982).

Proskauer (1961) points out that " [Mehra's] theory completely disregards, and is incompatible with, the ontogeny of the marchantialean chamber, where in the beginning there is a hole, and not the upgrowth of tissue from an uninterrupted thallus." After twenty years of additional collective study, Crandall-Stotler (1981) writes in her review that "there are no known developmental sequences of lamellar upgrowth, followed by epidermal overtopping, as should be expected if the Mehra theory were correct." However, contrast this with a later passage in the same essay by Crandall-Stotler, [referring to gametangia in Sphaerocarpales and Ricciales (i.e. riccioid Marchantiales), respectively] she writes, "with flask-shaped involucres surrounding each... in the former..., and epidermal **upgrowths** enclosing them in pits in the latter." (page 352).

Whether these observations have any bearing at all on the phylogenetic origin of the marchantialean air chamber is unknown. Air chambers are preserved in fossil marchantioids by the Triassic (Schuster 1984); it is questionable whether the ontogeny of derivative tissues in highly specialized modern taxa can still be trusted to say anything definitive about such an ancient event.

In *Sphaerocarpos*, the vegetative plant body is extremely abbreviated; gametangia are produced immediately near the apical meristem where they are seated and remain in a sessile position on the dorsal surface of the thallus. In the acarpocephalate marchantialean genus *Riccia* we also see a very rapid apical production of gametangia. Schuster (1992a) has diagrammed various marchantioid thallus-models; in both the 'solid' *Riccia* model and the 'spongy' *Ricciella* model (Figure 901:3,5), gametangia are depicted as sessile on the *thallus proper* which is progressively concealed by tissue upgrowth that progressively buries the true (ancestral?) thallus surface and any sessile organs.

An alternative interpretation of thallus ontogeny (one that accommodates both schizogeny and tissue upgrowth) is that compact tissues present at the apical meristem region in modern complex-thalloid taxa are in fact **secondarily** solid by compaction of ancestrally lamellar tissues. Such an argument would grant that the origin of chambers

is schizogenous; however, cleavage is secondary, in such a case, because it occurs between cells that were once free in the ancestral condition.

4.4. Putative ancestral types

Extant marchantioid liverworts (Marchantiopsida: consisting of Monocleales, Sphaerocarpales and Marchantiales) are the heterogeneous terminal taxa of an extremely old lineage. Monophyly and long phylogenetic isolation of Marchantiopsida from other extant bryophyte stem groups (jungermannioid liverworts, mosses and hornworts) is strongly supported by several recent molecular phylogenetic analyses (Waters et al. 1992; Capesius 1995; Bopp and Capesius 1996; Capesius and Bopp 1997; Lewis et al. 1997; Wheeler, in prep., Chapter 2). Analyses that sample Monocleales (*Monoclea*) position this taxon within Marchantiales *sensu stricto* (Lewis et al. 1997; Wheeler, in prep.); affinity of extant Sphaerocarpales to basal Marchantiales is well supported.

Monoclea (Monocleales) ostensibly exhibits a mixture of jungermannioid and marchantioid features (Schuster 1984; p.1040). On the weight of evidence, Schuster established the order Monocleales to account for only two species, *Monoclea fosteri* and *Monoclea gottschei*; however, there is no lack of precedent in the historical literature for the concept of placing *Monoclea* within Marchantiales (e.g. Campbell 1898; Müller 1939; Burgeff 1943; Proskauer 1951; Hässel de Menendez 1962). Affinity of *Monoclea* with the carpocephalate marchantialean genus *Dumortiera* is strongly supported by *rbc*L data (Lewis et al.1997), consistent with the hypotheses of some earlier authors. Following a careful morphological examination of semi-aquatic *Monoclea* specimens collected in Jamaica, Johnson (1904) concluded that the "absence of air chambers and ventral scales is probably due to the nearly aquatic habit of the plant". Extreme simplification and/or reduction of both air pores and ventral scales is seen in certain extant marchantialean taxa such as *Dumortiera* (Schuster 1992b) and *Cyathodium* (Srivastava and Dixit 1996). Given the reductive morphological specializations seen in other extant taxa, a super-specialized *Monoclea* derived from within Marchantiales

sensu stricto seems plausible. Independent placement of *Monoclea* within extant Marchantiales with chloroplast *rbc*L (Lewis et al. paper) and with nuclear LSU rDNA and chloroplast *trn*L-region data (Wheeler, in prep., Chapter 2) suggests that ordinal status is unwarranted.

Elimination of *Monoclea* as a primitive prototype greatly simplifies any further discussion of protomarchantioid evolution. Even Schuster confesses that "...attempts to 'wedge' *Monoclea* into a sequence going from a bilateral, leafy, *Sphaerocarpos*-like type to a marchantioid, complex thallus, to accommodate this genus [i.e. *Monoclea*] into the 'Mehra hypothesis', any such attempt is probably futile." (Schuster 1992a: page 766). The putatively archaic morphology seen in extant *Monoclea* is apparently secondary, the consequence of retrograde adaptation to shady, mesic habitats; therefore, a model for the proto-marchantioid should be sought elsewhere.

Blepharoplast features indicate that among extant jungermannioids examined to date, only *Blasia* resembles sampled Marchantiopsida (Rushing et al. 1995; Brown et al. 1995; Pass and Renzaglia 1995). Pass and Renzaglia (1995) recommend elevating *Blasia* (and *Cavicularia*) to the Order Blasiales; moreover, these authors also recommend realigning Blasiales into the marchantioid stem. Renzaglia (1982) states that this species is "one of the most interesting and complex of the Metzgeriales." *Blasia* (and sister genus *Cavicularia*) differ from all other metzgerioids by the presence of two-ranked ventral scales, a feature seen in many marchantioid taxa. *Blasia* can develop leaf-like lobes on elongate thalli but typically occurs as a thalloid plant with a discrete-rosette habit (Renzaglia 1982; personal observation). Schuster (1992a) writes, "As Leitgeb emphasized, distinctive for *Blasia* are the clearly alternate lateral 'leaves,' whose origin, as **lappet**-like structures, very close to the growing point are evident". If Blasiales does indeed belong to the Marchantioid stem, then perhaps the hypothetical protomarchantioid can be imagined as a synthetic model exhibiting features common to both Blasiales and Sphaerocarpales. [note: 'lappet' is a general term used to denote any kind of unistratose free-standing flap-like or plate-like structures with unknown or unclear function].

The order Sphaerocarpales consists of three extant genera (*Sphaerocarpos*, *Geothallus*, *Riella*) and perhaps one Triassic fossil genus (*Naiadita*). Collectively, these

taxa (Sphaerocarpales) are known as the "bottle hepatics" by virtue of synapomorphous bottle- or flask-like involucres that contain the gametangia. Most taxa are dioecious and heterothallic but a few monoecious species (in the genus *Riella*) are known. Unlike Marchantiales (and Monocleales), the photosynthetic lamina in Sphaerocarpales is always delicate, unistratose and translucent. Topographically-dimorphic pegged rhizoids (a derived feature common to Marchantiales and Monocleales; Schuster 1992a: figure 904) apparently never occur in Sphaerocarpales where simple smooth rhizoids are the rule.

4.5. Overview of sphaerocarpalean morphology

The genus *Riella* (about 18 species worldwide) is unique among all liverworts in its obligate-aquatic habit. Species occur in ephemeral habitats (e.g. vernal pools, playas, and seasonal steams). Species are collectively very unusual and specialized for aquatic life; the plant body is alga-like with an erect undulating wing-like thallus that is typically anchored to the substrate only at the holdfast-like base. Structural fusion has been a main theme in the evolutionary history of *Riella*. For example, antheridial involucres are arranged in a linear series along the free margin of the wing. Each involucre of the series is separate but imbedded into the continuous wing lamina; involucral walls are continuous (fused) with surrounding wing tissue.

The monotype *Geothallus tuberosus* is known from a few sites in southern California. The species is associated with vernal pool habitats where it is seasonally active but perenniates much of the year as drought-resistant tubers in the soil. The plant is mildly heterothallic, unisexual and resembles a large *Sphaerocarpos*. Doyle (1962) chronicles a rather loose morphogenesis in *Geothallus* with relatively frequent morphological aberrations such as free-standing dorsal **lappets**, lobe-fusion and the formation of occasional air-pockets in the lamina.

The genus *Sphaerocarpos* (8-12 species worldwide) occurs as strongly-heterothallic, unisexual ephemerals on early-successional mineral soils. Meiospores are permanently-united in most species as tetrads. Extreme crowding of involucres under

natural (high-light) conditions, tends to obscure the underlying thallus morphology but in etiolated material, or material grown in culture, the branching pattern is revealed; Proskauer (1954: Figure 3) clearly illustrates a forking thallus with alternating succubously-inserted leaf-like lobes. In *Sphaerocarpos*, we also see a rather loose control of morphogenesis; numerous culture studies document a propensity in the genus for the expression of aberrant morphologies such as fusion of parts, free-standing dorsal lobes (lappets), plates, scales and ridges, free-standing dorsal cell pillars (cilia), solid multistratose columns, deformed sterile involucres and deformed fertile involucres (Rickett 1920; Allen 1924; Allen 1925; Wolfson 1925; Allen 1935; Dillar, Fulford and Kersten 1955a, 1955b).

4.6. The implications of air chamber orientation

Mehra emphasizes the pattern of air chamber orientation that is obvious in many cleared modern Marchantialean thalli (Mehra 1957a: e.g. Figure 10, page 510); of paramount importance to him is the spatial organization of chambers into "basic lamellae" which ostensibly reflect the original lamellae of the *Petalophyllum*-like ancestor in a truly homologous sense. In cleared thalli, chambers do indeed extend outward and forward in neat arrays suggestive of obliquely-oriented parallel lamellae; to conclude that these arrays represent the modern form of primordial lamellae is certainly reasonable.

But the argument seems to rest or fall on whether there is homology in the strict phylogenetic sense between the parallel forward-extending chamber arrays seen in cleared modern complex-thalloids and the parallel (forward-extending) lamellae envisioned in the hypothetical ancestor (Figure 4.1). Strict phylogenetic homology between these structures is brought into question by the figures of Burgeff (1943). Of particular importance, in this light, is a figure carefully drawn to depict the dorsal view of the apical meristem and its immediate cell derivatives (Figure 19: page 18). In this figure, Burgeff attempts to follow the course of air chambers as they are sequentially generated from the apical region, implying that the chambers themselves form discreet

lineages that in turn reflect common meristematic initials or sets of initials. In his Figure 19, air chambers are traced back through morphological time from older to younger tissues following a series of backward-sweeping arcuate trajectories (Figure 4.2).

The concept of backward-sweeping arcuate lineages of air chambers, as interpreted by Burgeff, is clearly at odds with the concept of the forward-extending arrays explicit in Mehra's concept (and then followed by Schuster).

4.7. Dorsal lappets

Previous authors have argued that lappets and various other elaborations seen on the dorsal surface of cultured *Sphaerocarpos* specimens may actually represent deformed or degenerate involucral tissues. Wolfson (1925) notes that " there are also peculiar upgrowths from the thickened parts of the thallus. These are very variable in size and shape... the position of these upgrowths on the thallus leads to the conclusion that they may be distorted involucres" (page 322). Allen (1925), while describing antheridia in the mutant clone *polycladous*, observed that " involucres at times seem to be entirely absent; at other times they are saucer-shaped, or laterally expanded and then leaf-like, lacerate, or ciliate. The dorsal cilia previously referred to probably often represent reduced and dissected involucres" (page 2). Rickett (1920) writes, "in plants grown in culture, the involucres are often broadly open at the tip and show various irregularities in form. Dorsal lobes [lappets] were observed in several cases, and there are gradations between these dorsal lobes and the normal involucres" (page 191).

Doyle (1962) writes, " [in *Geothallus*] the dorsal lappets sometimes occur among the bottles on the surface of the midrib, but more commonly that are present on the midribs of sterile plants, where they are often so abundant as to obscure them" (page 196). Doyle (Figure 3.k) maps the orderly sequential production of dorsal lappet primordia behind the apical meristematic region in *Geothallus*. These primordia begin as concave arcuate ridges; "these ridges develop into dorsal lappets" (page 194). Another striking feature occasionally seen in *Geothallus* (and *Sphaerocarpos*) is the

propensity for morphological fusion of normally free parts; in *Geothallus,* fusion of low upgrowths can result in the formation of weak reticula on the dorsal surface (Doyle 1962: Figure 3.1).

4.8. An alternative modular hypothesis

What conceivable chain of events might have led to the modern complex chambered-thallus (e.g. *Marchantia*) from a *Geothallus*-like or *Sphaerocarpos*-like (perhaps even *Blasia*-like) ancestral form? I imagine the following chain of events (implicit in Figure 4.3):

1). Evolution of an ancestral taxon with regular (integrated) production of arcuate dorsal lappet-modules,

2). Further regularization of lappet-modules into neat bilateral dorsal files; each file of lappets arising from a common initial or set of initials in the apical meristem,

3). Lateral duplication into multiple dorsal files on either side of the midline (lateral expansion of the thallus) ± simultaneous with,

4). Consolidation/ fusion of lappet-modules within a file and among adjacent files to form a regular reticulum of 'air chambers' (walls of the reticulum equivalent and homologous to the sidewalls of air chambers in modern taxa),

5). Followed by the subsequent evolution of chamber roofing, chamber filaments and elaborate air pores seen in modern taxa.

The above scenario is consistent with the observations of Burgeff (1943). His backward-sweeping arcuate lineages of air chambers (Figure 4.2) can be envisioned as the product of backward sweeping arcuate lineages of fused lappet-modules (Figure 4.3). Tightly controlled, regularized fusion of lappet-modules (Figure 4.3) would result in a reticulum of chambers that are each bordered by unistratose sidewalls; this is exactly consistent with the basic pattern seen in modern chambered thalli.

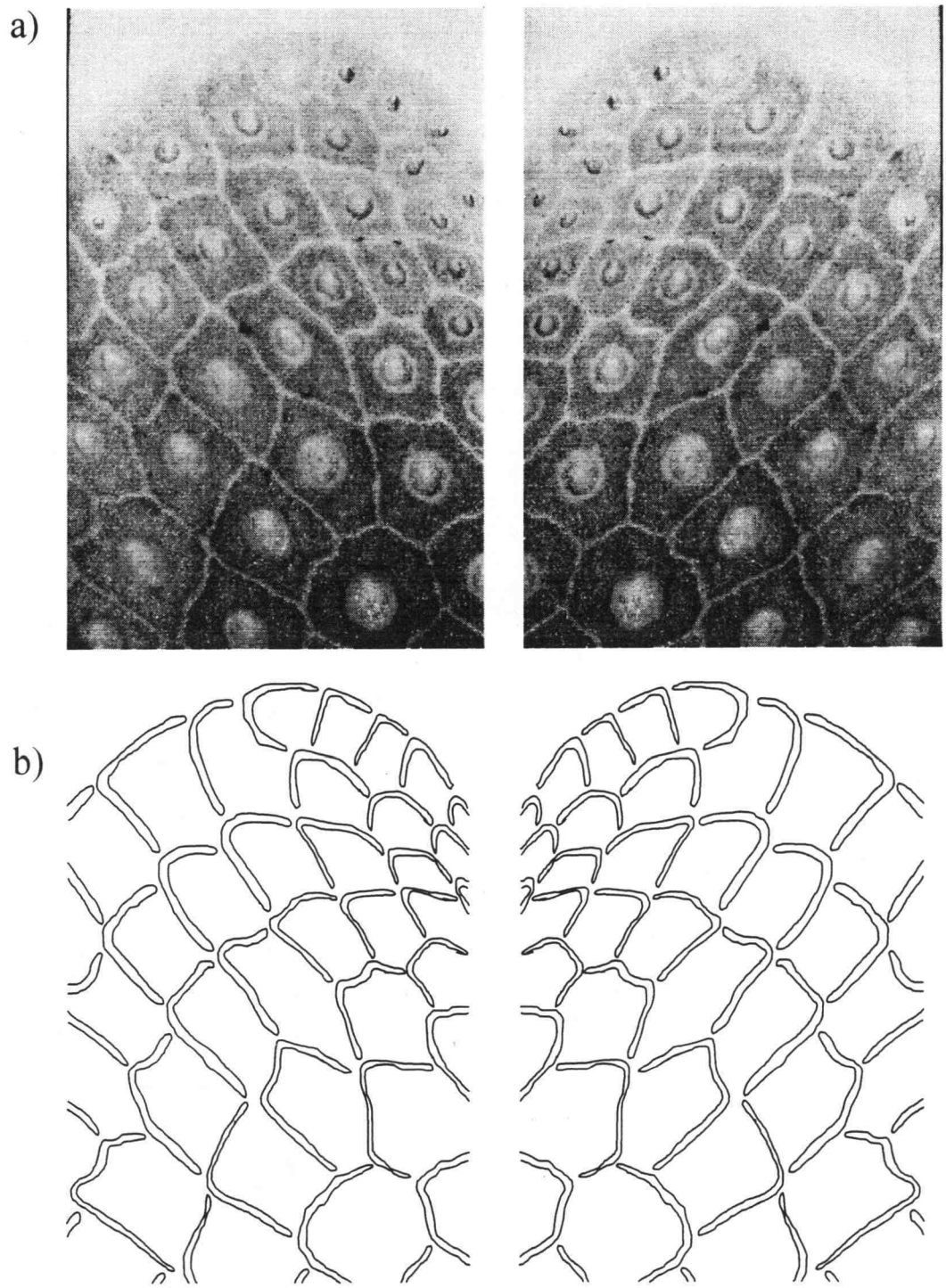

Figure 4.3. Schematic illustrating a lappet-modular model for the evolution of a complex thallus in Marchantiales. **a**.) The apical meristematic region of *Conocephalum conicum* visualized schematically as **b**.) a fusion-network of free-standing arcuate dorsal lappets .

The lappet-module concept presented here could also be invoked to account for the origin of elaborate air pores, a prominent feature in so many modern complex-thalloid taxa. As discussed above, in modern sphaerocarpalean taxa the dorsal lappet probably represents a modified involucral unit. Proskauer (1954) has demonstrated marked differentiation of cells at the mouth of the involucre in *Sphaerocarpos stipitatus*. Could the elaborate air pores of complex-thalloid liverworts ultimately trace back to ancient genes that originally evolved to govern expression of orifice morphology in the involucre? (i.e. genes that were present before the involucre was modified and appropriated as a modular building-block (lappet) during evolution of the modern chambered thallus). The utilization of pre-existing genes during air-pore evolution seems more parsimonious than does their de novo creation.

4.9. Future research

The lappet-module hypothesis presented here is a synthesis largely derived from the concepts of Burgeff (1943) and Doyle (1962) and attempts to reconcile the novel observations of both workers. Both Burgeff and Doyle focused their attention on the apical meristem. A careful reinvestigation and survey of apical meristems in diverse marchantioid liverworts, in light of a possible lappet-modular explanation, seems warranted. In particular, a careful study of the developmental trajectory of cell / chamber lineages in indicated.

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Chapter 5

Conclusions

A long history (well-supported branch) unites all Marchantiopsida sampled in this study and isolates this clade from other liverworts and bryophytes. This long branch may suggest extensive extinction of proto- and eomarchantioid forms that led to modern taxa. A major theme of topologies presented here is the unresolved marchantioid polytomy that follows the well-supported basal nodes. I speculate that this polytomy corresponds to an explosive radiation of marchantioid forms coincident with extreme conditions of the Permo-Triassic. However, the origin of Marchantiopsida probably occurred much earlier; amidst, perhaps, a series of long-extinct *Blasia*-like ancestors that colonized and innovated on any of various xeric surfaces (either cool or warm) that were available throughout embryophyte history in the Paleozoic.

Independent placement of *Monoclea* **within** extant Marchantiales with chloroplast *rbc*L (Lewis et al. 1996) and with both nuclear LSU rDNA and chloroplast *trn*L-region data (this study) suggests that ordinal status is unwarranted and that a model for the proto-marchantioid should be sought elsewhere.

The possibility that *Riccia* might be a polyphyletic catch-all, derived from independently reduced marchantioid lineages is not supported. All analyses point to a monophyletic *Riccia*. All topologies imply that this genus was derived, ultimately, from a carpocephalate ancestor (assuming a single origin of the carpocephalum).

Striking morphological divergence within well-supported terminal clades, suggests a propensity in *Riccia* for volatile morphology that is not reflected in the underlying genetic history. The topologies presented here suggest that morphology might be positively misleading in *Riccia*. The results of this study suggest that characters emphasized in prior systematic treatments are unstable and therefore largely

unreliable for the purpose of discriminating phylogenetically meaningful higher-level intrageneric taxa.

In all phylogenetic topologies presented here (nuclear, plastid and combined), resolution and/or support for basal relationships within *Riccia* is weak. Weak support at the base of *Riccia* may be the hallmark of an actual explosive initial radiation. If apomorphies accumulate at a relatively consistent rate over geologic time then weak support at the base of a large radiation might be explained as the inevitable consequence of that rapid evolution, i.e. there was simply insufficient time for documentation of the rapid cladogenesis (in the form of synapomorphic signatures).

Existing hypotheses regarding the origin of a complex marchantialean thallus seem at odds with actual air chamber ontogeny. An alternative lappet-modular hypothesis presented here seems more parsimonious in view of recent well-supported phylogenetic reconstructions (within Marchantiopsida) and more compatible with the actual thallus morphology of extant Marchantialean taxa.

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Appendices

Appendix 1. Sequence alignment (905 bp) for the full (all 48 taxa) nuclear data set.

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                1           11          21          31          41          50
                |           |           |           |           |           |
R-soroca        TAAGCGGAGG  AAAAGAA-CT  AACAAGGATT  CCCTTAGTAG  CGGCGAGCGA    49
R-gouget        ..........  ..........  ..........  ..........  ..........    50
R-macroc        .........A  ..........  ..........  ...C......  ..........    50
R-nigrel        .........A  ..........  ..........  ..........  ..........    50
R-atroma        ..........  ..........  ..........  ...C......  ..........    50
R-tricho        ..........  ..........  ..........  ..........  ..........    50
R-beyric        ..........  ..........  ..........  ...C......  ..........    50
R-frosti        .........-  ..........  ..........  ..........  ..........    49
R-cavern        ..........  ..........  ..........  ...C......  ..........    50
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R-natan2        ..........  ..........  ..........  ...C......  ..........    50
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O-crista        ..........  ..........  ..........  ..........  ..........    50
Peltolep        ...-......  ..........  ..........  ...C......  ..........    49
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Cryptomi        ..........  ..........  ..........  ..........  ..........    50
Plagioch        ..........  ..........  ...G......  ...C......  ..........    50
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  Blasia        .........n  ..........  ..........  T..C......  ..........    50
Fossombr        ..........  .......A..  ..........  T........A  ..........    50
  Pellia        ..........  .......A..  ...G......  T..C.....A  ..........    50
Dendroal        ..........  .G.....A..  ...C......  T..C.....A  ..........    50
Metaneck        ..........  .G.....A..  ...C......  T..C.....A  ..........    50
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                51          61          71          81          91         100
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R-macroc        ..........  ..........  ..........  ..........  ..........  100
R-nigrel        ..........  ..........  .G........  ..........  ..........  100
R-atroma        ..........  ..........  ..........  ..........  ..........  100
R-tricho        ..........  ..........  ..........  ..........  ..........  100
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R-schelp        ..........  ..........  ..........  ..........  ..........  100
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Cryptomi        .......GA.  ..........  .........T  ..........  ..........  100
Plagioch        .......GA.  ..........  .........T  .....A....  ..........  100
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  Blasia        .....A....  ..........  .G.......G  G...CC....  .A........  100
Fossombr        .....A....  ..........  .......T.G  G...CA.A..  .A........  100
  Pellia        .....A..TG  ..........  ......T...  G...C.T...  .A........  100
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Coleocha        ........A.  .....A....  G.....TG.G  G...CCTC..  .A....n...   99
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                101         111         121         131         141         150
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R-macroc        ..........  ..........  ..........  ..........  ..........  150
R-nigrel        ..........  ..........  ..........  ..........  ..........  150
R-atroma        ..........  ..........  ..........  ..........  ..........  150
R-tricho        ..........  ..........  ..........  ..........  ..........  150
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  Blasia        ........C.  ..........  ......G...  ......T...  ..........  150
Fossombr        ........C.  ..........  ......G...  ......T...  ......C...  150
  Pellia        ........C.  ..........  ......G...  ......T...  ......C...  150
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Metaneck        ........GT  C.......GA  ..CG..G...  ......T...  ..........  150
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R-macroc        .......... .......... .......... .......... ..........  200
R-nigrel        .......... .......... .......... .......... ..........  200
R-atroma        .......... .......... .......... .......... ..........  200
R-tricho        .......... .......... .......... .......... ..........  200
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R-natan2        .....A.... .......... .......... .......... ..........  200
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  Blasia        A......... .......... .......... A..TC..... ..........  200
Fossombr        A......... .......... G......... A..TC..... ..........  200
  Pellia        A......... .......... G......... A..TC..... ..........  200
Dendroal        A....ATC.. ........C. ........C. C...C...GT GC.TC.....  200
Metaneck        A....ATC.. ........C. ........C. C...C...A. GC.TCT....  200
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Sphagnum        A..AGA...A .......... T......... A...C..... ...A......  200
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A-punct1        A....n.... .......... G......... A..TC..... ...T......  200
Coleocha        A....A.... C......... G......... AT..C..... .....A....  199
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                201         211         221         231         241         250
                |           |           |           |           |           |
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R-gouget        ..........  ..........  ..........  ..........  .......C..  250
R-macroc        ..........  ..........  ..........  ..........  .......C..  250
R-nigrel        ..........  ..........  ..........  ..........  .......C..  250
R-atroma        ..........  ..........  ..........  ..........  ..........  250
R-tricho        ..........  ..........  ..........  ..........  .......C..  250
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R-natan2        ..........  ...T......  ..........  ..........  .......C..  250
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Cryptomi        ..........  ..........  ..........  ..........  .......A..  250
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  Blasia        ..........  ....G.....  ..........  ..........  .......C..  250
Fossombr        ..........  ....G.....  ..........  ..........  .......C..  250
  Pellia        ..........  ....T.....  ..........  ..........  .......C..  250
Dendroal        ......AC..  ...TG.....  ..........  ..........  ......TC..  250
Metaneck        ......AC..  ...TG.....  ..........  ..........  .......C..  250
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                251         261         271         281         291         300
                 |           |           |           |           |           |
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R-tricho        .......... .......... .......... .......... ..........  300
R-beyric        .......... .......... .......C.. .......... ..........  300
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R-lamell        .......... .......... .......C.. .......... ..........  299
R-papulo        .......... .......... .......CG. .......... ..........  299
R-hueben        .......... .......... .......CG. .......... ..........  300
R-toment        .......... .......... .......... .......... ..........  300
R-schelp        .......... .......... .......... .......... ..........  300
R-alboli        .......... .......... .......... .......... ..........  300
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R-natan2        .......... .......... .......CT. .......... ..........  300
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Cryptomi        .......... .......... .......CG. .......... ..........  300
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  Riella        .......... .......... .......CG. .......... ..........  300
  Blasia        .T........ ..A....... .......CG. ..A....... ..........  300
Fossombr        .T........ ..A....... .......CG. ..AA...... ..........  300
  Pellia        .T........ ..A....... .......CG. ..AA...... .....T....  300
Dendroal        .......... .......... ........G. ..AA...... ..........  300
Metaneck        .......... .......... ........G. ..AA...... ..........  300
Tetraphi        .T.....N.. ..A....... ........T. ..AA...... ..........  300
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A-punct1        .T........ ..A....... .......CT. ..AA...... ..........  300
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            |           |           |           |           |           |
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Athalami    .........T .......... T......... .......... ..........  350
Cryptomi    .......... .......... .......... .......... ..........  350
Plagioch    .......... .......... .......... .......... ..........  350
Reboulia    .......... .......... .......... .......... ..........  348
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Corsinia    .......... .......... .......... .......... ...C...G..  348
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S-texan1    .......... .......... .......... .......... .......G..  350
  Riella    .......... .......... .......... .......... .......G..  350
  Blasia    .......... .......... .......... .......... .......G..  350
Fossombr    .......... .......... .......... .......... .......G..  350
  Pellia    .......... .......... .......... .......... .......G..  350
Dendroal    .......... .......... .......... .......... ..........  350
Metaneck    .......... .......... .......... .......... ..........  350
Tetraphi    .......... .......... .......... .......... ..........  350
Sphagnum    .......... .......... .......... .......... .......G..  350
A-punct2    .......... .......... .......... .......... ...G...G..  347
A-punct1    .......... .......... .......... .......... ...G...G..  350
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                351         361         371         381         391         400
                |           |           |           |           |           |
R-soroca        TGCTTGAAAT  TGCTGGGAAG  GAAGCGAATG  GAAGCCTCGT  GTGCGCCCCG  395
R-gouget        ..........  ........G.  ..........  ..-.......  ..........  399
R-macroc        ..........  ........G.  ..........  ..........  ..........  399
R-nigrel        ..........  ........G.  ..........  .........G  ..........  399
R-atroma        ..........  ........G.  ..........  ..........  ..........  400
R-tricho        ..........  ........G.  ..........  ..........  ..........  400
R-beyric        ..........  ........G.  ..........  .........G  ..........  400
R-frosti        ..........  ........G.  ..........  ..G.......  ..........  399
R-cavern        ..........  ........G.  ..........  ..G.......  ..........  400
R-membra        ..........  ........G.  ..........  ..G.......  T.........  398
R-villos        ..........  ........G.  ..........  ..G.......  ..........  400
R-albida        ..........  ........G.  ..........  ..G.......  ..........  400
R-lamell        ..........  ........G.  ..........  ..G.......  ..........  399
R-papulo        ..........  ........G.  ..........  ..G.......  ..........  399
R-hueben        ..........  ........G.  ..........  ..G.......  ..........  400
R-toment        ..........  ........G.  ..........  ..G.......  ..........  400
R-schelp        ..........  ..........  .....C....  ....G.C.C.  T.TT......  400
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R-natan1        ..........  ........G.  ..........  ..G.......  ....A.....  400
R-natan2        ..........  ........G.  ..........  ..G.......  ....A.....  400
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Peltolep        ..........  ........G.  ..........  ..G.......  ..........  399
Athalami        ..........  ........-.  ...A......  .........A.  ..........  399
Cryptomi        ..........  ........G.  ..........  ..G.......  ..........  400
Plagioch        ..........  ........-.  ..........  ..G.......  ..........  399
Reboulia        ..........  ........G.  ..........  ..-.......  ..........  397
A-gracil        ..........  ........G.  ..........  ..G.......  ..........  399
A-boland        ..........  ........G.  ..........  ..G.......  ..........  397
A-califo        ..........  ........G.  ..........  ..G.......  ..........  399
Marchant        ..........  ........G.  ..........  ..G.......  ..........  399
Targioni        ..........  ........G.  ..........  ..-....G..  ..........  399
Monoclea        ..........  ........G.  ..........  ..G.......  ..........  400
Corsinia        ..........  ........G.  ...n......  ..-.....AC  ..........  397
Lunulari        ..........  ........G.  ..........  ..-.....C.  ..........  398
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S-texan1        ..........  ........G.  ..........  ..G.......  ..........  400
  Riella        ..........  ........G.  ..........  ..G......G  .C........  400
  Blasia        ..........  ........G.  ..a.......  ..G..TG..G  ..........  400
Fossombr        ..........  ........G.  ..........  ..G..TG...  ..........  400
  Pellia        ..........  ........G.  ..........  ..G..TG..G  ..........  400
Dendroal        ..........  ........G.  ..........  .....TG..G  ..........  400
Metaneck        ..........  ........G.  ..........  .....TG..G  ..........  400
Tetraphi        ..........  ........G.  .........T  .....TGN.G  ..........  400
Sphagnum        ..........  ........G.  .........T  .....TG..G  T.........  400
A-punct2        ..........  .....A..G.  g...c.....  .....TGc.G  ...c......  397
A-punct1        ..........  .....A..G.  ..........  .....TG..G  .......T..  400
Coleocha        ..........  ..T..A.GG.  ..........  .TG..AGT.A  ....A..G..  399
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                401         411         421         431         441         450
                |           |           |           |           |           |
R-soroca        GTCGGATGCG  GAACGGCTGG  TCCGCCGCT-  CGACGCGGGG  -CGCTGGTCG   443
R-gouget        ..........  ..........  ..........  ....N.....  ..N.......-   448
R-macroc        ..........  ..........  ..........  ..........  ..........   449
R-nigrel        ..........  ..........  ..........  ..........  ..........   449
R-atroma        ..........  ..........  ..........  ..........  ..........   450
R-tricho        ..........  ..........  ..........  ....-.....  ..........   449
R-beyric        ..........  ..........  ..........  ..........  .........C   450
R-frosti        ..........  ..........  ..........  ..........  .........C   449
R-cavern        ..........  ..........  ..........  ..........  ..........   450
R-membra        ..........  ..........  ..........  ..........  ..........   448
R-villos        ..........  ..........  ..........  ....n.C...  .........-   449
R-albida        ..........  ..........  ..........  ..........  ..........   450
R-lamell        ..........  ..........  ..........  ..........  ..........   449
R-papulo        ..........  ..........  ..........  ..........  ..........   449
R-hueben        ..........  ..........  ..........  ..........  ..........   450
R-toment        ..........  ..........  ..........  ..........  ..........   450
R-schelp        ....N..C..  ..........  ..........  .A..C.....  ..........   450
R-alboli        ..........  ..........  ..........  ..........  ..........   450
R-natan1        ..........  ..........  ..........  ..........  TT........   450
R-natan2        ..........  ..........  ..........  ..........  TT........   450
O-incras        ..........  ..........  ..........  ....C.....  ..........   449
O-crista        ..........  ..........  ..........  ..........  ..........   450
Peltolep        ..........  g.........  ..........  ......C...  ..........   449
Athalami        ..........  ..........  ..........  ....A.C...  .....T...C   449
Cryptomi        ..........  ..........  ..........  ..........  .T........   450
Plagioch        ..........  ..........  ..........  ..........  ..........   449
Reboulia        .........n  ..........  ..........  .N........  .........N   447
A-gracil        ..........  ..........  ..........  ..........  .T........   449
A-boland        ..........  ..........  ..........  ..........  ..........   447
A-califo        ..........  ..........  ..........  ..........  .T........   449
Marchant        ..........  ..........  ..........  ..........  .T.......A   449
Targioni        ..........  ..........  ..........  ..........  .T.......A   449
Monoclea        ..........  ..........  ..........  ....n.....  .T........   450
Corsinia        ..........  ..........  ..........  ....C.....  ....CT....   447
Lunulari        ...C......  ..........  ..........  ...AN.C...  .........A   448
S-texan2        ..........  ..........  ..........  ..........  .....T...A   449
S-texan1        ..........  ......G...  ..........  ..........  .....T...A   450
  Riella        ..........  ..........  ..........  ......C...  ....C....A   450
  Blasia        ..T.......  ..........  ..........  .A........  .........A   450
Fossombr        ..T.......  .........T  .......N..  .A........  .........A   450
  Pellia        ..T.......  ......T...  ..........  .A........  .T.......A   450
Dendroal        ..T.....T.  ..........  ..........  .A........  .G.......A   450
Metaneck        ..T.....T.  ..........  ..........  .A........  .G.......A   450
Tetraphi        ..T.....T.  ..........  .......G.T  .A........  ......T..A   450
Sphagnum        ..T.......  .G....G.T.  .......-G.T  .A........  .G.-.T...A   448
A-punct2        ..TC......  ........T.  ..........  .A........  .T...T...A   447
A-punct1        ..TC...A..  ..........  .....T.A..  TA........  .........A   450
Coleocha        ..T.T...T.  .G...C....  C....TAA..  .A.....A..  .T...T...A   449
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            451         461         471         481         491         500
            |           |           |           |           |           |
R-soroca    GCGTGGGCTG GCGGGA-TAA AAG-TGGCCG GCCTTGCCGT CGGGGAGG-C  490
R-gouget    ....N..... .......... ......N... ...G.A.... .....G.N..  498
R-macroc    .......... .......... .......... ...G...... .....-A...  498
R-nigrel    .......... .......... .......... ...G...... .....-A...  498
R-atroma    .......... .......... .......... ...G.C...C .....G....  500
R-tricho    ....n..... .......... .......... .......... .....-....  498
R-beyric    ....T..... .......... .......... ...G...... ....-.A...  499
R-frosti    ....T..... .......... .......... A..G...... ..........  499
R-cavern    .......... .......... .......... ...G...... ..........  500
R-membra    .......... .......... .......... A..G...... ......A...  498
R-villos    ....T..... .......... ..A....... ...G....T. ..........  499
R-albida    .......... .......... .......... ...C...... ..........  500
R-lamell    .......... .......... .......... ...G...... .C....A...  499
R-papulo    .......... .......... .......... ...G...... ......A...  499
R-hueben    ....N..... .N........ .......N.. ...G...... .........G  500
R-toment    .......... .......... ..A....... ...G....T. ......A...  500
R-schelp    ..A....... .......... .......... ..GG...TC. ......-...  499
R-alboli    .......... .......... .......... ...G...... ......A...  500
R-natan1    ....T..... .......... ......A... A..G.A..T. .......T.T  500
R-natan2    ....T..... .......... .......... ...G...... ..........  500
O-incras    .......... .......... .......... ...C...... ........TA.  499
O-crista    .......... .......... .......... ...G...... ..........  500
Peltolep    .......... A......... .......... ...G...... .N........  499
Athalami    ....T..... .......... .-T......C ...G...GT. .C....A...  498
Cryptomi    .......... .......C.. ........A. ...G...... .TA.......  500
Plagioch    .......... .......C.. .......T.. C..G...... ..AA.G.T..  499
Reboulia    .......... .......C.. .......... ...G....N. .CAA..A...  497
A-gracil    A......... .......C.. .G.....T.. ..TG.C.... ..AA.G....  499
A-boland    .......... .......C.. .......... ...G...... ..A.......  497
A-califo    .......... .......C.. .......... ...G...... .C.AN.A...  499
Marchant    .......... .N.....G.. ......TN.. CG.G.AT.C. .TC.CN.C.N  499
Targioni    .......... .......... .C...C...A ...G...... ..........  499
Monoclea    .......... .......G.. .......... ...G...... ..A...A...  500
Corsinia    .......... ........G. .C.G...GGC .T.G.C.... ..A..G.C..  497
Lunulari    C.N.T..... .......C.. .C........ A....A.... .C...T.A..  498
S-texan2    .......... .......C.. .C......G. .n........ ....AT....  499
S-texan1    .......... .......C.. .C....CGG. .......... ....AT....  500
  Riella    T........- .......C.. .......... ..AG...... .....C....  499
  Blasia    .......c.. .......C.. G...C.C.T. -......... ..A..T....  499
Fossombr    .......... .......C.. G...CT..TT .......... T.A.AT....  500
  Pellia    ......T... .......CG. G...C..NTC .....C...N ....AT...N  500
Dendroal    ........C. C......C.. G...CTT..T .T.......G T.C..T....  500
Metaneck    ........C. C......C.. G...CTT..T T........G T.C..T....  500
Tetraphi    ........C. .......C.. G...CC..T. .......... T.T..T....  500
Sphagnum    .-......C. ......AC.. G...CT.... ...G...... T.T..C....  497
A-punct2    ........C. .......C.. G...CT.... .......... G.C.TT....  497
A-punct1    ........C. .......C.. C...CT...A .......... T.C.TT....  500
Coleocha    ....TT.G.. ......GA.. ....CTA..T ..GC.CGGC- -.C.TT.A..  497
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                501         511         521         531         541         550
                |           |           |           |           |           |
R-soroca        CGAGGAAGTA  AGCGCGCGCA  -CCGGCGCGC  TCGGGACGT-  CGGCGTAGTG  538
R-gouget        .......AG.  ..........  ...C......  ..........  ..........  548
R-macroc        .....T.A..  .......C..  ...A......  ..........  .....C....  548
R-nigrel        .......A..  ..........  ...C......  ..........  N....C....  548
R-atroma        ..........  ..........  ..........  ......T...  .....C....  550
R-tricho        ...A......  ..........  ...A......  ..........  ..........  548
R-beyric        ...A...AG.  ..........  ...C......  .......C.C  .....C....  549
R-frosti        ........G.  ...T......  ...C......  ..........  .....C....  549
R-cavern        ........G.  ..........  ...C......  ..........  .....C....  550
R-membra        ......TAG.  ..........  ...T......  .T....A...  T....C....  548
R-villos        ........G.  ..........  ...T......  ..........  .....C....  549
R-albida        G......CG.  ..........  ...T......  ..........  .....C....  550
R-lamell        ...A...CG.  ..........  ...T......  ..........  ..........  549
R-papulo        .......C..  .........G  ...A......  ..........  .....C....  549
R-hueben        .......A..  .....C.C..  ...A......  ..........  .....C....  550
R-toment        .......CG.  ........N.  ...C.G....  ..........  .....C....  550
R-schelp        .......CG.  ..........  ...C......  ..........  .....C....  549
R-alboli        ...A...CG.  ..........  ...C......  ..........  .....C....  550
R-natan1        .......CG.  ..TT......  ...T....A.  ........C.  ..........  550
R-natan2        .......CG.  ..T.......  ...T....A.  ........C.  ..........  550
O-incras        .......CG.  ..T..A....  ...T....A.  ........C.  ..........  549
O-crista        ...A...CG.  .-T.......  ...T....A.  ........C.  ..........  549
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Athalami        .C.A...T--  .ATT......  ...T....A.  ........N.  ..........  546
Cryptomi        .......AG.  ..T.......  ...T....A.  ...A....C.  ..........  550
Plagioch        .......A..  ..T.......  ...T....A.  ........C.  ..........  549
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A-boland        .......CG.  ..T.......  ...T....A.  ........C.  ..........  547
A-califo        .......CG.  .AT...T.TT  ...T...TA.  ........-C  .......T..  548
Marchant        ..N...NAGC  .NN.......  ...T......  ...A....C.  T.........  549
Targioni        T......TG.  ..T.......  ...T...TA.  ........C.  T.....T...  549
Monoclea        T.....TCC.  .TT.......  T..N...A..  ..........  ..........  550
Corsinia        ....nC.AG.  ..........  ...T......  ......T.C.  ....ACG.C.  547
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S-texan1        .......CG.  ..........  ...T...T..  ........C.  T.........  550
  Riella        .......CG.  ..........  ...T..A...  ........C.  T.....T...  549
  Blasia        .......CG.  ..........  ...T......  ........C.  T......C..  549
Fossombr        .......CG.  ........G.  ..NT......  ........C.  T......T..  550
  Pellia        .....T.CG.  ........G.  ...T.T....  ...A......  T.C..C.T..  550
Dendroal        .......CG.  ..TATA....  .A.T..AT..  ........CT  T......T..  550
Metaneck        ....A..CG.  ..........  .A.T......  ........C.  T......T..  550
Tetraphi        .......CG.  ....T.-...  ...T..A...  ...A....C.  T......T..  549
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            551         561         571         581         591         600
            |           |           |           |           |           |
R-soroca    GGCTTTCCAT CC-GACCCGT CTTGAAACAC GGACCAAGGA GTCTAACATG  587
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R-macroc    .......... .......... .......... .......... ..........  598
R-nigrel    .......... .......... .......... .......... ..........  598
R-atroma    .......... .......... .......... .......... ..........  600
R-tricho    ......T... .......... .......... .......... ..........  598
R-beyric    .A........ .......... .......... .......... ..........  599
R-frosti    ......T... .......... .......... .......... ..........  599
R-cavern    ......T... .......... .......... .......... ..........  600
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R-papulo    ......T... .......... .......... .......... ..........  599
R-hueben    A.....T... .......... ........G. .......... ..........  600
R-toment    ......T... .......T.. .......... .......... ..........  600
R-schelp    ......T... .......... .......... .......... ..........  599
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R-natan1    ......T... .......... .......... .......... ..........  600
R-natan2    ......T... .......... .......... .......... ..........  600
O-incras    .......... .......... .......... .......... ..........  599
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Corsinia    .C....T... .......... .......... .......... ..........  597
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S-texan1    ......T... .......... .......... .......... ..........  600
  Riella    ......T... .......... .......... .......... ..A.......  599
  Blasia    A......... .......... .......... .......... ..........  599
Fossombr    A......... .......... .......... .......... ..........  600
  Pellia    AT........ .......... .......... .......... ..........  600
Dendroal    A...C.T... ..C.....AC T........A .......... ..........  600
Metaneck    A...C.T... .....A..T. .......... .......... ..........  600
Tetraphi    A...C..... .......... .......... .......... ..........  599
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A-punct2    A...C..... .......... .......... .......... ..........  597
A-punct1    A...C..... .......... .......... .......... ..........  600
Coleocha    --.-C..... .......... .......... .......... ..........  592
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            601         611         621         631         641         650
            |           |           |           |           |           |
R-soroca    CA--TGCGA-  GCCGGTGGGC  GGCAAACCCA  GAGGCGCAAA  TAACTTGAGG  634
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R-macroc    ....C.....  ..........  ..........  ..........  ..........  648
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Cryptomi    ..........  ..........  ..........  ......A...  ..........  650
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A-boland    ..........  ..........  ..........  ..........  ......A...  647
A-califo    ......GA..  A.........  ..GC......  ..A...A...  ..........  648
Marchant    ..........  C.........  ..........  ..........  ......A...  649
Targioni    ..........  ..........  ..........  ..........  ..........  649
Monoclea    ....C.....  .T........  ..........  ......A...  ..........  650
Corsinia    ..........  ..........  .C........  ..........  ..........  647
Lunulari    ..........  C........T  ..........  ..........  ..........  648
S-texan2    ....C.....  ..........  ..........  ..........  G..A......  649
S-texan1    ....C.....  ..........  ..........  ..........  G..A......  650
  Riella    ..........  ..........  ..........  ..........  G..A......  649
  Blasia    T.........  .........T  ..........  .........G  ....C.....  649
Fossombr    T.........  .........T  ..........  .........G  ...AC.....  650
  Pellia    T.........  .........T  ..........  ......T.GG  ....CC....  650
Dendroal    T....C.T..  ........C.  .C........  .....A...G  ...AC.....  650
Metaneck    T.AT.CGA..  ........C.  .C........  .........G  ...AC.....  650
Tetraphi    T.........  ..........  ..........  .........G  G...C.....  649
Sphagnum    T.........  ..........  ..........  ......T..G  G...C.....  646
A-punct2    T.........  .........T  ..........  .........G  G..-C.....  646
A-punct1    T.........  A........T  .C........  .........G  G...C.....  650
Coleocha    T.........  .........T  ..A.......  .........G  ....C.....  642
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                651         661         671         681         691         700
                |           |           |           |           |           |
R-soroca        TGCGATGTG-  -CAGCATCGA  CCGACCATGA  TCTTCTGTGA  AAGGTTCGAG   682
R-gouget        ..........  ..........  ..........  ..........  ..........   698
R-macroc        ..........  ..........  ..........  ..........  ..........   698
R-nigrel        ..........  ..........  ..........  ..........  ..........   698
R-atroma        ..........  ..........  ..........  ..........  ..........   700
R-tricho        ..........  ..........  ..........  ..........  ..........   698
R-beyric        ..........  .........C  ..........  ..........  ..........   699
R-frosti        ..........  ..........  ..........  ..........  ..........   699
R-cavern        ..........  ..........  ..........  ..........  ..........   700
R-membra        ..........  ..........  ..........  ..........  ..........   698
R-villos        ..........  ..........  ..........  ..........  ..........   699
R-albida        ..T.......  ...A......  ..........  ....T.....  ..........   700
R-lamell        ..T.......  ...A......  ..........  ....T.....  ..........   698
R-papulo        ..T.......  ...A......  ..........  ..........  ..........   699
R-hueben        ..T.......  ...A......  ..........  ..........  ..........   700
R-toment        ..T.......  ...A......  ..........  ....T.....  ..........   700
R-schelp        ..T.......  ...A......  ..........  ....T.....  ..........   699
R-alboli        ..T.......  ...A......  ..........  ..........  ..........   700
R-natan1        ..T.......  ...A......  ..........  ..........  ..........   700
R-natan2        ..T.......  ...A......  ..........  ..........  ..........   700
O-incras        ..T.......  ...A......  ..........  ..........  ..........   699
O-crista        ..T.......  ...A......  ..........  ..........  ..........   699
Peltolep        ..T.......  ...A......  ....G.....  ..........  ..........   699
Athalami        ..T.......  ...A......  ..........  ..........  ..........   696
Cryptomi        ..T.......  ...A......  ..........  ..........  ..........   700
Plagioch        ..T.......  ...A......  ..........  ..........  ..........   699
Reboulia        ..T.......  ...A......  ..........  ....N.....  ..........   697
A-gracil        ..T.......  ...A......  ..........  ..........  ..........   699
A-boland        ..T.......  ...A......  ..........  ..........  ..........   697
A-califo        ..T.......  ...A......  ..........  ..........  ..........   698
Marchant        ..T.......  ...A......  ..........  G......C..  ..........   699
Targioni        ..T.......  ...A......  ..........  ..........  ..........   699
Monoclea        A.T.......  ...A......  ..........  ..........  ..........   700
Corsinia        ..T.......  ...A......  ......T...  ..........  ..........   697
Lunulari        ..T.......  ...A......  ..........  ..........  ..........   698
S-texan2        ..T.......  ...A......  ..........  ..........  ..........   699
S-texan1        ..T.......  ...A......  ..........  ..........  ..........   700
  Riella        ..T.......  ...A......  ..........  ..........  ..........   699
  Blasia        ..G....A..  ...C......  ..........  ..........  ..........   699
Fossombr        ..G....A..  ...C......  ..........  ..........  ......T...   700
  Pellia        ..G....A..  ...C......  ..........  ....T.....  ......T...   700
Dendroal        ..G..C.A..  ...C......  ...T..G...  ..G.......  ......T...   700
Metaneck        ..G..C.A..  ...C......  ......G...  ..........  ......T...   700
Tetraphi        ..G....A..  ...C......  ......G...  ..........  ......T...   699
Sphagnum        ..G....A..  ...C......  ......G...  ..........  ......T...   696
A-punct2        ..G....A..  ...C......  ..........  ..........  ......T...   696
A-punct1        ..G....A..  ...C......  ..........  ..........  ......T...   700
Coleocha        ..G....A.T  G..C......  ..........  ..........  ......T...   692
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                701         711         721         731         741         750
                |           |           |           |           |           |
R-soroca        TACGAGCATG CCTGTTGGGA CCCGAAAGAT GGTGAACTAT GCCTGAGCAG  732
R-gouget        .......... .......... .......... .......... ..........  748
R-macroc        ..A....G.. .......... .......... .......... ..........  748
R-nigrel        ..A....G.. .......... .......... .......... ......A...  748
R-atroma        ..A....... .......... .......... .......... ..........  750
R-tricho        ..A....... .......... .......... .......... ..........  748
R-beyric        .GA....... .......... .......... .......... ..........  749
R-frosti        .GA....... .......... N......... .......... ..........  749
R-cavern        .GA....... .......... .......... .......... ..........  750
R-membra        .GA....... .......... .......... .......... ..........  748
R-villos        ..A....... .......... .......... .......... ..........  749
R-albida        ..A....... .......... .......... .......... ..........  750
R-lamell        ..A....... .......... .......... .......... ..........  748
R-papulo        ..A....... .......... .......... .......... ..........  749
R-hueben        ..A..T.... .......... .....C.... .......... ..........  750
R-toment        ..A....... .......... .......... .......... ..........  750
R-schelp        ..A....... .......... .......... .......... ..........  749
R-alboli        ..G....... .......... .......... .......... ..........  750
R-natan1        ..G....... .......... .......... .......... ..........  750
R-natan2        ..G....... .......... .......... .......... ..........  750
O-incras        ..A....... .......... .......... .......... ..........  749
O-crista        ..A....... .......... .......... .......... ..........  749
Peltolep        ..A....... .......... .......... .......... ..........  749
Athalami        ..A......C .......... .......... .......... ......A...  746
Cryptomi        ..A....... .......... .......... .......... ..........  750
Plagioch        ..A....... .......... .......... .......... ..........  749
Reboulia        ..A....... .......... .......... .......... ..........  747
A-gracil        ..A....... .......... .......... .......... ..........  749
A-boland        ..A....... .......... .......... .......... ..........  747
A-califo        ..A....... .......... .......... .......... ..........  748
Marchant        ..A....... .......... .......... .......... ..........  749
Targioni        ..A....... .......... .......... .......... ..........  749
Monoclea        .GA....G.. .......... .......... .......... ..........  750
Corsinia        ..A....... .......... .......... .......... ..........  747
Lunulari        ..A....... .......... .......... .......... ..........  748
S-texan2        ..A....G.. ....A..... .......... .......... ..........  749
S-texan1        ..A....G.. ....A..... .......... .......... ..........  750
  Riella        .GA....... ....A..... .......... .......... ..........  749
  Blasia        .G.......A ....A..... .......... .......... ..........  749
Fossombr        .GT......A ....C..... .......... .......... ..........  750
  Pellia        .GT....... ....C..... .......... .......... ..........  750
Dendroal        .GT..C...A .......... .......... .....T.... ..........  750
Metaneck        .GT..C...A .......... .....T.... .......... ..........  750
Tetraphi        .GT......A ....C..... .......... .......... ..........  749
Sphagnum        .G........ ..C....... .......... .......... ..........  746
A-punct2        .GT......A ....C..... .......... .......... ..........  746
A-punct1        .G.......A ....C..... .......... .......... ..........  750
Coleocha        .G.......A .......... .......... .......... ..........  742
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                751         761         771         781         791         800
                |           |           |           |           |           |
R-soroca        GGCGAAGCCA  GAGGAAACTC  TGGTGGAGGC  TCGTAGCGAT  ACTGACGTGC  782
R-gouget        ..........  ..........  ..........  ..........  ..........  798
R-macroc        ..........  ..........  ..........  ..........  ..........  798
R-nigrel        ..........  ..........  ..........  ..........  ..........  798
R-atroma        ..........  ..........  ..........  ..........  ..........  800
R-tricho        ..........  ..........  ..........  ..........  ..........  798
R-beyric        ..........  ..........  ..........  ..........  ..........  799
R-frosti        .......T..  ..........  ..........  ..........  ..........  799
R-cavern        .......T..  ..........  ..........  ..........  ..........  800
R-membra        .......T..  ..........  ..........  ..........  ..........  798
R-villos        .......T..  ..........  ..........  ..........  ..........  799
R-albida        ..........  ..........  ..........  ..........  ..........  800
R-lamell        ..........  ..........  ..........  ..........  ..........  798
R-papulo        .......T..  ..........  ..........  ..........  ..........  799
R-hueben        .......T..  ..N.......  ..........  ..........  ..........  800
R-toment        .......T..  ..........  .........T  ..........  ..........  800
R-schelp        .......T..  .......T..  .........T  ..........  ..........  799
R-alboli        .......T..  ..........  ..........  ..........  ..........  800
R-natan1        .......T..  ..........  ..........  ..........  ..........  800
R-natan2        .......T..  ..........  ..........  ..........  ..........  800
O-incras        ..........  ..........  ..........  ..........  ..........  799
O-crista        .......T..  ..........  ..........  ..........  ..........  799
Peltolep        ..........  ..........  ..........  ..........  ..........  799
Athalami        ..........  ..........  ..........  ..........  ..........  796
Cryptomi        .......T..  ..........  ..........  ..........  ..........  800
Plagioch        .......T..  ..........  ..........  ..........  ..........  799
Reboulia        .......T..  ..........  ..........  ..........  ..........  797
A-gracil        .......T..  ..........  ..........  ..........  ..........  799
A-boland        .......T..  ..........  ..........  ..........  ..........  797
A-califo        .......T..  ..........  ..........  ..........  ..........  798
Marchant        .......T..  ..........  ..........  ..........  ..........  799
Targioni        ..........  ..........  ..........  ..........  ..........  799
Monoclea        ..........  ..........  ..........  ..........  ..........  800
Corsinia        ......N...  ..........  ..........  ..........  ..........  797
Lunulari        .......T..  ..........  ..........  ..........  ..........  798
S-texan2        .......T..  ..........  ..........  ..........  ..........  799
S-texan1        .......T..  ..........  ..........  ..........  ..........  800
  Riella        .......T..  ..........  ..........  ..........  ..........  799
  Blasia        ..........  ..........  ..........  ..........  ..........  799
Fossombr        ..........  ..........  ..........  ..........  ..........  800
  Pellia        ..........  ..........  ..........  ..........  ..........  800
Dendroal        ..........  ..........  ..........  ..........  ..........  800
Metaneck        ......T...  ..........  ..........  ..........  ..........  800
Tetraphi        ..........  ..........  ..........  ..........  ..........  799
Sphagnum        ..........  ..........  ..........  ..........  ..........  796
A-punct2        ..........  ..........  .-........  ..........  ..........  795
A-punct1        ..........  ..........  ..........  ..........  ..........  800
Coleocha        ..........  ..........  ..........  ..........  .........n.  792
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                801         811         821         831         841         850
                |           |           |           |           |           |
R-soroca        AAATCGTTCG  TCAGACTCGG  GTATAGGGGC  GAAAGACTAA  TCGAACCATC  832
R-gouget        ..........  ..........  ..........  ..........  ..........  848
R-macroc        ..........  ..........  ..........  ..........  ..........  848
R-nigrel        ..........  ..........  ..........  ..........  ..........  848
R-atroma        ..........  ..........  ..........  ..........  ..........  850
R-tricho        ..........  ..........  ..........  ..........  ..........  848
R-beyric        ..........  ..........  ..........  .....T....  ..........  849
R-frosti        ..........  .......T..  ..........  ..........  ..........  849
R-cavern        ..........  .......T..  ..........  ..........  ..........  850
R-membra        ..........  ..........  ..........  ..........  ..........  848
R-villos        ..........  ..........  ..........  ..........  ..........  849
R-albida        ..........  .......T..  ..........  ..........  ..........  850
R-lamell        ..........  .......T..  ..........  ..........  ..........  848
R-papulo        ..........  ..........  ..........  ..........  ..........  849
R-hueben        ..........  ..........  ..........  ..........  ..........  850
R-toment        ..........  ..........  ..........  ..........  ..........  850
R-schelp        ..........  ..........  ..........  ..........  ..........  849
R-alboli        ..........  ..........  ..........  ..........  ..........  850
R-natan1        ..........  ..........  ..........  ..........  ..........  850
R-natan2        ..........  ..........  ..........  ..........  ..........  850
O-incras        ..........  ..........  ..........  ..........  ..........  849
O-crista        ..........  .......T..  ..........  ..........  ..........  849
Peltolep        ..........  ..........  ..........  ..........  ..........  849
Athalami        ..........  ..........  ..........  .....T....  ..........  846
Cryptomi        ..........  ..........  ..........  ..........  ..........  850
Plagioch        ..........  ..........  ..........  ..........  ..........  849
Reboulia        ..........  ..........  ..........  ..........  ..........  847
A-gracil        ..........  ..........  ..........  ..........  ..........  849
A-boland        ..........  ..........  ..........  ..........  ..........  847
A-califo        ..........  ..........  ..........  ..........  ..........  848
Marchant        ..........  .......T..  ..........  ..........  ..........  849
Targioni        ..........  .......T..  ..........  ..........  ..........  849
Monoclea        ..........  ..........  ..........  ..........  ..........  850
Corsinia        ..........  ..........  ..........  ..........  ..........  847
Lunulari        ..........  .......T..  ..........  ..........  ..........  848
S-texan2        ..........  .......T..  ..........  ..........  ..........  849
S-texan1        ..........  .......T..  ..........  ..........  ..........  850
  Riella        ..........  .......T..  ..........  ..........  ..........  849
  Blasia        ..........  .......T..  ..........  ..........  ..........  849
Fossombr        ..........  .......T..  ..........  ..........  ..........  850
  Pellia        ..........  .......T..  ..........  ..........  ..........  850
Dendroal        ..........  .......T..  .........T.  ..........  ..........  850
Metaneck        ..........  .......T..  .........T.  ..........  ..........  850
Tetraphi        ..........  .......T..  ..........  ..........  ..........  849
Sphagnum        ..........  .......T..  ..........  ..........  ..........  846
A-punct2        ..........  .......T..  ..........  ..........  ..........  845
A-punct1        ..........  .......T..  ..........  ..........  ..........  850
Coleocha        ..........  .......T..  ..........  ..........  ..........  842
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                851         861         871         881         891         900
                |           |           |           |           |           |
R-soroca        TAGTAGCTGG  TTCCCTCCGA  AGTTTCCCTC  AGGATAGCCG  GAGCACAGTT  882
R-gouget        ..........  ..........  ..........  ..........  ..........  898
R-macroc        ..........  ..........  ..........  ..........  ..........  898
R-nigrel        ..........  ..........  ..........  ..........  ..........  898
R-atroma        ..........  ..........  ..........  ..........  ..........  900
R-tricho        ..........  ..........  ..........  ..........  ..........  898
R-beyric        ..........  ..........  ..........  ..........  ..........  899
R-frosti        ..........  ..........  ..........  ..........  ..........  899
R-cavern        ..........  ..........  ..........  ..........  ..........  900
R-membra        ..........  ..........  ..........  ..........  ..........  898
R-villos        ..........  ..........  ..........  ..........  ..........  899
R-albida        ..........  ..........  ..........  ..........  ..........  900
R-lamell        ..........  ..........  ..........  ..........  ..........  898
R-papulo        ..........  ..........  ..........  ..........  ..........  899
R-hueben        ..........  ..........  ..........  ..........  C...G.....  900
R-toment        ..........  ..........  ..........  ..........  ..........  900
R-schelp        ..........  ....T.....  ..........  ..........  ..........  899
R-alboli        ..........  ..........  ..........  ..........  ..........  900
R-natan1        ..........  ..........  ..........  ..........  ..........  900
R-natan2        ..........  ..........  ..........  ..........  ..........  900
O-incras        ..........  ..........  ..........  ..........  ..........  899
O-crista        ..........  ..........  ..........  ..........  ..........  899
Peltolep        ..........  ..........  ..........  .........T  ..........  899
Athalami        ..........  ..........  ..........  .........T  ..........  896
Cryptomi        ..........  ..........  ..........  .........T  ..........  900
Plagioch        ..........  ..........  ..........  .........T  ..........  899
Reboulia        ..........  ..........  ..........  .........T  ..........  897
A-gracil        ..........  ..........  ..........  .........T  ..........  899
A-boland        ..........  ..........  ..........  .........T  ..........  897
A-califo        ..........  ..........  ..........  .........T  ..........  898
Marchant        ..........  ..........  ..........  .........T  ..........  899
Targioni        ..........  ..........  ..........  ..........  .....-....  898
Monoclea        ..........  ..........  ..........  ..........  .....G....  900
Corsinia        ..........  ..........  ..........  ..........  ...G......  897
Lunulari        ..........  ..........  ..........  .........T  ..........  898
S-texan2        ..........  ..........  ..........  .........T  ..........  899
S-texan1        ..........  ..........  ..........  .........T  ..........  900
  Riella        ..........  ..........  ..........  .........T  ..........  899
  Blasia        ..........  ..........  ..........  .........T  ....T.....  899
Fossombr        ..........  ..........  ..........  .........T  ....T.....  900
  Pellia        ..........  ..........  ..........  .........T  ....T.....  900
Dendroal        ..........  ..........  ..........  .........T  ....T.....  900
Metaneck        ..........  ..........  ..........  .........T  A...T.....  900
Tetraphi        ..........  ..........  ..........  .........T  ....T.....  899
Sphagnum        ..........  ..........  ..........  .........T  ....T.....  896
A-punct2        ..........  ..........  ..........  .........T  ....T.....  895
A-punct1        ..........  ..........  ..........  .........T  ....T.....  900
Coleocha        ..........  ..........  ..........  .........T  ....T.....  892
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           901
           |
R-soroca   TCATC                                                887
R-gouget   .....                                                903
R-macroc   .....                                                903
R-nigrel   .....                                                903
R-atroma   .....                                                905
R-tricho   .....                                                903
R-beyric   .....                                                904
R-frosti   .....                                                904
R-cavern   .....                                                905
R-membra   .....                                                903
R-villos   .....                                                904
R-albida   .....                                                905
R-lamell   .....                                                903
R-papulo   .....                                                904
R-hueben   .....                                                905
R-toment   .....                                                905
R-schelp   .....                                                904
R-alboli   .....                                                905
R-natan1   .....                                                905
R-natan2   .....                                                905
O-incras   .....                                                904
O-crista   .....                                                904
Peltolep   .....                                                904
Athalami   .....                                                901
Cryptomi   .....                                                905
Plagioch   .....                                                904
Reboulia   .....                                                902
A-gracil   .....                                                904
A-boland   .....                                                902
A-califo   .....                                                903
Marchant   .....                                                904
Targioni   .....                                                903
Monoclea   ..G..                                                905
Corsinia   ..G.G                                                902
Lunulari   .....                                                903
S-texan2   .T...                                                904
S-texan1   .T...                                                905
  Riella   .T...                                                904
  Blasia   .T...                                                904
Fossombr   .T...                                                905
  Pellia   .T...                                                905
Dendroal   .T...                                                905
Metaneck   .T...                                                905
Tetraphi   .T...                                                904
Sphagnum   .T...                                                901
A-punct2   .T...                                                900
A-punct1   .T...                                                905
Coleocha   .T...                                                897
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Appendix 2. Sequence alignment (348 bp) for the marchantioid plastid data set.

```
           1          11         21         31         41         50
           |          |          |          |          |          |
R-soroca   GACTTAAATT AATTGAGCTT TTGTTGAGAA ATCAACTAAA TGATTGTTTT   50
R-gouget   .........A .......... .A........ .......... .........-   49
R-macroc   .......T.. .......... .......... .......... ..........   50
R-nigrel   .........G .......... .......... .......... .........-   49
R-atroma   .......... .......... .......... .......... ..........   50
R-tricho   .......... .......... .......... .......... ..........   50
R-beyric   .......... .......... .......... .......... .........-   49
R-frosti   .......T.. .......... .......... .........- .........-   48
R-cavern   .......T.. .......... .......... .........- .........-   48
R-membra   .......T.. .......... .C........ .........- .........-   48
R-villos   .......T.. .......... .......... .......... ..........   50
R-albida   .......-.. .......... .......... .......... ..T.......   49
R-lamell   .......... .......... .C........ .........C ..........   50
R-hueben   ......TT.. ..G....... .......... .......... ..........   50
R-toment   .....N.T.. .......... .C........ .......... .........-   49
R-shelpe   .......T.. .......... .C........ .......... .........-   49
R-alboli   .......T.. .......... .C........ .......... ..........   50
R-natans   .......T.. .......... .A........ ...G...... .........-   49
O-incras   .......T.- .......... .A........ .......... ..........   49
O-crista   .......T.. .......... .A........ ..A....... ..........   50
Peltolep   .......T.. .......... .A..C..... .......... ..........   50
Athalami   .......T.. .......... .A..C..... ...C...... ...C.....-   49
Cryptomi   .......T.. .......... .A........ ..TG...... .........-   49
Plagioch   .......T.. .......... .A..C..... ...C...... ..........   50
Reboulia   .......T.. .......... .A..C..... .......... .........-   49
A-gracil   .......T.. .......... .A........ .......... ..........   50
A-boland   .......T.. .......... .A..C..... .......... ..........   50
A-califo   .......T.. .......... .A..C..... .......... ..........   50
Marchant   .......T.. .......... .A........ ..TT...... ..........   50
Targioni   .......T.. .......... .A..C..... .......... ..........   50
Monoclea   .......T.. .......... .A........ ..T....... ..........   50
Corsinia   .......T.. .......... .A..A..... ...T.....- .........-   48
Lunulari   .......T.. .......... .A........ ...T...... .........-   49
Sphaeroc   .......... .-........ .A..A..... ..T......G .........-   48
  Riella   .......T.. .......... .A........ ...T...... .........-   49
  Blasia   ---------- -T....C.CA .A..AA.... -.A......G ..........   38
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                51          61          71          81          91          100
                |           |           |           |           |           |
R-soroca        CAAATTCAGG  GAAACTTAGG  ATGAAACAAA  AGAAAATTTA  GGCAATCCTG  100
R-gouget        ..........  .....C....  ..........  ..........  ..........   99
R-macroc        ..........  .....C....  ..........  ..........  ..........  100
R-nigrel        ..........  .....C....  ..........  ..........  ..........   99
R-atroma        ..........  .....C....  ..........  ..........  ..........  100
R-tricho        .........A  .....C....  ..........  ..........  ..........  100
R-beyric        ..........  .....C....  ..........  ..........  ..........   99
R-frosti        ....G.....  .....C....  G.........  ...C......  ..........   98
R-cavern        ....G.....  .....C....  G.........  ...C......  ..........   98
R-membra        ..........  ..........  G.....-...  .T.G......  ..........   97
R-villos        ..........  .....C....  G.........  .-........  ..........   99
R-albida        ..........  .....C.C..  G.........  ..........  ..T.......   99
R-lamell        ..........  .....C.C..  G.........  ..........  ..........  100
R-hueben        ..........  .....C....  G.........  ..........  ..........  100
R-toment        ..........  ...C.C....  G.........  ..........  ..........   99
R-shelpe        ..........  ...C.C....  G.........  ...T......  ..........   99
R-alboli        ..........  ...C.C....  G.........  ........A.  ..........  100
R-natans        ..........  ..........  G.....A...  .T...T....  ..T.......   99
O-incras        ..........  .G..TC....  ......A...  .....-....  ..T..C....   98
O-crista        ..........  ....TC....  G.....A...  .....-....  ..T.......   99
Peltolep        ..........  .....C....  ......A...  -T...GA...  ..T.......   99
Athalami        ..........  .....C....  T.....A...  .A...GA...  ..T.-.....   98
Cryptomi        ..........  .....C.T..  .....CA...  .------...  ..T.......   93
Plagioch        ..........  .....C.T..  ......A...  .T...GA...  ..T.......  100
Reboulia        ..........  .....C.TCT  ......A...  .T...GA...  ..T.-.....   98
A-gracil        ..........  .....C.T..  ......A...  -T...GA...  ..T.......   99
A-boland        ..........  .....C.T..  ..A...A...  .C...GA...  ..T.C.....  100
A-califo        ..........  .....C.T..  ......A...  .....GA...  ..T.......  100
Marchant        ..........  .....C....  T..C..A...  .T....A...  ..T.......  100
Targioni        ..........  .....C....  ......A...  -T...TA...  ..T...T...   99
Monoclea        ..........  .....C....  ......A...  .------...  ..T....T..   94
Corsinia        ..........  A....C....  C.....A...  TT...T....  ..T...T...   98
Lunulari        ..........  .....C....  ...C..A...  .------...  ..T.......   93
Sphaeroc        ..........  .....C....  T.....A.TC  T------...  ..T.......   92
  Riella        ..........  ....TC....  G...T.A...  T------.C.  ..T.......   93
  Blasia        ..T......A  .....C....  T.....A...  .-----A...  ..T.......   83
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            101         111         121         131         141        150
            |           |           |           |           |          |
R-soroca    AGCCAAATTT  TGTGTAAACA  AAATAGGTGC  AGAGACTCAA  AGAAAACTGT  150
R-gouget    ..........  ...A......  ..........  ..........  ..........  149
R-macroc    ..........  ..........  ..........  ..........  ..........  150
R-nigrel    ..........  ..........  ..........  ..........  ..........  149
R-atroma    ..........  ..........  ..........  ..........  ..........  150
R-tricho    ..........  ..........  ..........  ..........  ..........  150
R-beyric    ..........  ..........  ..........  ..........  .....G....  149
R-frosti    ..........  ..........  ..........  ..........  ..........  148
R-cavern    ..........  ..........  ..........  ..........  ..........  148
R-membra    ..........  ..........  ..........  ..........  ..........  147
R-villos    ..........  ...A......  ..........  ..........  ..........  149
R-albida    ..........  ..........  ..........  ..........  ..........  149
R-lamell    ..........  ..........  ..........  ..........  ..........  150
R-hueben    ..........  ..........  ..........  ..........  ........A.  150
R-toment    ..........  ..........  ...C......  ..........  ..........  149
R-shelpe    ..........  ..........  ...C......  ..........  ..........  149
R-alboli    ......T...  ..........  ...C......  ..........  ..........  150
R-natans    ..........  ..........  ..........  ..........  ........A.  149
O-incras    ..........  ..........  ..........  ...A......  ..........  148
O-crista    ..........  ...A......  ..........  ...A......  ..........  149
Peltolep    ..........  ...A..G...  ..........  ..........  ........A.  149
Athalami    ..........  ..........  ..........  ..........  ........A.  148
Cryptomi    ..........  ..........  ..........  ..........  ........A.  143
Plagioch    ..........  ..........  ..........  ..........  ........A.  150
Reboulia    ..........  ..........  ..........  ..........  ........A.  148
A-gracil    ..........  ..........  ..........  ..........  ........A.  149
A-boland    ..........  ...A......  ..........  ..........  ........A.  150
A-califo    ..........  ..........  ..........  ..........  ........A.  150
Marchant    ..........  ...T......  ...G......  ..........  ........A.  150
Targioni    ...-......  ...A..G...  ..........  ..........  ........A.  148
Monoclea    ..........  ...A......  ..........  ..........  ........A.  144
Corsinia    ..........  ...TA.....  ..........  ..........  ........A.  148
Lunulari    ..........  ...T......  ..........  ..........  ........A.  143
Sphaeroc    .........C  .A.T....T.  GC........  ..........  ........A.  142
  Riella    .........A  ...T......  ..........  ..........  ........A.  143
  Blasia    ..........  ...T....T.  ..........  ..........  ..G.....A.  133
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                151         161         171         181         191         200
                |           |           |           |           |           |
R-soroca        CCTAACGAAT  TTATTGTAGA  CGAGGATAAA  GATAGAGTCC  GTTTTTACAA  200
R-gouget        ..........  ..........  ..........  ..........  ..........  199
R-macroc        ..........  .......T..  ..........  ..........  A.........  200
R-nigrel        ..........  ..........  ..........  ..........  ..........  199
R-atroma        .........C  ..........  ..........  ..........  ..........  200
R-tricho        ..........  ..........  ..........  ..........  ..........  200
R-beyric        ..........  ..........  ..........  ..........  ..........  199
R-frosti        ..........  .......-..  ..........  ..........  A.........  197
R-cavern        ..........  ..........  ..........  ..........  A.........  198
R-membra        ..........  .......-..  ..........  ..........  A.........  196
R-villos        ..........  ..........  ..........  ..........  ..........  199
R-albida        ..........  ..........  ..........  ..........  ..........  199
R-lamell        ..........  ..........  ..........  ..........  A.........  200
R-hueben        ..........  .....A.N..  ..........  ..........  A.........  200
R-toment        ..........  ..........  ..........  ..........  A.........  199
R-shelpe        ..........  ..........  ..........  ..........  A.........  199
R-alboli        ..........  .......T..  ..........  ..........  A.........  200
R-natans        ..........  ....G.C...  ..........  ..........  ..........  199
O-incras        ..........  ..........  .A........  ..........  .........T  198
O-crista        ..........  ......C...  .A........  ..........  .........T  199
Peltolep        .........-  --...A....  ..........  ..........  ..........  196
Athalami        .........A  .....A....  ..........  ..........  ..........  198
Cryptomi        .......TTA  ..-..A....  ..........  ..........  A.........  192
Plagioch        .........A  .....A....  ..........  ..........  A.........  200
Reboulia        .........A  .....A....  ..........  ..........  A.........  198
A-gracil        .........A  ..C..A....  ..........  ..........  A.........  199
A-boland        .........A  ..C..N....  ..........  ..........  A.........  200
A-califo        .........A  ..C..A....  ..........  ..........  A....-....  199
Marchant        .........A  ..T..A....  ..........  ..........  ..........  200
Targioni        .........A  .....A....  ..........  ..........  ..........  198
Monoclea        ..........  ..T..AC...  ..........  ..........  C.........  194
Corsinia        .........A  .....AC...  ..........  ..........  AA........  198
Lunulari        .........A  ..T..A...G  ..........  ..........  ..........  193
Sphaeroc        T........A  ..T..A...G  ..........  ..........  ..........  192
  Riella        .........A  ..T..A....  -.........  ..........  ..........  192
  Blasia        .........A  ..T.AA.N..  .....G....  ..........  A.........C 183
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                201         211         221         231         241         250
                |           |           |           |           |           |
R-soroca        GTTAAAAATT  G-TAGTAAAA  TGAAAATCCG  TTGGCTTTAA  AAACCGTGAG  249
R-gouget        ..........  ..........  ..........  ..........  ..........  249
R-macroc        T.........  ..........  ..........  ..........  ..........  250
R-nigrel        ..........  ..........  ..........  ..........  ..........  249
R-atroma        ..........  ..........  ..........  ..........  ..........  250
R-tricho        ..........  ..........  ..........  ..........  ..........  250
R-beyric        ..........  ..........  ..........  ..........  ..........  249
R-frosti        ..........  ..........  ..........  ..........  .G........  247
R-cavern        ..........  ..........  ..........  ..........  .G........  248
R-membra        ..........  ..........  ..........  ..........  .G........  246
R-villos        ..........  ..........  ..........  ..........  ..........  249
R-albida        ..........  ..........  ..........  ..........  .G........  249
R-lamell        ..........  ..........  ..........  ..........  .G........  250
R-hueben        .......G..  ........G.  ..........  ..........  .G........  250
R-toment        ..G.......  ..........  ..........  ..........  .G........  249
R-shelpe        ..G.......  ..........  ..........  ..........  .G........  249
R-alboli        ..G.......  ..........  ..........  ..........  .G........  250
R-natans        ..........  .G........  ..........  ..........  .G........  249
O-incras        ..........  ...G......  ..........  ..........  .G........  248
O-crista        ...T......  ..........  ..........  ..........  .G........  249
Peltolep        ..........  ..........  ..........  ..........  .G........  246
Athalami        A.G.......  .GC.......  ..........  ..........  .G........  248
Cryptomi        ..........  ......C...  ..........  ..........  .G........  242
Plagioch        ..........  ..........  ..........  ..........  .G........  250
Reboulia        ..........  ..........  ..........  ..........  .G........  248
A-gracil        ..........  ..........  ..........  ..........  .G........  249
A-boland        ..........  ....A.....  ..........  ..........  .G........  250
A-califo        ..........  ..........  ..........  ..........  .G........  249
Marchant        ..........  ..........  ..........  ..........  .G........  250
Targioni        ..........  ..........  ..........  ..........  .G........  248
Monoclea        ..........  ..C.......  ..........  ..........  .G........  244
Corsinia        ..........  ..........  ..........  ........C.  .G........  248
Lunulari        ..........  ..A.......  C.........  ..........  .G........  243
Sphaeroc        ..........  ......G...  C.........  ..........  .G........  242
  Riella        ..........  ..........  C.........  ........T.  .G........  242
  Blasia        ..........  ..........  ..........  .........T  .G........  233
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            251         261         271         281         291         300
            |           |           |           |           |           |
R-soroca    GGTTCAAGTC  CCTCTACCCC  CAATTTTTTC  TTTTTATGTT  TCGCCGGGAT  299
R-gouget    .......... .......... ..G......A .......... .......... 299
R-macroc    .......... .......... .......... .......... .......... 300
R-nigrel    .......... .......... .......... .......... .......... 299
R-atroma    .......... .......... .......... .......... .......... 300
R-tricho    .......... .......... .......... .......... .......... 300
R-beyric    .......... .......... -......... .......... .......... 298
R-frosti    .......... .......... .......... .......... .......... 297
R-cavern    .......... .......... .......... .......... .......... 298
R-membra    .......... .......... .........T .......... .......... 296
R-villos    .......... .......... .......... .......... .T........ 299
R-albida    .......... .......... .........A AA........ .T........ 299
R-lamell    .......... .......... .......... .......... .T........ 300
R-hueben    .......... .......... .......... .......... .T........ 300
R-toment    .......... .......... .......... .......... .......... 299
R-shelpe    .......... .......... .......... .......... .......... 299
R-alboli    .......... .......... ..C....... .......... .......... 300
R-natans    .......... .......... .........- -......... .T........ 297
O-incras    .......... .......... .........T A......... .T.....A.. 298
O-crista    .......... .......... ........C. C......... .T.....A.. 299
Peltolep    .......... .......... .......... .........C. .T........ 296
Athalami    .......... .......... .......... .......... .T........ 298
Cryptomi    .......... .......... ...C.....T ..A....... -T.....A.. 291
Plagioch    .......... .......... .........A C......... -T........ 299
Reboulia    .......... .......... .........A .......... -T........ 297
A-gracil    .......... .......... ..T......A .......... -T........ 298
A-boland    .......... .......... .........A .......... -T........ 299
A-califo    .......... .......... .........A .......... -T........ 298
Marchant    .......... .......... ........G. .A........ .T........ 300
Targioni    .......... .......... .......... .......... .T........ 298
Monoclea    .......... .......... .......... .......---- A-........ 289
Corsinia    .......... .......... ..-.A....- -.......-.. .T........ 294
Lunulari    .......... .......... ..G......A .A......C. .T.....A.. 293
Sphaeroc    .......... .......... ......G.GA .......... .T........ 292
  Riella    .......... .......... ........GA ........C. .T........ 292
  Blasia    .......... .......... ...AA....A A......... .T......-. 282
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            301         311         321         331         341         350
            |           |           |           |           |           |
R-soroca    AGCTCAGTTG GTAGAGCAGA AGACTGAAAA TCCTCGTGTC ACCAGTTCAA  349
R-gouget    .........G. .......... .......... .......... ..........  349
R-macroc    .......... .....A.... .......... .......... ..........  350
R-nigrel    .......... .......... G......... .......... ..........  349
R-atroma    .......... .......... G......... .......... ..........  350
R-tricho    .......... .......... .......... .......... ..........  350
R-beyric    .......... .......... .......... .......... ..........  348
R-frosti    .......... .......... .......... .......... ..........  347
R-cavern    .......... .......... G......... .......... ..........  348
R-membra    .A....T... .....A.... G......... .......... ..........  346
R-villos    .......... .......... G......... .......... ..........  349
R-albida    .......... .......... G......... .......... ..........  349
R-lamell    .......... .......... .......... .......... ..........  350
R-hueben    .......... .......... .......... .......... ..........  350
R-toment    .......... .......... .......... .......... ..........  349
R-shelpe    .......... .......... .......... .......... ..........  349
R-alboli    .......... .......... .......... .......... ..........  350
R-natans    .A....T... .......... G......... .......... ..........  347
O-incras    .......... .......... G......... .......... ..........  348
O-crista    .......... .......... G......... .......... ..........  349
Peltolep    .......... .......... .......... .......... ..........  346
Athalami    .......... .......... .......... .......... ..........  348
Cryptomi    .......... .......... .......... .......... ..........  341
Plagioch    .......... .......... G......... .......... ..........  349
Reboulia    .......... .......... .......... .......... ..........  347
A-gracil    .......... .......... G......... .......... ..........  348
A-boland    .......... .......... G......... .......... ..........  349
A-califo    .......... .......... .......... .......... ..........  348
Marchant    .......... .......... G......... .......... ..........  350
Targioni    .......... .......... .......... .......... ..........  348
Monoclea    .......... .......... .......... .......... ..........  339
Corsinia    .......... .......... .......... .......... ..........  344
Lunulari    .......... .......... .......... .......... ..........  343
Sphaeroc    .......... .......... G......... .......... ..........  342
  Riella    .......... .......... .......... .......... ..........  342
  Blasia    .......... .CG...---- ---------- ---------- ----------  298
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Appendix 3. Sequence alignment (949 bp) for the riccioid (21 taxa) nuclear data set.

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                1           11          21          31          41          50
                |           |           |           |           |           |
R-soroca        TAAGCGGAGG AAAAGAA-CT AACAAGGATT CCCTTAGTAG CGGCGAGCGA    49
R-gouget        .......... .......... .......... .......... ..........    50
R-macroc        .........A ..........  .......... ...C...... ..........    50
R-nigrel        .........A ..........  .......... .......... ..........    50
R-atroma        .......... .......... .......... ...C...... ..........    50
R-tricho        .......... .......... .......... .......... ..........    50
R-beyric        .......... .......... .......... ...C...... ..........    50
R-frosti        .........- .......... .......... .......... ..........    49
R-cavern        .......... .......... .......... ...C...... ..........    50
R-membra        .........- ..........- ...G...... .......... ..........    48
R-villos        .......... .......... .......... .......... ..........    50
R-albida        .......... .......... .......... .......... ..........    50
R-lamell        ...-...... .......... .......... .......... ..........    49
R-papulo        ...-...... .......... .......... .......... ..........    49
R-hueben        .......G.. .......A.. .......... .......... ..........    50
R-toment        .......... .......... .......... .......... ..........    50
R-schelp        .......... .......... .......... ...C...... ..........    50
R-alboli        .......... .......... .......... .......... ..........    50
R-natans        .......... .......... .......... ...C...... ..........    50
O-incras        .........A ..........  .......... ...C...... ..........    50
O-crista        .......... .......... .......... .......... ..........    50

                51          61          71          81          91          100
                |           |           |           |           |           |
R-soroca        ACCGGGAAGA GCCCAGCTTG AAAATCGCGC CG--CGCGGC GCGAGTTGTA    97
R-gouget        .......... .......... .......... .......... ..........   100
R-macroc        .......... .......... .......... .......... ..........   100
R-nigrel        .......... .......... .G........ .......... ..........   100
R-atroma        .......... .......... .......... ....T..... ..........   100
R-tricho        .......... .......... .......... .......... ..........   100
R-beyric        .......... .......... .......... .......... ..........   100
R-frosti        .......... .......... .......... ...CG..... ..........    99
R-cavern        .......... .......... .......... ...TG..... ..........   100
R-membra        .......T.. .......... .......... .......... ..........    98
R-villos        .......... .......... .......... .......... ..........   100
R-albida        .........A ..........  .......... .A....T... ..........   100
R-lamell        ..T....... .......... .......... .......... ..........    99
R-papulo        .......... .......... .......... ...CG..... ..........    99
R-hueben        .......... .......... .......... ...TG..... ..........   100
R-toment        .......... .......... .......... ...TG..... ..........   100
R-schelp        .......... .......... .......... ...TG..... ..........   100
R-alboli        .......... .......... .......... ...TG..... ..........   100
R-natans        .........A ..........  .......T.. ..TT...... ..........   100
O-incras        .......GA. .......... .......... .......... ..........   100
O-crista        .......GA. .......... .........G ...TG...A. ..........   100
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            101         111         121         131         141         150
            |           |           |           |           |           |
R-soroca    GTCTGGAGAA  GTGTCCTCTG  CAGCGGACCC  GGCCCAAGTC  C-CCTGGAAA  146
R-gouget    N.........  ..........  ..........  ..........  ..........  150
R-macroc    ..........  ..........  ..........  ..........  ..........  150
R-nigrel    ..........  ..........  ..........  ..........  ..........  150
R-atroma    ..........  ..........  ..........  ..........  ..........  150
R-tricho    ..........  ..........  ..........  ..........  ..........  150
R-beyric    A.........  ..........  ..........  ..........  ..........  150
R-frosti    ..........  ..........  ..........  ..........  ..........  149
R-cavern    ..........  ..........  ..........  ....T.....  ..........  150
R-membra    ..........  .C........  ..........  ....T.....  ..........  148
R-villos    ..........  ..........  ..........  ....T.....  ..........  150
R-albida    ..........  ..........  ..........  ....T.....  ..........  150
R-lamell    .......n..  ..........  ..........  ..........  ..........  149
R-papulo    n.........  .C........  ..........  ....T..n..  ..........  149
R-hueben    ..........  .C........  ..........  ....T.....  ..........  150
R-toment    .......A..  ..........  ..........  ....T.....  ..........  150
R-schelp    .......A..  n.........  ..........  ....T.....  ..........  150
R-alboli    ..........  ..........  ..........  .T..T.....  ..........  150
R-natans    ..........  .C........  ..........  .T..T.....  ..........  150
O-incras    n.........  ..........  ..........  ....T.....  ..........  150
O-crista    ..........  ..........  ..........  ....T.....  ..........  150

            151         161         171         181         191         200
            |           |           |           |           |           |
R-soroca    GGGGCGTCGG  A-GAGGGTGA  GAACCCCGTC  GGGCCGGGAC  CCTGCTGCTC  195
R-gouget    ..........  ..........  ..........  ..........  ..........  200
R-macroc    ..........  ..........  ..........  ..........  ..........  200
R-nigrel    ..........  ..........  ..........  ..........  ..........  200
R-atroma    ..........  ..........  ..........  ..........  ..........  200
R-tricho    ..........  ..........  ..........  ..........  ..........  200
R-beyric    ..........  ..........  ..........  ..........  ..........  200
R-frosti    ..........  ..........  ..........  ..........  ..........  199
R-cavern    ..........  ..........  ..........  ..........  ..........  200
R-membra    ..........  ..........  ..........  ..........  ..........  198
R-villos    ..........  ..........  ..........  ..........  ..........  200
R-albida    ..........  ..........  ..........  ..........  ..........  200
R-lamell    ..........  ..........  ..........  ..........  ..........  199
R-papulo    ..........  ..........  ..........  ..........  ..........  199
R-hueben    ..........  .G........  ..........  ..........  ..........  200
R-toment    ..........  ..........  ..........  ..........  ..........  200
R-schelp    ..........  ....A.....  ..........  ..........  ..........  200
R-alboli    ..........  ..........  ..........  ..........  ..........  200
R-natans    ........A.  ..........  ..........  ..........  ..........  200
O-incras    ........A.  ..........  ..........  ..........  ..........  200
O-crista    ........A.  ..........  ..........  ..........  ..........  200
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            201         211         221         231         241         250
            |           |           |           |           |           |
R-soroca    CACGAGGCGC TGTCGACGAG TCGGGCTGTT TGGGAATGCA GCCCTAAGTG  245
R-gouget    .......... .......... .......... .......... ........C.  250
R-macroc    .......... .......... .......... .......... ........C.  250
R-nigrel    .......... .......... .......... .......... ........C.  250
R-atroma    .......... .......... .......... .......... ..........  250
R-tricho    .......... .......... .......... .......... ........C.  250
R-beyric    .......... .......... .......... .......... ........C.  250
R-frosti    .......... .......... .......... .......... .......AC.  249
R-cavern    .......... .......... .......... .......... ..........  250
R-membra    .......... .T........ .......... .......... ........C.  248
R-villos    .......... .......... .......... .......... ........C.  250
R-albida    .......... .......... .......... .......... ........A.  250
R-lamell    .......... .......... .......... .......... ..........  249
R-papulo    .......... .......... .......... .......... ..........  249
R-hueben    .......... .......... .......... .......... ..........  250
R-toment    .......... .......... .......... .......... ..........  250
R-schelp    .......... .......... .......... .......... ..........  250
R-alboli    .......... .......... .......... .......... ..........  250
R-natans    .......... ....T..... .......... .......... ........C.  250
O-incras    .......... .......... .......... .......... ........C.  250
O-crista    .......... .......... .......... .......... ........C.  250

            251         261         271         281         291         300
            |           |           |           |           |           |
R-soroca    GGAGGTAAAT TCCTTCCAAG GCTAAATATC GGCGGGAGAC CGATAGCGAA  295
R-gouget    .......... .......... ........C. .......... ..........  300
R-macroc    ...-...... .......... .......... .......... ..........  299
R-nigrel    .......... -......... .......... .......... ..........  299
R-atroma    .......... .......... .......... .......... ..........  300
R-tricho    .......... .......... .......... .......... ..........  300
R-beyric    .......... .......... ........C. .......... ..........  300
R-frosti    .......... .......... .......... .......... ..........  299
R-cavern    .......... .......... .......... .......... ..........  300
R-membra    .......... .......... ........CG .......... ..........  298
R-villos    .......... .......... .......... .......... ..........  300
R-albida    .......... .......... ........C. .......... ..........  300
R-lamell    .......... .......... ........C. .......... ..........  299
R-papulo    .......... .......... ........CG .......... ..........  299
R-hueben    .......... .......... ........CG .......... ..........  300
R-toment    .......... .......... .......... .......... ..........  300
R-schelp    .......... .......... .......... .......... ..........  300
R-alboli    .......... .......... .......... .......... ..........  300
R-natans    .......... .......... ........CT .......... ..........  300
O-incras    ...-...... .......... ........CG C......... ..........  299
O-crista    .......... .......... ........CG .......... ..........  300
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            301         311         321         331         341         350
            |           |           |           |           |           |
R-soroca    CAAGTACCGC GAGGGAAAGA TGAAAAGGAC TTTGAAAAGA GAGTTAAAAA  345
R-gouget    .......... .......... .......... .......... ..........  350
R-macroc    .......... .......... .......... .......... ..........  349
R-nigrel    .......... .......... .......... .......... ..........  349
R-atroma    .......... .......... .......... .......... ..........  350
R-tricho    .......... .......... .......... .......... ..........  350
R-beyric    .......... .......... .......... .......... ..........  350
R-frosti    .......... .......... .......... .......... ..........  349
R-cavern    .......... .......... .......... .......... ..........  350
R-membra    .......... .......... .......... .......... ........G.  348
R-villos    .......... .......... .......... .......... ..........  350
R-albida    .......... .......... .......... .......... ..........  350
R-lamell    .......... .......... .......... .......... ..........  349
R-papulo    .......... .......... .......... .......... ..........  349
R-hueben    .......... .......... .......... .......... ..........  350
R-toment    .......... .......... .......... .......... .....T....  350
R-schelp    .....N.... .......... .......... .......... ........T..  350
R-alboli    .......... .......... .......... .......... ..........  350
R-natans    .......... .......... .......... .......... ........G.  350
O-incras    .......... .......... .......... .......... ..........  349
O-crista    .......... .......... .......... .......... ..........  350

            351         361         371         381         391         400
            |           |           |           |           |           |
R-soroca    GTGCTTGAAA TTGCTGGGAA GGAAGCGAAT GGAAGCCTCG TGTGCGCCCC  395
R-gouget    .......... .........G .......... ...-...... ..........  399
R-macroc    .......... .........G .......... .......... ..........  399
R-nigrel    .......... .........G .......... .......... G.........  399
R-atroma    .......... .........G .......... .......... ..........  400
R-tricho    .......... .........G .......... .......... ..........  400
R-beyric    .......... .........G .......... .......... G.........  400
R-frosti    .......... .........G .......... ...G...... ..........  399
R-cavern    .......... .........G .......... ...G...... ..........  400
R-membra    .......... .........G .......... ...G...... .T........  398
R-villos    .......... .........G .......... ...G...... ..........  400
R-albida    .......... .........G .......... ...G...... ..........  400
R-lamell    .......... .........G .......... ...G...... ..........  399
R-papulo    .......... .........G .......... ...G...... ..........  399
R-hueben    .......... .........G .......... ...G...... ..........  400
R-toment    .......... .........G .......... ...G...... ..........  400
R-schelp    .......... .......... ......C... .....G.C.C .T.TT.....  400
R-alboli    .......... .........G .......... ...G...... ..........  400
R-natans    .......... .........G .......... ...G...... .....A....  400
O-incras    .......... .........G .......... ...G...... ..........  399
O-crista    .......... .........G .......... ...G...... ..........  400
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            401         411         421         431         441         450
            |           |           |           |           |           |
R-soroca    GGTCGGATGC  GGAACGGCTG  -C-GAAGCTG  GTCCGCCGCT  CGACGCGGGG   443
R-gouget    ..........  .........-  T..-......  ..........  ....N.....   447
R-macroc    ..........  ..........  T.AA......  ..........  ..........   449
R-nigrel    ..........  .........-  T..A......  ..........  ..........   448
R-atroma    ..........  .........-  T..A......  ..........  ..........   449
R-tricho    ..........  .........-  T..A......  ..........  ....-.....   448
R-beyric    ..........  ..........  CG.-......  ..........  ..........   449
R-frosti    ..........  .........-  TG.A......  ..........  ..........   448
R-cavern    ..........  .........-  T..A......  ..........  ..........   449
R-membra    ..........  .........-  T..AG.....  ..........  ..........   447
R-villos    ..........  .........-  T..-..-...  ..........  ....n.C...   447
R-albida    ..........  .........-  C..-......  ..........  ..........   448
R-lamell    ..........  .........-  TT.-TT....  ..........  ..........   447
R-papulo    ..........  .........-  T..-......  ..........  ..........   447
R-hueben    ..........  .........-  T..-......  ..........  ..........   448
R-toment    ..........  .........-  T..A......  ..........  ..........   449
R-schelp    .....N..C.  .........T  T..A..C...  ..........  .A..C.....   449
R-alboli    ..........  .........-  T..A......  ..........  ..........   449
R-natans    ..........  .........-  CG.--.....  ..........  ..........   447
O-incras    ..........  .........-  TG.-.T....  ..........  ....C.....   447
O-crista    ..........  .........-  TG.-......  ..........  ..........   448

            451         461         471         481         491         500
            |           |           |           |           |           |
R-soroca    -CGCTGGTCG  GCGTGGGCTT  CCCCGGCGGG  ATAAAAGTCG  GCCTT-GGCC   491
R-gouget    ..N......-  ....N.....  ..........  ..........  .....C.N..   496
R-macroc    ..........  ..........  ..........  ..........  .....T....   499
R-nigrel    ..........  ..........  ..........  ..........  .....T....   498
R-atroma    ..........  ..........  ..........  ..........  C....T....   499
R-tricho    ..........  ....n....C  ..T.......  ..........  .....C....   498
R-beyric    .........C  ....T.....  ..........  ..........  -....T....   498
R-frosti    .........C  ....T.....  ..........  ..........  .....C....   498
R-cavern    ..........  ..........  ..........  ..........  .....T....   499
R-membra    ..........  ..........  ..........  ..........  -....C....   496
R-villos    ..........-  ....T.....  ..........  ......A...  ..-..C....   495
R-albida    ..........  ..........  ..........  ..........  .n...T....   498
R-lamell    ..........  ..........  ..........  ..........  .....T....   497
R-papulo    ..........  ..........  G.........  ..........  .....T....   497
R-hueben    ..........  ....N.....  ......N...  ..........  .N...T..N.   498
R-toment    ..........  ..........  ..........  ......A..C  ...A.T....   499
R-schelp    ..........  ..A.......  ...-......  ..........  ...A.C....   498
R-alboli    ..........  ..........  ..........  ..........  ...A.C....   499
R-natans    TT........  ....T.....  ..........  ..........  C....G....   497
O-incras    ..........  ..........  .T........  .........A  A....T....   497
O-crista    ..........  ..........  ..........  ..........  .....T....   498
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            501         511         521         531         541         550
            |           |           |           |           |           |
R-soroca    GGCCTATGCC  GTCGGGGAGG  -CCGAGGAAT  AAGCGCGCGC  CCGGGGCA-C  539
R-gouget    ....G..A..  .......G.N  .........G  ..........  ..........  546
R-macroc    ....G.....  .........-.  .......T..  ........C.  ..........  548
R-nigrel    ....G.....  .........-.  ..........  ..........  .T........  547
R-atroma    ....G..C..  .C.....G..  ..........  ..........  ..........  549
R-tricho    ..........  .......-..  .....A....  ..........  ..C.......  547
R-beyric    ....G.....  ......-.A.  .....A...G  ..........  ..........  547
R-frosti    .A..G.....  ..........  .........G  ....T.....  ....AA....  548
R-cavern    ....G.....  ..........  .........G  .........T  T.........  549
R-membra    .A..G.....  ........A.  .........TG  .........T  ....T.....  546
R-villos    ....G.....  T.........  .........G  ..........  ..........  545
R-albida    ....C.....  ..........  ..G......G  ..........  ..........  548
R-lamell    ....G.....  ...C....A.  .....A...G  ..........  ..........  547
R-papulo    ....G.....  ........A.  ..........  ..........  .......G..  547
R-hueben    ....G.....  ..........  .G........  ......C.C.  ..........  548
R-toment    ....G.....  T.......A.  .........G  ..........  ..C...N...  549
R-schelp    ...GG....T  C.......-.  .........G  ..........  ..........  547
R-alboli    ....G.....  ........A.  .....A...G  ..........  ..........  549
R-natans    ....G.....  ..........  .........G  ...T......  ..........  547
O-incras    ....C.....  .........T  A........G  ...T..A...  ..........  547
O-crista    ....G.....  ..........  .....A...G  ..-T......  ..........  547

            551         561         571         581         591         600
            |           |           |           |           |           |
R-soroca    CGGCGCGCTC  GGGACGT-CG  GCGTAGTGGG  CTTTCCATCC  GACCCGTCTT  588
R-gouget    .C........  ..........  ..........  ....T.....  ..........  596
R-macroc    .A........  ..........  ...C......  ..........  ..........  598
R-nigrel    .C........  ........N.  ...C......  ..........  ..........  597
R-atroma    ..........  ....T.....  ...C......  ..........  ..........  599
R-tricho    .A........  ..........  ..........  ....T.....  ..........  597
R-beyric    .C........  .....C.C..  ...C.....A  ..........  ..........  597
R-frosti    .C........  ..........  ...C......  ....T.....  ..........  598
R-cavern    .C........  ..........  ...C......  ....T.....  ..........  599
R-membra    .T.......T  ....A...T.  ...C......  ....TT....  ..........  596
R-villos    .T........  ..........  ...C......  ....T.....  .G........  595
R-albida    .T........  ..........  ...C......  ....T.....  ..........  598
R-lamell    .T........  ..........  ..........  ....T.....  ..........  597
R-papulo    .A........  ..........  ...C......  ....T.....  ..........  597
R-hueben    .A........  ..........  ...C....A.  ....T.....  ..........  598
R-toment    .C.G......  ..........  ...C......  ....T.....  ....T.....  599
R-schelp    .C........  ..........  ...C......  ....T.....  ..........  597
R-alboli    .C........  ..........  ...C......  ....T.....  ..........  599
R-natans    .T....A...  ......C...  ..........  ....T.....  ..........  597
O-incras    .T....A...  ......C...  ..........  ..........  ..........  597
O-crista    .T....A...  ......C...  ..........  ....T.....  ..........  597
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                601         611         621         631         641         650
                |           |           |           |           |           |
R-soroca        GAAACACGGA CCAAGGAGTC TAACATGCAT GCGA-GCCGG TGGGCGGCAA  637
R-gouget        .......... .......... .......... .......... ..........  646
R-macroc        .......... .......... .........C .......... ..........  648
R-nigrel        .......... .......... .........C C......... ......C...  647
R-atroma        .......... .......... .......... .......T.. ..........  649
R-tricho        .......... .......... .......... .......... ..........  647
R-beyric        .......... .......... .......... .......... ..........  647
R-frosti        .......... .......... .......... .......... ......C...  648
R-cavern        .......... .......... .......... .......... ..........  649
R-membra        .......... .........T .......... .......... ..........  646
R-villos        .......... .......... .......... .......... ..........  645
R-albida        .......... .......... .......... .......... ..........  648
R-lamell        .......... ......-... .......... .......... ..........  646
R-papulo        .......... .......... .......... .......... ..........  647
R-hueben        .....G.... .......... .......... ....TT.... ...C......  648
R-toment        .......... .......... .......... .......... ..........  649
R-schelp        .......... .......... .......... .......... ..........  647
R-alboli        .......... .......... .......... .......... ..........  649
R-natans        .......... .......... .......... .......... ..........  647
O-incras        .......... .......... .......... .......... ......C...  647
O-crista        .......... .......... .......... .......... ..........  647

                651         661         671         681         691         700
                |           |           |           |           |           |
R-soroca        ACCCACGCGA GGCGCAAATA ACTTGAGGGT GCGATCCTCC TTCGAGGGGT  687
R-gouget        .......... .......... ......A... .......... .C..C.....  696
R-macroc        .......... .......... .......... .......... .C........  698
R-nigrel        ......T... .......... .......... .......... .C........  697
R-atroma        .......... .......... ......-... .......... .C........  698
R-tricho        .......... .......... .......... .......... .C........  697
R-beyric        .......... .......... .......... .......... .C........  697
R-frosti        .......... .......... ......-... .......... .C........  697
R-cavern        ......AT.. .......... ......A... .......... .C........  699
R-membra        .....A.... .......... .A........ .......... .C........  696
R-villos        .......... .......... .......... .......... .C........  695
R-albida        .......A.. .......... .......A... .T........ .C........  698
R-lamell        .......... .......... .......A... .T........ .C........  696
R-papulo        .......... .......... .......A... .T........ .C........  697
R-hueben        .......... .......... .......A... .T........ CC........  698
R-toment        .......G.. .......... .......... .T........ CC........  699
R-schelp        .......... .......... .......... .T........ CC........  697
R-alboli        .......... .......... .......... .T........ CC........  699
R-natans        .......A.. ....A..... ......-... .T........ .C..C.....  696
O-incras        ......TG.. ....A..... ......-... .T........ .C..C.....  696
O-crista        ......TG.. .......... .......... .T........ .C..C.....  697
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                701         711         721         731         741         750
                |           |           |           |           |           |
R-soroca        GCAGCATCGA CCGACCATGA TCTTCTGTGA AAGGTTCGAG TACGAGCATG  737
R-gouget        .......... .......... .......... .......... ..........  746
R-macroc        .......... .......... .......... .......... ..A....G..  748
R-nigrel        .......... .......... .......... .......... ..A....G..  747
R-atroma        .......... .......... .......... .......... ..A.......  748
R-tricho        .......... .......... .......... .......... ..A.......  747
R-beyric        .........C .......... .......... .......... .GA.......  747
R-frosti        .......... .......... .......... .......... .GA.......  747
R-cavern        .......... .......... .......... .......... .GA.......  749
R-membra        .......... .......... .......... .......... .GA.......  746
R-villos        .......... .......... .......... .......... ..A.......  745
R-albida        ...A...... .......... ....T..... .......... ..A.......  748
R-lamell        ...A...... .......... ....T..... .......... ..A.......  746
R-papulo        ...A...... .......... .......... .......... ..A.......  747
R-hueben        ...A...... .......... .......... .......... ..A..T....  748
R-toment        ...A...... .......... ....T..... .......... ..A.......  749
R-schelp        ...A...... .......... ....T..... .......... ..A.......  747
R-alboli        ...A...... .......... .......... .......... ..G.......  749
R-natans        ...A...... .......... .......... .......... ..G.......  746
O-incras        ...A...... .......... .......... .......... ..A.......  746
O-crista        ...A...... .......... .......... .......... ..A.......  747

                751         761         771         781         791         800
                |           |           |           |           |           |
R-soroca        CCTGTTGGGA CCCGAAAGAT GGTGAACTAT GCCTGAGCAG GGCGAAGCCA  787
R-gouget        .......... .......... .......... .......... ..........  796
R-macroc        .......... .......... .......... .......... ..........  798
R-nigrel        .......... .......... .......... ......A... ..........  797
R-atroma        .......... .......... .......... .......... ..........  798
R-tricho        .......... .......... .......... .......... ..........  797
R-beyric        .......... .......... .......... .......... ..........  797
R-frosti        .......... N......... .......... .......... .......T..  797
R-cavern        .......... .......... .......... .......... .......T..  799
R-membra        .......... .......... .......... .......... .......T..  796
R-villos        .......... .......... .......... .......... .......T..  795
R-albida        .......... .......... .......... .......... ..........  798
R-lamell        .......... .......... .......... .......... ..........  796
R-papulo        .......... .......... .......... .......... .......T..  797
R-hueben        .......... .....C.... .......... .......... .......T..  798
R-toment        .......... .......... .......... .......... .......T..  799
R-schelp        .......... .......... .......... .......... .......T..  797
R-alboli        .......... .......... .......... .......... .......T..  799
R-natans        .......... .......... .......... .......... .......T..  796
O-incras        .......... .......... .......... .......... ..........  796
O-crista        .......... .......... .......... .......... .......T..  797
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            801         811         821         831         841         850
            |           |           |           |           |           |
R-soroca    GAGGAAACTC  TGGTGGAGGC  TCGTAGCGAT  ACTGACGTGC  AAATCGTTCG   837
R-gouget    ..........  ..........  ..........  ..........  ..........   846
R-macroc    ..........  ..........  ..........  ..........  ..........   848
R-nigrel    ..........  ..........  ..........  ..........  ..........   847
R-atroma    ..........  ..........  ..........  ..........  ..........   848
R-tricho    ..........  ..........  ..........  ..........  ..........   847
R-beyric    ..........  ..........  ..........  ..........  ..........   847
R-frosti    ..........  ..........  ..........  ..........  ..........   847
R-cavern    ..........  ..........  ..........  ..........  ..........   849
R-membra    ..........  ..........  ..........  ..........  ..........   846
R-villos    ..........  ..........  ..........  ..........  ..........   845
R-albida    ..........  ..........  ..........  ..........  ..........   848
R-lamell    ..........  ..........  ..........  ..........  ..........   846
R-papulo    ..........  ..........  ..........  ..........  ..........   847
R-hueben    ..N.......  ..........  ..........  ..........  ..........   848
R-toment    ..........  .........T  ..........  ..........  ..........   849
R-schelp    .......T..  .........T  ..........  ..........  ..........   847
R-alboli    ..........  ..........  ..........  ..........  ..........   849
R-natans    ..........  ..........  ..........  ..........  ..........   846
O-incras    ..........  ..........  ..........  ..........  ..........   846
O-crista    ..........  ..........  ..........  ..........  ..........   847

            851         861         871         881         891         900
            |           |           |           |           |           |
R-soroca    TCAGACTCGG  GTATAGGGGC  GAAAGACTAA  TCGAACCATC  TAGTAGCTGG   887
R-gouget    ..........  ..........  ..........  ..........  ..........   896
R-macroc    ..........  ..........  ..........  ..........  ..........   898
R-nigrel    ..........  ..........  ..........  ..........  ..........   897
R-atroma    ..........  ..........  ..........  ..........  ..........   898
R-tricho    ..........  ..........  ..........  ..........  ..........   897
R-beyric    ..........  ..........  .....T....  ..........  ..........   897
R-frosti    .......T..  ..........  ..........  ..........  ..........   897
R-cavern    .......T..  ..........  ..........  ..........  ..........   899
R-membra    ..........  ..........  ..........  ..........  ..........   896
R-villos    ..........  ..........  ..........  ..........  ..........   895
R-albida    .......T..  ..........  ..........  ..........  ..........   898
R-lamell    .......T..  ..........  ..........  ..........  ..........   896
R-papulo    ..........  ..........  ..........  ..........  ..........   897
R-hueben    ..........  ..........  ..........  ..........  ..........   898
R-toment    ..........  ..........  ..........  ..........  ..........   899
R-schelp    ..........  ..........  ..........  ..........  ..........   897
R-alboli    ..........  ..........  ..........  ..........  ..........   899
R-natans    ..........  ..........  ..........  ..........  ..........   896
O-incras    ..........  ..........  ..........  ..........  ..........   896
O-crista    .......T..  ..........  ..........  ..........  ..........   897
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           901         911         921         931         941
           |           |           |           |           |
R-soroca   TTCCCTCCGA AGTTTCCCTC AGGATAGCCG GAGCACGGGG AGTTTCATC    936
R-gouget   .......... .......... .......... .......A.. .........   945
R-macroc   .......... .......... .......... .......... .........   947
R-nigrel   .......... .......... .......... .......... .........   946
R-atroma   .......... .......... .......... .......... .........   947
R-tricho   .......... .......... .......... .......... .........   946
R-beyric   .......... .......... .......... .......... .........   946
R-frosti   .......... .......... .......... .......A.. .........   946
R-cavern   .......... .......... .......... .......A.. .........   948
R-membra   .......... .......... .......... .......A.. .........   945
R-villos   .......... .......... .......... .......A.. .........   944
R-albida   .......... .......... .......... .......A.. .........   947
R-lamell   .......... .......... .......... .......A.. .........   945
R-papulo   .......... .......... .......... .......... .........   946
R-hueben   .......... .......... .......... C...G..A.. .........   947
R-toment   .......... .......... .......... .......A.. .........   948
R-schelp   ....T..... .......... .......... .......A.. .........   946
R-alboli   .......... .......... .......... .......A.. .........   948
R-natans   .......... .......... .......... .......A.. .........   945
O-incras   .......... .......... .......... .......A.. .........   945
O-crista   .......... .......... .......... .......A.. .........   946
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Appendix 4. Sequence alignment (479 bp) for the riccioid (20 taxa) plastid data set (*Riccia papulosa* missing).

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                1          11         21         31         41         50
                |          |          |          |          |          |
R-soroca        GACTTAAATT AATTGAGCTT TTGTTGAGAA ATCAACTAAA TGATTGTTTT   50
R-gouget        .........A .......... .A........ .......... ..........-  49
R-macroc        .......T.. .......... .......... .......... ..........   50
R-nigrel        .........G .......... .......... .......... ..........-  49
R-atroma        .......... .......... .......... .......... ..........   50
R-tricho        .......... .......... .......... .......... ..........   50
R-beyric        .......... .......... .......... .......... ..........-  49
R-frosti        .......T.. .......... .......... .........- ..........-  48
R-cavern        .......T.. .......... .......... .........- ..........-  48
R-membra        .......T.. .......... .C........ .........- ..........-  48
R-villos        .......T.. .......... .......... .......... ..........   50
R-albida        .......-.. .......... .......... .......... ..T.......   49
R-lamell        .......... .......... .C........ .........C ..........   50
R-hueben        ......TT.. ..G....... .......... .......... ..........   50
R-toment        .....N.T.. .......... .C........ .......... ..........-  49
R-schelp        .......T.. .......... .C........ .......... ..........-  49
R-alboli        .......T.. .......... .C........ .......... ..........   50
Riccioca        .......T.. .......... .A........ ...G...... ..........-  49
O-incras        .......T.- .......... .A........ .......... ..........   49
O-crista        .......T.. .......... .A........ ..A....... ..........   50

                51         61         71         81         91         100
                |          |          |          |          |          |
R-soroca        CAAATTCAGG GAAACTTAGG ATGAAACAAA GA-AAATTTA GGCAATCCTG   99
R-gouget        .......... .....C.... .......... .......... ..........   99
R-macroc        .......... .....C.... .......... .......... ..........  100
R-nigrel        .......... .....C.... .......... .......... ..........   99
R-atroma        .......... .....C.... .......... .......... ..........  100
R-tricho        .........A .....C.... .......... .......... ..........  100
R-beyric        .......... .....C.... .......... .......... ..........   99
R-frosti        ....G..... .....C.... G......... ...C...... ..........   98
R-cavern        ....G..... .....C.... G......... ...C...... ..........   98
R-membra        .......... .......... G.....-... T..G...... ..........   97
R-villos        .......... .....C.... G......... -......... ..........   99
R-albida        .......... .....C.C.. G......... .......... ..T.......   99
R-lamell        .......... .....C.C.. G......... .......... ..........  100
R-hueben        .......... .....C.... G......... .......... ..........  100
R-toment        .......... ...C.C.... G......... .......... ..........   99
R-schelp        .......... ...C.C.... G......... ...T...... ..........   99
R-alboli        .......... ...C.C.... G......... .........A. ..........  100
Riccioca        .......... .......... G.....A... T....T.... ..T.......   99
O-incras        .......... .G..TC.... ......A... ..G....... ..T..C....   99
O-crista        .......... ....TC.... G.....A... ..G....... ..T.......  100
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            101         111         121         131         141        150
            |           |           |           |           |          |
R-soroca    AGCCAAATTT  TGTGTACTAA  AACAAAATAG  GTGCAGAGAC  TCAAAGAAAA  149
R-gouget    ..........  ...A......  ..........  ..........  ..........  149
R-macroc    ..........  .....G....  ..........  ..........  ..........  150
R-nigrel    ..........  .....G....  ..........  ..........  ..........  149
R-atroma    ..........  ..........  ..........  ..........  ..........  150
R-tricho    ..........  ..........  ..........  ..........  ..........  150
R-beyric    ..........  ..........  ..........  ..........  .........G  149
R-frosti    ..........  ..........  ..........  ..........  ..........  148
R-cavern    ..........  ..........  ..........  ..........  ..........  148
R-membra    ..........  ..........  ..........  ..........  ..........  147
R-villos    ..........  ...A......  ..........  ..........  ..........  149
R-albida    ..........  ..........  ..........  ..........  ..........  149
R-lamell    ..........  ..........  ..........  ..........  ..........  150
R-hueben    ..........  ..........  ..........  ..........  ..........  150
R-toment    ..........  ......T...  ........C..  ..........  ..........  149
R-schelp    ..........  ......T...  ........C..  ..........  ..........  149
R-alboli    ......T...  ..........  ........C..  ..........  ..........  150
Riccioca    ..........  .....G....  ..........  ..........  ..........  149
O-incras    ..........  .....G....  ..........  ........A..  ..........  149
O-crista    ..........  ...A.GT...  ..........  ........A..  ..........  150

            151         161         171         181         191        200
            |           |           |           |           |          |
R-soroca    CTGTCCTAAC  GAATTTATTA  TCTAAAAAAG  ATAAAAAATT  GCACTAA-TA  198
R-gouget    ..........  ..........  ..........  ..........  .......-...  198
R-macroc    ..........  ..........  ---.......  ..........  T......A..  197
R-nigrel    ..........  ..........  ..........  ..........  ........A..  199
R-atroma    ..........  ...C......  ..........  ..........  .........G.  200
R-tricho    ..........  ..........  .......G.A  ..........  ...A..-...  199
R-beyric    ..........  ..........  ..........  ..........  ..........  199
R-frosti    ..........  ..........  ......T.G.  .........G  ...T...AG.  198
R-cavern    ..........  ..........  ......T.G.  .........G  ...T...AG.  198
R-membra    ..........  ..........  ..........  ..........  A......A..  197
R-villos    ..........  ..........  ......-...  ..........  ....G..A.-  197
R-albida    ..........  ..........  ......T.G.  ..........  ...G...A..  199
R-lamell    ..........  ..........  ......T.G.  .G........  ........A..  200
R-hueben    ..A.......  ..........  ......-...  .........A  A......A..  199
R-toment    ..........  ..........  ......T...  ..........  ........A..  199
R-schelp    ..........  ..........  ......T...  ..........  ........A..  199
R-alboli    ..........  ..........  ......T...  ..........  ........A..  200
Riccioca    ..A.......  ........G.  ..A...T---  --........  ........A..  194
O-incras    ..........  ..........  .TA...TG..  ..........  ...T...A..  199
O-crista    ..........  ..........  ..A...T...  ..........  ........A..  200
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            201         211         221         231         241         250
            |           |           |           |           |           |
R-soroca    GTAAGAAAAT CTTTTAAAAG TTTTTCAATT ATTATGACGA GGATAAAGAT  248
R-gouget    .......... .C.......A ........C. .......... ..........  248
R-macroc    ....T...C. .C.......T ........C. .......... ..........  247
R-nigrel    .......... .C.......C ........C. .......... ..........  249
R-atroma    .......... A.......... ........C. .......... ..........  250
R-tricho    ....T..... TA...C.... ........C. .......... ..........  249
R-beyric    .......... .C........ ........C. .......... ..........  249
R-frosti    ....T..... TA....TTCA .....T..C. .......... ..........  248
R-cavern    ....T..... TA....TT.A .....T..C. .......... ..........  248
R-membra    ....T..... TA..G....A ..A.....C. .......... ..........  247
R-villos    ....T..... .A...TC..A ........C. .......... ..........  247
R-albida    ....T..... TA..G....A A.......C. .......... ..........  249
R-lamell    ....T..... TA..C....T .C......C. ...T...... ..........  250
R-hueben    ....T..... .AG..C...A .....T-.C. .GA.C..... ..........  248
R-toment    ....T..... TA.......A .....T..C. .......... ..........  249
R-schelp    -...T..... TA.......A .....G..C. .......... ..........  248
R-alboli    ....T..... TA..G....A ........C. .......... ..........  250
Riccioca    ....C..... TA........ C.C..A..C. .......... ..........  244
O-incras    ....T..... AA.......A C.C..A..C. ...G....A. ..........  249
O-crista    ....T..... .A.......A C.C..A..C. ...G....A. ..........  250

            251         261         271         281         291         300
            |           |           |           |           |           |
R-soroca    AGAGTCCGTT TTTACAAGTT AATTTTAAAA ACAATGCAAA TTGTAGTAAA  298
R-gouget    .......... .......... ........C. .......... ..........  298
R-macroc    .......A.. .......T.. ........C. .......... ..........  297
R-nigrel    .......... .......... ........C. .......... ..........  299
R-atroma    .......... .......... ........C. .......... ..........  300
R-tricho    .......... .......... ........C. .......... ..........  299
R-beyric    .......... .......... ........C. .......... ..........  299
R-frosti    .......A.. .......... ........CT ......T... ..........  298
R-cavern    .......A.. .......... ........CT ......T... ..........  298
R-membra    .......A.. .......... ........C. .......... ..........  297
R-villos    .......... .......... .....A..T. .......... ..........  297
R-albida    .......... .......... ........C. .......... ..........  299
R-lamell    .......A.. .......... ........C. ....G..... ..........  300
R-hueben    .......A.. .......... ........C. .........G ........G  298
R-toment    .......A.. .........G ........C. .......... ..........  299
R-schelp    .......A.. .........G ........C. .......... ..........  298
R-alboli    .......A.. .........G ........C. .......... ..........  300
Riccioca    .......... .......... ........C. .......... ..........  294
O-incras    .......... ......T... ........C. .......... ....G.....  299
O-crista    .......... ......T... T.......C. ...G...... ..........  300
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            301         311         321         331         341         350
            |           |           |           |           |           |
R-soroca    ATGAAAATCC  GTTGGCTTTA  AAAACCGTGA  GGGTTCAAGT  CCCTCTACCC  348
R-gouget    ..........  ..........  ..........  ..........  ..........  348
R-macroc    ..........  ..........  ..........  ..........  ..........  347
R-nigrel    ..........  ..........  ..........  ..........  ..........  349
R-atroma    ..........  ..........  ..........  ..........  ..........  350
R-tricho    ..........  ..........  ..........  ..........  ..........  349
R-beyric    ..........  ..........  ..........  ..........  ..........  349
R-frosti    ..........  ..........  ..G.......  ..........  ..........  348
R-cavern    ..........  ..........  ..G.......  ..........  ..........  348
R-membra    ..........  ..........  ..G.......  ..........  ..........  347
R-villos    ..........  ..........  ..........  ..........  ..........  347
R-albida    ..........  ..........  ..G.......  ..........  ..........  349
R-lamell    ..........  ..........  ..G.......  ..........  ..........  350
R-hueben    ..........  ..........  ..G.......  ..........  ..........  348
R-toment    ..........  ..........  ..G.......  ..........  ..........  349
R-schelp    ..........  ..........  ..G.......  ..........  ..........  348
R-alboli    ..........  ..........  ..G.......  ..........  ..........  350
Riccioca    ..........  ..........  ..G.......  ..........  ..........  344
O-incras    ..........  ..........  ..G.......  ..........  ..........  349
O-crista    ..........  ..........  ..G.......  ..........  ..........  350

            351         361         371         381         391         400
            |           |           |           |           |           |
R-soroca    CCATTTTTAG  AAAATTTGAA  TAAAAAGTTG  ACACATTTTT  TTTTTATGTT  398
R-gouget    .........C  ..........  ..........  ....G.....  ..........  398
R-macroc    .........C  ..........  G.........  ..........  ..........  397
R-nigrel    .........C  ..........  G.........  ..........  ..........  399
R-atroma    .........C  ..........  G.........  ..........  ..........  400
R-tricho    .........C  ...G......  G.........  ..........  ..........  399
R-beyric    .-.......T  ..........  A.........  ..........  ..........  398
R-frosti    .........C  .........G  ..........  ..........  ..........  398
R-cavern    .........C  .........G  ..........  ..........  ..........  398
R-membra    .........C  ..........  .........T  ..........  ..........  397
R-villos    .......G.T  ..........  ..........  ..........  ..........  397
R-albida    .........C  ..........  ..........  ..........  .A........  399
R-lamell    ......GG.C  ..........  ..........  ..........  ..........  400
R-hueben    .........A  .....G.T..  ..........  ...T......  ..........  398
R-toment    .........C  ..........  ..........  ..........  ..........  399
R-schelp    .......G.C  ..........  ..........  ..........  ..........  398
R-alboli    .........T  ..........  ..........  ....C.....  ..........  400
Riccioca    ........GA  ..........  ..........  ..........  ..........  394
O-incras    .........T  ....A.....  ..........  ..........  ..........  399
O-crista    .......G.T  ..........  ..........  ..........  C.........  400
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            401         411         421         431         441         450
            |           |           |           |           |           |
R-soroca    AAAATGACAA  AAAATAAAAT  CGCCGGGATA  GCTCAGTTGG  TAGAGCAGAA  448
R-gouget    .......T..  ..........  ..........  .......G..  ..........  448
R-macroc    ..........  ..........  ..........  ..........  ....A.....  447
R-nigrel    ..........  ..........  ..........  ..........  .........G  449
R-atroma    ..........  ..........  ..........  ..........  .........G  450
R-tricho    ..........  ......C...  ..........  ..........  ..........  449
R-beyric    ..........  ..........  ..........  ..........  ..........  448
R-frosti    ........G.  ....G.....  ..........  ..........  ..........  448
R-cavern    ........G.  ....G.....  ..........  ..........  .........G  448
R-membra    .....C...G  ....G.....  ..........  A....T....  ....A....G  447
R-villos    ..........  ....G.....  T.........  ..........  .........G  447
R-albida    .......G..  ...-G.....  T.........  ..........  .........G  448
R-lamell    .....C.G..  ....G.....  T.........  ..........  ..........  450
R-hueben    ..........  ......T...  T.........  ..........  ..........  448
R-toment    ..........  ...TG.G...  ..........  ..........  ..........  449
R-schelp    ..........  ...TG.G...  ..........  ..........  ..........  448
R-alboli    .....T....  ....G.....  ..........  ..........  ..........  450
Riccioca    .....A.T..  ....G.....  T.........  A....T....  .........G  444
O-incras    ..T..A.T..  ....-.....  T.....A...  ..........  .........G  448
O-crista    T....A.T..  ....-.....  T.....A...  ..........  .........G  449

            451         461         471
            |           |           |
R-soroca    GACTGAAAAT  CCTCGTGTCA  CCAGTTCAA                            477
R-gouget    ..........  ..........  .........                            477
R-macroc    ..........  ..........  .........                            476
R-nigrel    ..........  ..........  .........                            478
R-atroma    ..........  ..........  .........                            479
R-tricho    ..........  ..........  .........                            478
R-beyric    ..........  ..........  .........                            477
R-frosti    ..........  ..........  .........                            477
R-cavern    ..........  ..........  .........                            477
R-membra    ..........  ..........  .........                            476
R-villos    ..........  ..........  .........                            476
R-albida    ..........  ..........  .........                            477
R-lamell    ..........  ..........  .........                            479
R-hueben    ..........  ..........  .........                            477
R-toment    ..........  ..........  .........                            478
R-schelp    ..........  ..........  .........                            477
R-alboli    ..........  ..........  .........                            479
Riccioca    ..........  ..........  .........                            473
O-incras    ..........  ..........  .........                            477
O-crista    ..........  ..........  .........                            478
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