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Phylogeny and classification of the Grimmiaceae/Ptychomitriaceae complex (Bryophyta) inferred from cpDNA

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Abstract

Phylogenetic relationships within the Grimmiaceae/Ptychomitriaceae were studied using a plastid tRNA cluster, including four tRNAs (*trnS*, *trnT*, *trnL*, *trnF*), a fast evolving gene (*rps4*), four spacers separating the coding regions, as well as one group I intron. Secondary structure analyses of the spacers as well as the *trnL* intron P8 domain identified several homoplastic inversions. Tracing the structural evolution of P8 we were able to identify lineage specific modifications that are mainly explained by inversions often in combination with large indel events. Phylogenetic analyses using maximum parsimony, maximum likelihood, and Bayesian methods indicate that *Jaffueliobryum* and *Indusiella* are closely related to *Ptychomitrium* and form the Ptychomitriaceae s. str. As *Campylostelium* is neither resolved within Ptychomitriaceae s. str. nor Grimmiaceae s. str., we prefer to treat it in its own family, Campylosteliaceae De Not. The systematic position of *Glyphomitrium*, as also found by other authors, should be considered in a broader analysis of haplolepidous mosses as our analyses indicate that it is not part of Campylosteliaceae, Grimmiaceae, or Ptychomitriaceae. Within Grimmiaceae s. str., *Racomitrium* is recognized as a monophyletic group sister to a clade including *Dryptodon*, *Grimmia*, and *Schistidium*. *Coscinodon* species appear disperse in *Grimmia* s. str. next to species sharing the same gametophyte morphology, and thus the genus is synonymized with *Grimmia*. Finally, *Schistidium* is resolved monophyletic with high statistical support, and seems to represent a rapidly evolving group of species. Our results are not fully congruent with recently published treatments splitting Grimmiaceae in a fairly high number of genera, neither with a comprehensive *Grimmia* including *Dryptodon* and *Grimmia* s. str.

Keywords: Grimmiaceae; Ptychomitriaceae; Campylosteliaceae; *Schistidium*; *Racomitrium*; *Grimmia*; *Dryptodon*; *trnL*; Inversions; Group I intron; Secondary structure; Microstructural changes

1. Introduction

Among arthrodontous mosses the haplolepideous mosses have shown to represent a monophyletic lineage (e.g., La Farge et al., 2000; Beckert et al., 2001; Magombo, 2003; Werner et al., 2004) that traditionally has been recognized as the subclass Dicranidae (e.g., Vitt et al., 1998; Buck and Goffinet, 2000). In haplolepideous mosses the peristome consists only of an endostome that comprises a single row of teeth with externally undivided sides while the internal one is split in two asymmetric columns. One of the most speciose groups in the Dicranidae includes the families Grimmiaceae and Ptychomitriaceae, which form the core of the order Grimmiales. This order has been differently treated in the past, either with the Drummondiaceae and Scouleriaceae (Buck and Goffinet, 2000) included or without both, but Seligeriaceae included (Ochyra et al., 2003; Tsubota et al., 2003; Goffinet and Buck, 2004). Whatever the familial composition of the Grimmiales turns out to be in the near future (Hernández-Maqueda, in preparation), the latter families are usually

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considered more distantly related (Goffinet and Buck, 2004), and thus not a source of much dispute. But the generic composition of the Grimmiaceae and Ptychomitriaceae as well as the relationship between both families have been discussed controversially in the past and are still unresolved (Hernández-Maqueda et al., 2007). Whereas, some authors have lumped both families into a single one (Brotherus, 1901–1909; Dixon and Jameson, 1924; Jones, 1933; Lawton, 1971; Deguchi, 1978; Churchill, 1981; Deguchi, 1987; Noguchi, 1988; Gradstein et al., 2001; Allen, 2002; Tsubota et al., 2003; Allen, 2005), others treat them as independent families, either closely related (Nyholm, 1956, 1960; Scott et al., 1976; Ignatov and Afonina, 1992; Sharp et al., 1994; Buck and Goffinet, 2000; Li and Crosby, 2001; Gao and Crosby, 2003; Ochyra et al., 2003; Hedderson et al., 2004; Smith, 2004), or rather distant (Brotherus, 1924, 1925; Nyholm, 1979; Crum and Anderson, 1981).

The genera included in each family have varied considerably among authors. The most drastic change, with respect to the traditional view, was published by Churchill (1981) grouping *Racomitrium* within the subfam. Ptychomitrioideae solely based on peristome similarities. Table 1 summarizes the treatment of the Grimmiaceae/Ptychomitriaceae complex in several classification systems, however for a more detailed summary of Grimmiales systematics we refer to Tsubota et al. (2003).

In recent years, several studies at ordinal level or above using cpDNA sequences have helped to delimit the circumscription of Grimmiaceae and Ptychomitriaceae when combined with morphological traits, which alone failed to provide incontrovertible data at such scale (e.g., the inclusion of *Racomitrium* in subfam. Ptychomitrioideae based on peristome traits, cf. Churchill, 1981). Studies using the *rps4* gene (Goffinet et al., 2001; Hedderson et al., 2004), *rbcL* (Tsubota et al., 2003), or both combined with *trnL-F* (La Farge et al., 2000), rendered basically the same results, which can be summarized as: (1) Grimmiaceae and Ptychomitriaceae are sister groups; (2) closely related to Seligeriaceae; (3) *Glyphomitrium* does not pertain in Grimmiaceae or Ptychomitriaceae, a result also reached by Estébanez et al. (2002) using histochemical data, and according to Tsubota et al. (2003), this genus should be included in the Dicranaceae or Rhabdoweisiaceae; (4) the systematic position of *Campylostelium* is controversial, as revealed by Tsubota et al. (2003) and corroborated by Hernández-Maqueda et al. (2007); (5) neither *Scouleria* nor *Drummondia* pertain in the Grimmiales, being in fact basal to the core of the Dicranidae (further confirmed by Cox et al., 2000); (6) finally, the genus *Grimmia* is polyphyletic, and *Dryptodon* should be recognized as an independent genus to render the former monophyletic.

Although, in a recent phylogenetic study, we were able to confidently resolve the phylogenetic position of the former *Grimmia pitardii* using *rps4* and *trnL-F* (Hernández-Maqueda et al., 2007), the obtained trees showed that the phylogenetic relationships on generic level could not be confidently resolved using these markers only. Therefore, we explored more variable regions, namely the spacers between *rps4*, *trnT*, and *trnL* as additional phylogenetic markers. Whereas, *rps4* and *trnL-F* have been widely used in phylogenetic reconstructions at all classification levels, both spacers mentioned above have never been used to resolve phylogenies within bryophytes (Quandt and Stech, 2004; Stech, 2004). However, recently the molecular evolution of *trnT-L* spacer as well as the adjacent *trnL-F* region has been addressed by Quandt and Stech (2004), suggesting its suitability for this purpose.

As already stated, the aims of the previous molecular phylogenetic studies were to resolve the systematic relationships at ordinal classification level and above, and therefore they do not present extensive discussion on generic relationships within the families. The objective of the present study is thus to elucidate the phylogenetic relationships within the Grimmiaceae and Ptychomitriaceae, as well as between these two families. More specifically, we try to answer: (1) Do the non-coding parts of the plastid *trnS-F* region represent a useful marker at this classification level? (2) Which of the previously proposed familial schemes is supported by the DNA sequence data, if any? (3) Are the genera accepted for each family in such divergent treatments as Buck and Goffinet (2000) or Ochyra et al. (2003)—followed by Goffinet and Buck (2004)—monophyletic?

2. Materials and methods

2.1. Plant material

Plant vouchers are deposited in BCB, MA, MO, MUB, and S. GenBank accession numbers, herbarium number of the vouchers, as well as the geographical origin of the specimens are listed in Table 2.

2.2. DNA isolation amplifications and sequencing

Total DNA of gametophore tips from dried herbarium specimens or recent collections was isolated using the NaOH method following the protocol described by Werner et al. (2002), recommended for isolation of small quantities of dry material. PCRs of the total region were generally performed in three sets: (a) the *rps4* gene, including the *trnS–rps4* spacer, (b) the *rps4–trnL* region, and (c) the *trnL-F* region using the primers as indicated in Fig. 1. In some cases nested PCRs for the *rps4–trnL* region were performed with internal primers (compare Fig. 1). All amplifications were done in 50 µl-reactions containing 1.5 U *Taq* DNA polymerase, 1 mM dNTPs-Mix each 0.25 mM, 1× buffer, 1.5 mM $MgCl_2$, 10 pmol of each amplification primer, and 1 µl of DNA. Primer sequences and references are listed in Table 3. Amplification cycles for all reactions were as follows: 2 min at 94 °C, followed by 30 cycles each with 2 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C, and a final 7 min extension step at 72 °C. Amplified *trnS–rps4* and *trnL-F* products were directly cleaned using spin filter columns (PCR Clean-up DNA Purification Kit,

Table 1
Several systematic treatments of the Grimmiaceae/Ptychomitriaceae complex

Limpricht (1885–1890)	Brotherus (1901–1909)	Brotherus (1909, 1925)	Churchill (1981)	Buck and Goffinet (2000)	Goffinet and Buck (2004)	This study
Campylosteliaceae	Grimmiaceae	Grimmiales	Grimmiaceae	Grimmiales	Grimmiales	Grimmiales
Campylostelium	Orthotrichaceae	Grimmiaceae	Grimmioideae	Grimmiaceae	Grimmiaceae	Campylosteliaceae
Brachydontium	*Aulacomitrium*	Scoulerioideae	"Guembelia"	*Aligrimmia*	*Aligrimmia*	*Campylostelium*
Grimmiaceae	(=*Glyphomitrium*)	*Scouleria*	"Rhabdogrimmia"	*Coscinodon*	*Bucklandiella*	Grimmiaceae
Cinclidonteae	Ptychomitrieae	Grimmioideae	*Grimmia*	*Coscinodontella*	*Codriophorus*	*Dryptodon*
Cinclidotus	*Glyphomitrium*	*Coscinodon*	*Schistidium*	*Dryptodon*	*Coscinodon*	*Grimmia*
Grimmieae	*Ptychomitrium*	*Indusiella*	*Hydrogrimmia*	*Grimmia*	*Coscinodontella*	*Racomitrium*
Schistidium	*Euglyphomitrium*	*Aligrimmia*	Coscinodontoideae	*Indusiella*	*Dryptodon*	*Schistidium*
Coscinodon	(=*Glyphomitrium*)	*Grimmia*	*Coscinodon*	*Jaffueliobryum*	*Grimmia*	Ptychomitriaceae
Grimmia	*Campylostelium*	*Schistidium*	*Jaffueliobryum*	*Leucoperchaetium*	*Guembelia*	*Aligrimmia*
Dryptodon	Scoulerieae	*Racomitrium*	*Indusiella*	*Racomitrium*	*Hydrogrimmia*	*Indusiella*
Racomitrium	*Scouleria*	Isobryales	*Aligrimmia*	*Schistidium*	*Indusiella*	*Jaffueliobryum*
Ptychomitrieae	Grimmieae	Ptychomitriaceae	Ptychomitrioideae	Ptychomitriaceae	*Jaffueliobryum*	*Ptychomitrium*
Brachysteleum	*Coscinodon*	*Campylostelium*	*Racomitrium*	*Campylostelium*	*Leucoperchaetium*	*Incertae sedis*
(*Ptychomitrium*)	*Indusiella*	*Ptychomitrium*	*Campylostelium*	*Glyphomitrium*	*Niphotrichum*	*Glyphomitrium*
(*Glyphomitrium*)	*Grimmia*	*Glyphomitrium*	*Ptychomitrium*	*Ptychomitriopsis*	*Orthogrimmia*	*Leucoperichaetium*
	Grimmia		*Incertae sedis*	*Ptychomitrium*	*Racomitrium*	
	Schistidium		*Glyphomitrium*		*Schistidium*	
	Racomitrium				*Streptocolea*	
					Ptychomitriaceae	
					Campylostelium	
					Ptychomitriopsis	
					Ptychomitrium	

The systematic arrangement suggested by our data is presented as this study, with families arranged in alphabetical order. Under *incertae sedis* we include *Leucoperichaetium*, a very rare taxon not treated in this study, and *Glyphomitrium*, for which our results are not concluding. Goffinet and Buck (2004) treatment follows the systematic arrangement proposed by Ochyra et al. (2003) on a worldwide basis.

Table 2
List of the species included in the analysis with the voucher's reference and GenBank accession number for each particular molecular region, as well as the geographic origin of the specimens

Species	Voucher herbarium reference	GenBank Accession No.			Geographical origin
		rps4	*rps4–trnL*	*trnL-F*	
Campylostelium pitardii Corb.	MA 19752	DQ399605	**EU246870**	DQ399632	Spain: Almería
Campylostelium strictum (Solms) Kindb.	MA 4527	DQ399604	**EU246871**	DQ399631	Portugal: Marvao
Crossidium davidai Catches.	MUB 5349	DQ399626	**EU246874**	DQ399627	Spain: Canary Islands
Dryptodon (Grimmia) anomalus (Hampe) Loeske	MA 24709	**EU246852**	**EU246877**	**EU246912**	Russia Altay Republic
Dryptodon (Grimmia) austrofunalis (Müll. Hal.) Ochyra & Zarnowiec	MO 5211690	**EU246853**	**EU246878**	**EU246913**	Bolivia: La Paz
Dryptodon (Grimmia) decipiens (Schultz.) Loeske	MA 32764	**EU246855**	**EU246881**	**EU246915**	Spain: Toledo
Dryptodon (Grimmia) patens (Hedw.) Brid.	MO 5142675	**EU246857**	**EU246886**	**EU246917**	USA: Alaska
Dryptodon (Grimmia) torquatus (Drumm.) Brid.	MA 25588	**EU246858**	**EU246887**	**EU246918**	USA: California
Dryptodon (Grimmia) trichophyllus (Grev.) Brid.	MA 25700	DQ399624	**EU246888**	DQ399651	USA: California
Grimmia (Coscinodon) calyptrata (Drumm.) C.E.O. Jensen	MO 5126877	DQ399614	**EU246872**	DQ399641	USA: South Dakota
Grimmia (Coscinodon) cribrosa Spruce	MO 4441357	DQ399615	**EU246873**	DQ399642	USA: Maine
Glyphomitrium humillimum (Mitt.) Cardot	MA 32763	**EU246851**	**EU246875**	**EU246911**	Japan: Kyoto
Grimmia anodon Bruch & Schimp.	MA 25617	DQ399619	**EU246876**	DQ399646	USA: Nevada
Grimmia crinita Brid.	MA 22641	DQ399620	**EU246880**	DQ399647	Spain: Huesca
Grimmia (Hydrogrimmia) mollis Bruch & Schimp.	S B6791	**EU246856**	**EU246882**	**EU246916**	Austria: Tirol
Grimmia ovalis (Hedw.) Lindb.	MO 5217105	DQ399618	**EU246883**	DQ399645	USA: Nevada
Grimmia plagiopodia Hedw.	S B70024	DQ399616	**EU246884**	DQ399643	Sweden: Torne Lappmark
Grimmia pulvinata (Hedw.) Sm.	MA 25026	DQ399617	**EU246885**	DQ399644	USA: California
Grimmia caespiticia (Brid.) Jur.	MA 19713	**EU246854**	**EU246879**	**EU246914**	Spain: Ávila
Indusiella thianschanica Broth. & Müll. Hal.	MO 4435504	**EU246859**	**EU246889**	**EU246919**	China: Qinghai
Jaffueliobryum raui (Austin) Thér.	MO 4420291	**EU246860**	**EU246890**	**EU246920**	USA: New Mexico
Jaffueliobryum wrighti (Sull.) Thér.	MO 3684962	**EU246861**	**EU246891**	**EU246921**	USA: Nebraska
Ptychomitrium drummondii (Wilson) Sull.	MO 5123797	**EU246862**	**EU246892**	**EU246922**	USA: Arkansas
Ptychomitrium formosicum Broth. & Yosuda	MO 5219650	DQ399601	**EU246893**	DQ399628	Taiwan: Taichung Co
Ptychomitrium gardneri Lesq.	MO 5135689	DQ399602	**EU246894**	DQ399629	USA: Idaho
Ptychomitrium sellowianum (Müll. Hal.) A. Jaeger	MO 5215787	DQ399603	**EU246895**	DQ399630	Paraguay: Paraguarí
Racomitrium aciculare (Hedw.) Brid.	MA 22609	DQ399609	**EU246896**	DQ399636	Spain: Cantabria
Racomitrium aquaticum (Schrad.) Brid.	MA 22070	**EU246863**	**EU246897**	**EU246923**	Spain: Santander
Racomitrium carinatum Cardot	MA 21356	DQ399610	**EU246898**	DQ399637	South Korea: Kyonggi-do
Racomitrium crispipilum(Taylor) A. Jaeger	MA 14328	**EU246864**	**EU246899**	**EU246924**	Colombia: Usme
Racomitrium didymum (Mont.) Jaeger	MA 25251	**EU246865**	**EU246900**	**EU246925**	Chile: Región de los Lagos
Racomitrium elongatum Frisvoll	MA 13319	**EU246866**	**EU246901**	**EU246926**	Spain: Palencia
Racomitrium heterostichum (Hedw.) Brid.	MO 5125302	DQ399608	**EU246902**	DQ399635	USA: California
Schistidium apocarpum (Hedw.) Bruch & Schimp.	MA 13294	DQ399611	**EU246903**	DQ399638	Spain: León
Schistidium crassipilum H.H. Blom	MA 14862	**EU246867**	**EU246904**	**EU246927**	Spain: Granada
Schistidium lingulatum Blom	MA 26281	**EU246868**	**EU246905**	**EU246928**	USA: Washington
Schistidium papillosum Culm.	MA 26557	**EU246869**	**EU246906**	**EU246929**	Spain: Lérida
Schistidium rivulare (Brid.) Podp.	MA 20932	DQ399613	**EU246907**	DQ399640	Spain: Palencia
Schistidium trichodon (Brid.) Poelt	MA 7455	DQ399612	**EU246908**	DQ399639	Austria: Totes Gebirge
Syntrichia rigescens (Broth. & Geh.) Ochyra	MUB 11378	AF481037	**EU246909**	DQ400972	Morocco: High Atlas
Tortula atrovirens (Sm.) Lindb.	MUB 11352	AF480990	**EU246910**	AY651833	Spain: Sevilla

Sequences in bold were obtained for this study.

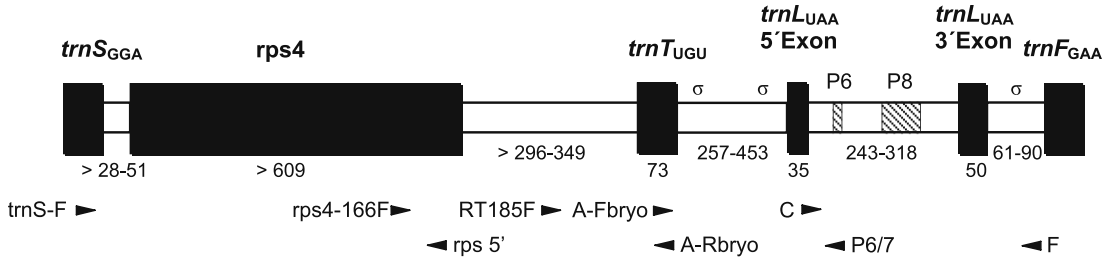

Fig. 1. Overview of the plastid *trnS–trnF* region. Black boxes indicate coding areas whereas the non-coding parts are represented by white boxes. Hatched boxes denote the location of the length variable P6 and P8 domains of the *trnL* intron. Locations of amplification and sequencing primers are specified below. Length variation of the region in the study group is shown below, putative promoter elements are indicate by σ (compare Quandt and Stech, 2003).

Table 3
Primers used in the study

Region amplified	Primer	Sequence 5′–3′	Reference
trnS–rps4	trnS-F	TAC CGA GGG TTC GAA TC	Souza-Chies et al. (1997)
trnS–rps4	rps 5′	ATG TCC CGT TAT CGA GGA CCT	Nadot et al. (1994)
trnL–F	C	CGA AAT CGG TAG ACG CTA CG	Taberlet et al. (1991)
trnL–F	F	ATT TGA ACT GGT GAC ACG AG	Taberlet et al. (1991)
rps4–trnL spacer	rps4-166F	CCA TAA TGA AAA CGT AAT TTT TG	This study
rps4–trnL spacer	P6/7	CAT YGA GTC TCT GCA CCT	Quandt et al. (2004)
rps4–trnL spacer*	RT185F	TCA AAA ACA TCA TAA CAT AAG AGA	This study
rps4–trnT spacer*	A-Rbryo	AGA GCA CCG CAC TTG TAA TG	This study
trnT–L spacer*	A-Fbryo	CAT TAC AAG TGC GGT GCT CT	This study (modification of Taberlet et al., 1991 primer A)

Sequencing primers and/or primers that have been used for nested PCR approaches in cases where the whole fragment could not be amplified are indicated by an ∗.

MoBIO Laboratories, California) following the manufacturers protocols. For the *rps4–trnL* region three to four products were pooled and gel cleaned. Cleaned products were directly sequenced using dye terminators (Big Dye Terminator v 2.0, Applied Biosystems, California). Unfortunately, the amplification of *Aligrimmia peruviana* R.S. Williams and *Indusiella bryanii* (R.S. Williams) S.P. Churchill extracts was unsuccessful, and *Ptychomitriopsis*, synonymized with *Ptychomitrium* by Churchill (1981), includes very rare species hardly ever collected, hence suitable material for DNA sequencing was unavailable.

2.3. Data analysis

Sequences were edited and manually aligned using PhyDE® (Müller et al., 2005) following alignment rules described in Kelchner (2000), Quandt and Stech (2005). Following the approach in Quandt et al. (2003a), Quandt and Stech (2004, 2005), the data matrix was screened for inversions using secondary structure models calculated with RNA structure 4.2 (Mathews et al., 2004). Detected inversions were positionally separated in the alignment. As discussed in Quandt et al. (2003a), Quandt and Stech (2004), presence or absence of detected inversions was not coded for the phylogenetic analyses. However, in order to gain information from substitutions within detected inversions, a second alignment file for the phylogenetic analyses was generated with the inversions included as reverse complemented. Alignments are available from www.treebase.com.

For phylogenetic inference, all characters were given equal weight and gaps were treated as missing data. Parsimony analyses were conducted using *win*PAUP∗4b10 (Swofford, 2002) and PRAP (Müller, 2004). The latter generates command files for PAUP that allow parsimony ratchet searches as designed by Nixon (1999) for analysis of large data sets. In the present study, 10 random addition cycles of 200 ratchet iterations each were used. Each iteration comprised two rounds of TBR branch swapping, one on a randomly re-weighted data set (25% of the positions), and the other on the original matrix saving one shortest tree. Since each random addition cycle rapidly converged to the same tree score, cycles were not extended to more than 200 iterations, nor were further cycles added. Shortest trees collected from the different tree islands were used to compute a strict consensus tree. Furthermore the data set was analyzed employing a simple indel coding (sic) approach as advocated by Simmons and Ochoterena (2000) using the PAUP command file generated by SeqState (Müller, 2005) and the same options in effect.

Internal branch support was estimated by heuristic bootstrap searches with 1000 replicates and 10 addition sequence replicates per bootstrap replicate. Decay values as a further measurement of support for the individual clades were obtained using PRAP in combination with PAUP with the same options in effect as for the ratchet.

Maximum likelihood analyses were executed assuming a general time reversible model (GTR + I + Γ), and rate variation among sites following a gamma distribution (four

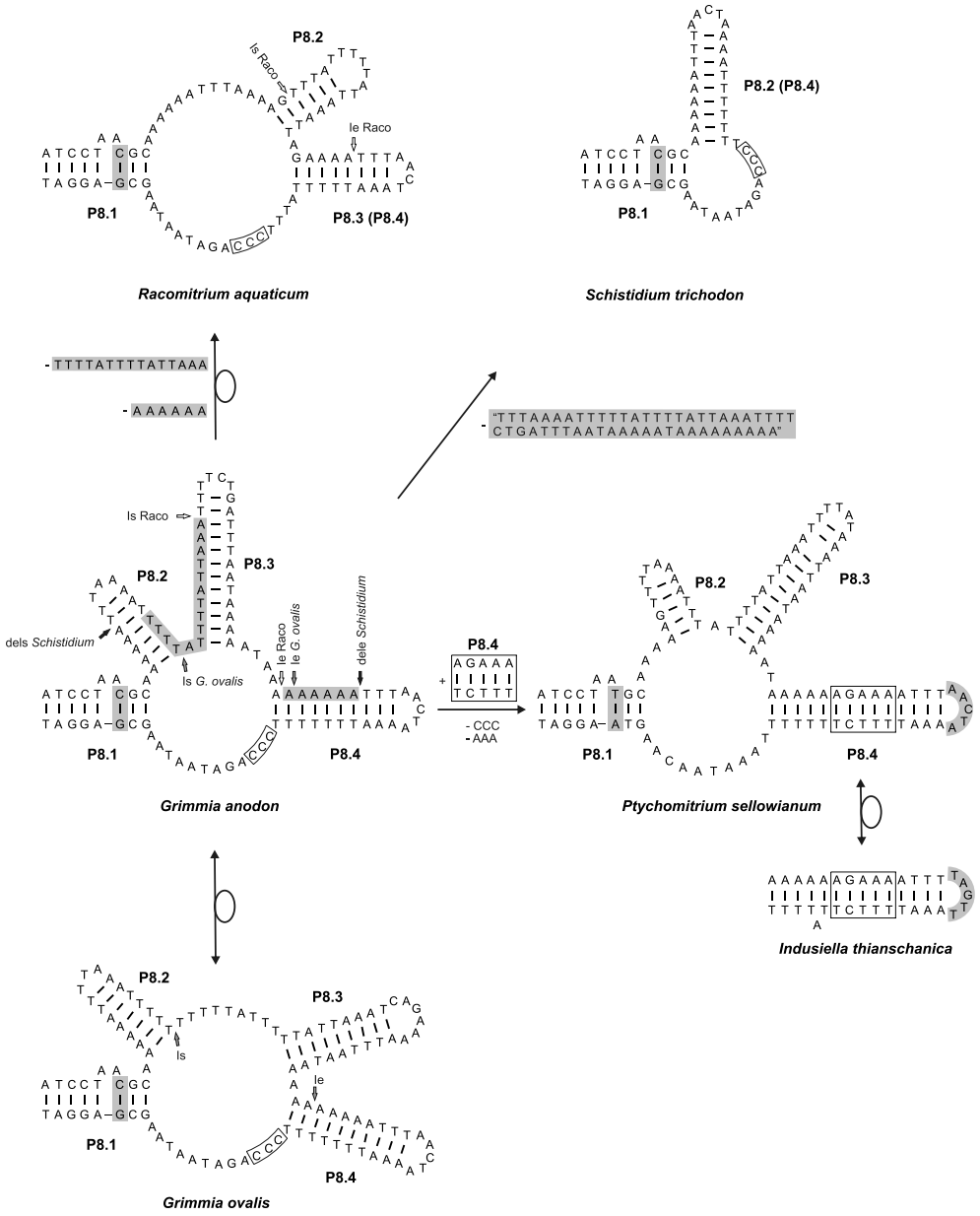

Fig. 2. Taxon or lineage specific P8 secondary structure models. All structures can be inferred by a few inversions, insertions, and deletions events or combinations thereof from the common and, according to the phylogenetic analyses, ancestral type shared by the outgroups and the majority of ingroup taxa. Arrows with a circle on top indicate inversion events. Paired regions annotations in brackets indicate the homolog paired region in the common structure. Is, inversion start; Ie, inversion end; dels, deletion start; dele, deletion end; Raco, *Racomitrium*.

categories represented by mean). GTR + I + Γ was chosen as the model that best fitted the data according to the Akaike Information Criterion by Modeltest v3.6 (Posada and Crandall, 1998) employing the Windows® interface MTgui (Nuin, 2005). The settings proposed by Modeltest v3.6 were executed in PAUP∗ 4.0b10. For the combined data set the following settings were used: BaseFreq = (0.4109 0.1016 0.1060), Nst = 6, Rmatrix = (0.7745 2.3907 0.2275 0.8774 2.3907), Shape = 1.2555, and Pinvar = 0.4614.

For further measurement of support, posterior probabilities were calculated using MrBayes v3.1 (Huelsenbeck and Ronquist, 2001) employing the GTR model of nucleotide substitution, assuming site-specific rate categories follow-

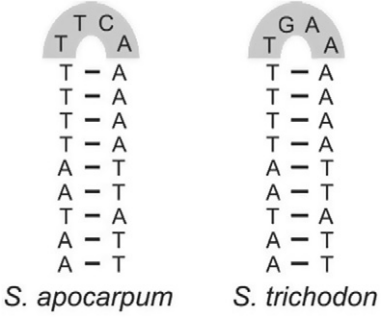

Fig. 3. Example of a hairpin associated inversion (inversion 1) as randomly found in *Schistidium* and *Grimmia* (compare Table 4 and Figs. 4 and 5).

ing a gamma distribution and a proportion of invariable sites. In addition, an independent analysis with an appended indel matrix was performed employing the binary model for the indel partition. The *a priori* probabilities supplied were those specified in the default settings of the program. Posterior probability (PP) distributions of trees were created using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method and the following search strategies suggested by Huelsenbeck et al. (2001, 2002). Four runs with four chains each were run simultaneously for 10^6 generations each run, with the temperature of the heated chains set to 0.2. Chains were sampled every

Table 4
Alignment and distribution of the inversions 1 and 4 detected in the data set

species	*trnT-L* spacer alignment position : 1280-1303	*trnL-F* spacer alignment position : 2023-2044
Glyphomitrium humillimum	----------TTTTAGTTAAA----------	
Crossidium davidai	----------	
Tortula atrovirens	----------	
Syntrichia rigescens	----------	
Jaffueliobryum wrightii	----------TTTTAGTTAAA----------	
Jaffueliobryum raui	----------TTTTAGTTAAA----------	
Indusiella thianschanica	----------TTTTAGTTAAA----------	
Ptychomitrium formosicum	----------TTTTAGTTAAA----------	
Ptychomitrium gardneri	----------TTTTAGTTAAA----------	
Ptychomitrium drummondii	----------TTTTAGTTAAA----------	
Ptychomitrium sellowianum	----------TTTAACTAAAA	
Campylostelium pitardii	----------TTTTAGTTAAA----------	
Campylostelium strictum	----------TTTTAGTTAAA----------	
Racomitrium heterostrichum	----------TTTAACTAAA-	
Racomitrium carinatum	----------TTTAACTAAA-	
Racomitrium aciculare	----------TTTAACTAAA-	
Racomitrium elongatum	----------TTTAACTAAA-	
Racomitrium crispipilum	----------TTTAACTAAA-	
Racomitrium didymum	----------TTTAACTAAA-	
Racomitrium aquaticum	----------TTTAACTAAA-	
Dryptodon austrofunalis	----------TTTAACTAAA-	
Dryptodon torquatus	----------TTTAACTAAA-	
Dryptodon decipiens	----------TTTAACTAAA-	
Dryptodon patens	----------TTTAACTAAA-	
Dryptodon trichophyllus	----------TTTAACTAAA-	
Dryptodon anomalus	----------TTTAACTAAA-	
Schistidium apocarpum	----------TTTTTTCAAAAA----------TTTAACTAAAA	
Schistidium trichodon	TTTTTGAAAAAA----------TTTAACTAAAA	
Schistidium rivulare	----------TTTTTTCAAAAA----------TTTAACTAAAA	
Schistidium lingulatum	----------TTTTTTCAAAAA----------TTTAACTAAAA	
Schistidium crassipilum	----------TTTTTTCAAAAA----------TTTAACTAAAA	
Schistidium papillosum	----------TTTTTTCAAAAA----------TTTAACTAAA-	
Grimmia cribrosa	TTTTTGAAAAAA----------TTTAACTAAAA	
Grimmia calyptrata	TTTTTGAAAAAA----------TTTAACTAAAA	
Grimmia mollis	----------TTTAACTAAAA	
Grimmia plagiopodia	----------TTTTTTCAAAAA----------TTTAACTAAAA	
Grimmia pulvinata	----------TTGTTTCAAAAA----------TTTAACTAAAA	
Grimmia ovalis	----------TTTTTTCAAAAA----------TTTAACTAAAA	
Grimmia anodon	TTTTTGAAAAAA----------TTTAACTAAAA	
Grimmia caespiticia	TTTTTTTAAAAA----------TTTAACTAAAA	
Grimmia crinita	----------TTTTTTCAAAAA----------TTTAATTAAAA	
	typ A typ B inversion 1	typ A typ B inversion 4

The alignment position for each inversion is indicated. In both cases the reverse complement of each particular block derivates in the subsequent particular block.

10 generations and the respective trees written to a tree file. Calculation of the consensus tree and of posterior probabilities of clades was based on the trees sampled after the burn-in (we used a 25% criterion as default). Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph (Müller and Müller, 2004).

3. Results

3.1. Molecular evolution

The combined aligned data set (*trnS–rps4–trnT–trnL–trnF*) comprised 2359 positions, with five observed inversion that were positionally separated in the original alignment. Three of the inversions were directly associated with structural changes of the P8 stem-loop region of the *trnL* intron as illustrated in Fig. 2, whereas the other two inversions are associated with hairpins located in the *trnT-L* (Fig. 3 and Table 4) or the *trnL-F* spacer (not shown), respectively. The inversion located in the *trnT-L* spacer (inversion 1, cf. Table 4 and Fig. 3) affected the *Grimmia–Hydrogrimmia–Schistidium–Coscinodon* complex, and included two reverse complementary sequences spanning 12 nucleotides (positions 1280–1303). Inversion 2, involving the alignment positions 1903–1935 and 1955–2013, was confined to the *Racomitrium* clade (Fig. 2); inversion 3 (positions 1944–2015) was autapomorphic for *Grimmia ovalis* and affected almost the complete P8 stem-loop region (Fig. 2); inversion 4 (positions 2023–2044) distinguished the Grimmiaceae from the remainder but was shared with

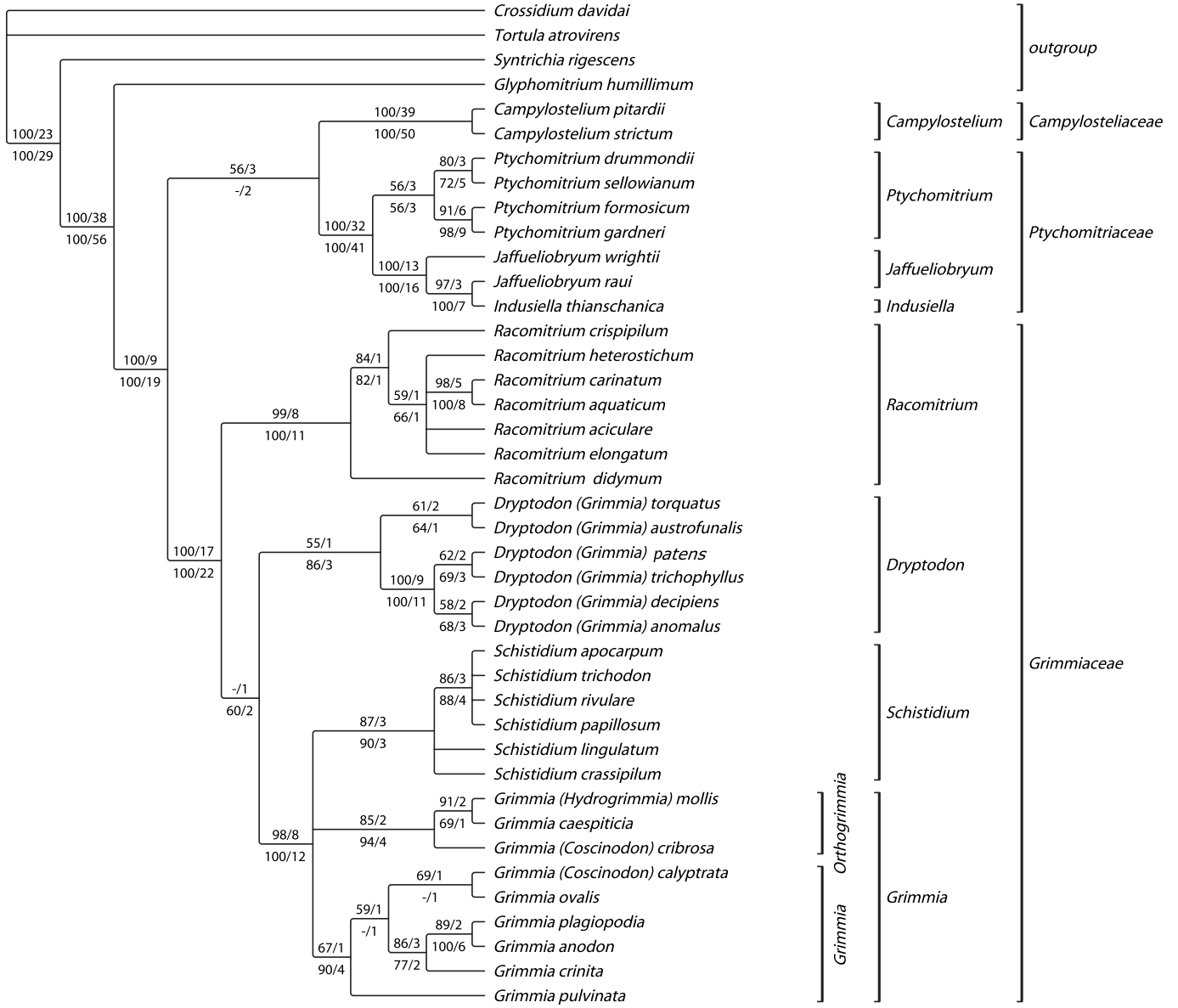

Fig. 4. Strict consensus tree of 79 most parsimonious trees (length = 1073, CI = 0.674, RI = 0.818, RC = 0.551). Bootstrap support (left) and decay values (right) without indel coding are shown above the branches, and with indel coding below the branches. Taxa indicated to the right follow the systematic arrangement proposed in this study.

Table 5
Summary of sequence length, divergence and proportional contribution of the different regions to the data matrix as well as ti/tv ratios number and distribution of indels and inversions

Character set	No. of characters	Length range	Mean	SD	Uncorrected		Corrected		Uncorrected		Corrected		Uncorrected	Corrected	Corrected	No. of indels	No. of inversions
					Divergence [%]	SE	Divergence [%]	SE	ti/tv	SE	ti/tv	SE	Variable [%]	Variable [%]	Informative [%]		
trnS–rps4 spacer*	75	28–51	37.5	7.05	11.833	2.925	11.833	3.103	1.004	0.519	1.004	0.532	28	28	14.667	8	0
rps4	609	609	609	0	4.082	0.407	4.082	0.387	3.926	0.838	3.926	0.936	23.153	23.153	15.435	0	0
rps4–trnT spacer	455	296–349	323.9	11.38	9.731	0.894	9.731	0.883	1.423	0.313	1.423	0.339	31.429	31.429	21.099	81	0
trnT	73	73	73	0	0.535	0.225	0.535	0.226	—	—	—	—	8.219	8.219	2.74	0	0
trnT–trnL spacer	445	257–445	291.3	26.79	6.722	0.713	6.723	0.706	1.06	0.272	1.063	0.243	27.133	27.865	17.287	75	1
trnL 5′exon	35	35	35	0	0.408	0.228	0.408	0.232	0.051	—	0.051	—	8.571	8.571	0	0	0
trnL-intron	371	243–318	290.6	20.16	5.042	0.684	5.069	0.646	2.408	0.67	2.288	0.618	20.814	24.259	12.896	64	3
trnL 3′exon	50	50	50	0	0.278	0.277	0.278	0.28	0	—	0	—	2	2	2	0	0
trnL–trnF spacer	111	62–90	65.6	4.68	13.615	2.288	13.675	2.184	1.991	0.791	1.689	0.723	30.081	30.631	24.39	18	1
*trnF**	40	40	40	0	1.17	0.657	1.17	0.66	0.216	—	0.216	—	10	10	5	5	0	0
	∑ 2264															**∑246**	**∑5**

The uncorrected values refer to the original alignment, whereas the corrected values are based on the matrix with the inversions included as reverse complement.

Ptychomitrium sellowianum, indicating the homoplastic nature of its occurence. Finally, inversion 5 (positions 2210–2256) was located directly after the *trnL* 3′exon in the *trnL-F* spacer and differs from the previously recorded *trnL-F* inversion observed in pleurocarpous mosses (Quandt et al., 2003b; Quandt and Stech, 2004). Interestingly, the inversion of the hairpin formed by the putative sigma promotor elements in front of *trnF* (Quandt and Stech, 2004; Quandt et al., 2004) was not observed in the present data set. Except inversion 2, defining the *Racomitrium* species, all inversions were homoplastic and thus reduced tree resolution, which is in agreement with previous results (Quandt et al., 2003b; Quandt and Stech, 2004).

Secondary structure calculations of the *trnL* intron P8 region revealed a simple multi-loop structure with, apart from the closing helix P8.1, three additional paired regions (P8.2–P8.4) generally common for all taxa included in the study that is represented by the structure calculated for *Grimmia anodon* (Fig. 2). Compared to the Grimmiaceae the outgroups as well as *Glyphomitrium humillimum*, Ptychomitriaceae and Campylosteliaceae lack a CCC element in the multi-loop structure that is specific to the Grimmiaceae (Fig. 2). Apart from the autapomorphic inversion found in *G. ovalis* that affected almost the entire P8, major deviations of the calculated structures are generally specific to inferred clades, such as for *Racomitrium*, *Schistidium*, or Ptychomitriaceae and can be explained as derivates from the common structure as represented in *G. anodon*. For example, in the Ptychomitriaceae basically the same structure as in the Grimmiaceae and Campylosteliaceae is found, but P8.4 is extended by the insertion of two paring repeats in the middle of the hairpin (Fig. 2) that according to the phylogenetic analyses (Fig. 4 and 5) were partly lost again in *Ptychomitrium formosicum* and *Jaffueliobryum wrightii*. *Indusiella* and *Jaffueliobryum* share the same P8 structure with the other Ptychomitriaceae. Here, the structure for *P. sellowianum* was chosen as it shares the inversion type B in the hairpin loop of P8.4 (inversion 4, Table 4) with the Grimmiaceae, whereas all other Ptychomitriaceae have the inversion type A (represented by *Indusiella thianschanica* below in Fig. 2 and Table 4). The structure for *Schistidium* is characterized by the loss of the original P8.2 and P8.3. Similarly, the *Racomitrium* structure can be explained by a large deletion plus an inversion of large parts of the original P8.3 resulting in the loss of the original P8.2 and P8.3, and the increase of the multi-loop together with the formation of a new P8.2 (Fig. 2). However, in all structures P8.4 (P8.2 in *Schistidium* and P8.3 in *Racomitrium*) is consistently retained. In addition to the observed indels and inversions a compensating base pair change (CBC) in P8.1 was observed (Fig. 2).

Although all non-coding regions displayed considerable length variation, resulting in numerous indels that provided additional information, the spacers displayed a higher relative variability in terms of substitutions as well as indel events compared to the group I intron in *trnL* (Table 5). Interest-

ingly, the relative amount of parsimony informative sites recorded for *trnL* was almost identical to the *rps4* values, indicating the fast evolving nature of the gene (Table 5).

3.2. Phylogenetics

Corrected for inversions the alignment comprised 2264 positions with 567 variable sites of which 371 have been parsimony informative, contributions of each region can be extracted from Table 5. After reverse complementing the inversions one parsimony informative site was lost. The simple indel coding approach yielded another 246 characters of which 152 were parsimony informative (61.79%).

The MP ratchet analysis retained 79 most parsimonious trees (MPT, length = 1073, CI = 0.674, RI = 0.818, RC = 0.551). Fig. 4 depicts the strict consensus tree, in which bootstrap support (left) and decay values (right) are shown above (without indel coding) and below (with indel coding) branches. The maximum likelihood tree (−ln 8887.86914) with bootstrap support indicated above the branches and posterior probabilities below (without/with indel coding) is depicted in Fig. 5. Coding of indels as characters according to Simmons and Ochoterena (2000) generally increased the statistical support for the clades especially at the tips of the tree as nicely illustrated by the example of *Racomitrium* (Fig. 6). Whereas the clade is largely unresolved in the MP analysis without indel coding, it is fully resolved and parts of the tree gain strong support with the sic-matrix appended.

Three clades are maximally supported in all analyses: the first one includes *Campylostelium* (Maximum Parsimony [MP]: 100/100 bootstrap support [bs], 39/50 decay value [dv]; Maximum Likelihood [ML]: 100 bootstrap support [bs]; Bayesian Inference [BI]: 100/100 posterior probability [pp]). It is defined by a 16 nucleotide insertion located at the end of the *trnS* spacer (positions 48–63 in the aligned matrix) and another 11 nucleotides insertion in the *rps4–trnT* spacer (positions 1030–1040). The second includes *Ptychomitrium*, *Jaffueliobryum*, and *Indusiella*

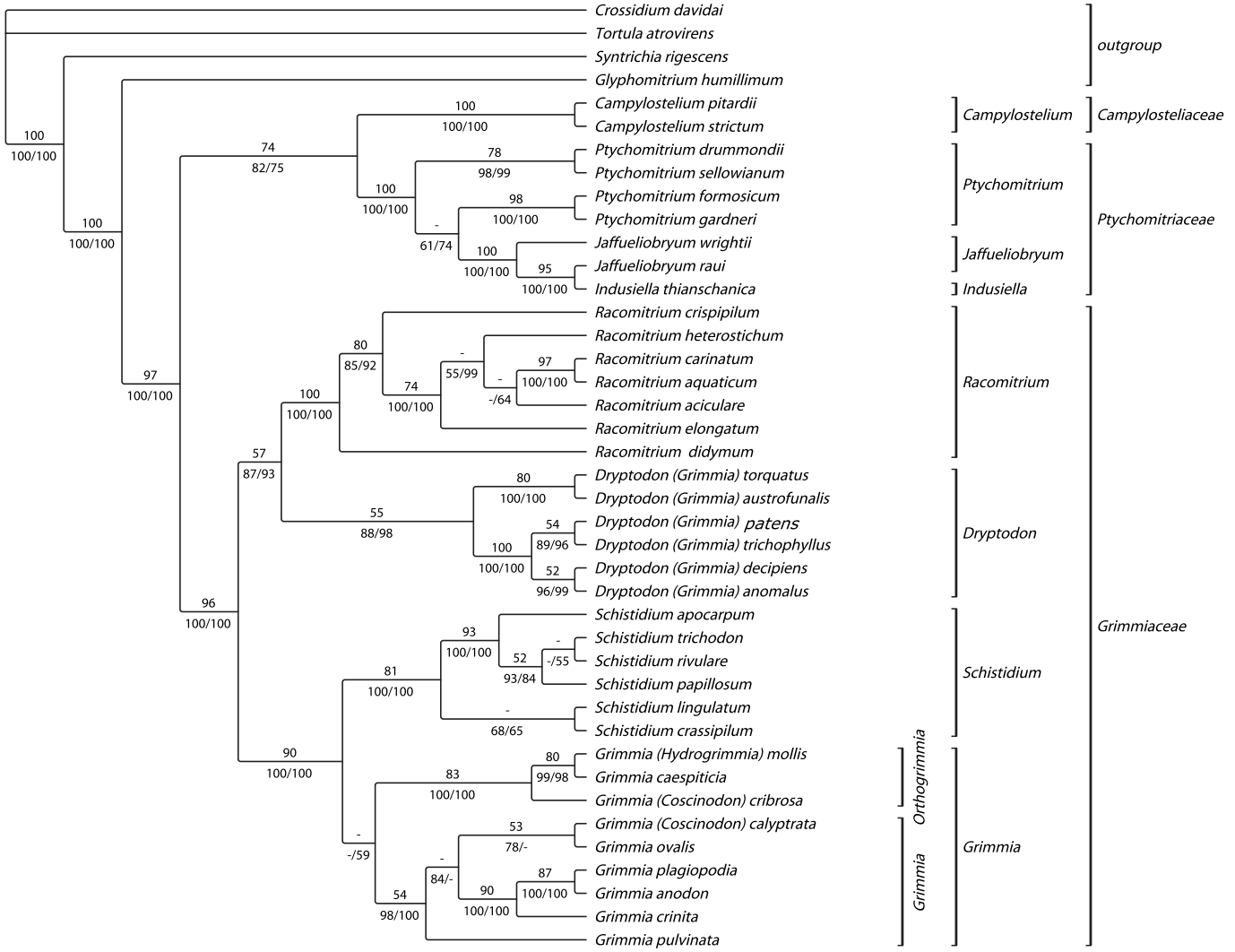

Fig. 5. The maximum likelihood tree (−ln 8887.86914). Numbers above the branches indicate bootstrap support (>50%), while numbers below branches indicate Bayesian posterior probabilities (>50%) with (right) and without (left) indel coding. Taxa indicated to the right follow the systematic arrangement proposed in this study.

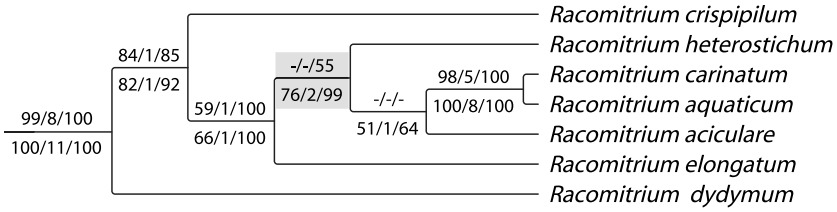

Fig. 6. Detailed summary of the *Racomitrium* clade showing the effect of indel coding on resolution and support values (BS/DV/PP). Support values above were inferred solely with the nucleotide matrix, whereas the values below are based on the nucleotide matrix with the indel matrix appended.

(MP: 100/100 bs, 32/41 dv; ML: 100 bs; BI: 100/100 pp). Finally, the third includes *Coscinodon*, *Grimmia*, *Racomitrium*, and *Schistidium* (MP: 100/100 bs, 17/22 dv; ML: 96 bs; BI: 100/100 pp).

Within Grimmiaceae (Figs. 4 and 5), *Racomitrium* is robustly resolved in a monophyletic clade (MP: 99/100 bs, 8/11 dv; ML: 100 bs; BI: 100/100 pp). The position of the *Dryptodon* clade depends on the analysis employed: with maximum parsimony it is resolved with the *Grimmia–Hydrogrimmia–Schistidium–Coscinodon* clade (Fig. 4), whilst with maximum likelihood or bayesian inference it branches with *Racomitrium* (Fig. 5).

The last clade is strongly supported (MP: 98/100 bs, 8/12 dv; ML: 90 bs; BI: 100/100 pp) in all the analyses. It includes as paraphyletic groups the remaining species of *Grimmia* and *Coscinodon*, with *Hydrogrimmia* nested within as well as a strongly supported monophyletic *Schistidium* clade (MP: 87/90 bs, 3/3 dv; ML: 81 bs; BI: 100/100 pp).

4. Discussion

As illustrated by Figs. 2 and 3 as well as Table 4 applying rapidly evolving non-coding molecular markers for phylogenetic reconstructions is not as straight forward as using rather slow evolving genes displaying low degrees of microstructural change. Length mutations and especially hairpin associated inversions considerably complicate the homology assessment and might mess up the phylogenetic structure of the data set leading to low resolution and unsupported and in the worst case to erroneous trees (cf. Kelchner, 2000; Quandt et al., 2003a). However, using alignment approaches based on repeat recognition (possibly guided by secondary structures) and applying mechanisms of molecular evolution as advocated by Kelchner (2000), Borsch et al. (2003), Quandt and Stech (2005) as well as Quandt et al. (2003b) in alignment construction enables the utilization of more complex evolving regions such as spacers and introns. Though more difficult to treat, the addition of both spacers (*rps4–trnT*, *trnT–trnL*) improved the tree resolution in comparison to a previous study by the same authors (Hernández-Maqueda et al., 2007), especially within the *Grimmia–Hydrogrimmia–Schistidium–Coscinodon* complex. Although we increased the number of taxa in the present study of the Grimmiaceae/Ptychomitriaceae complex, the use of the spacers between *rps4* and *trnL* in combination with *trnS–rps4* and *trnL-F* rendered a better structured and supported topology. Especially, the additional information gained from indels increased the number of parsimony informative sites considerably and overall resulted in higher support values as nicely illustrated in Fig. 4. In contrast to the observed inversions that are highly homoplastic in the present study indels seem to provide a high quality signal that is similar to substitutions (CI indels = 0.656; CI substitutions = 0.674; CI inversions = 0.455).

Our results corroborate previous findings that *Glyphomitrium* is not a member of the complex and suggest a different systematic arrangement of the genera in the Grimmiaceae/Ptychomitriaceae complex different to any previously proposed. In addition our results indicate the need of accepting Campylosteliaceae as an independent family, although its systematic affinities are not yet confidently resolved due reported incongruities when comparing different DNA regions, analysis techniques, and morphological traits around *Campylostelium* (Hernández-Maqueda et al., 2007).

4.1. Glyphomitrium

The exclusion of *Glyphomitrium* from either Grimmiaceae or Ptychomitriaceae is corroborated by our results, although we are not able to yet answer its phylogenetic relationships. Its familial placement has varied widely (Table 1), mostly due to its small size and paucity of distinct morphological characters that allow disentangling its phylogenetic relationships. Based on morphology, Churchill (1981) was the first in removing it from the Grimmiaceae/Ptychomitriaceae complex, although he did not formally propose any alternative placement. His views were corroborated using *rbcL* sequence data by Tsubota et al. (2003), who proposed a close relationship with *Arctoa* Bruch & Schimp. in the Dicranales not refuted yet.

4.2. Campylosteliaceae

In a previous study using *rps4* and *trnL-F*, Hernández-Maqueda et al. (2007) found a conflicting signal regarding the systematic position of *Campylostelium*. Using *trnL-F* *Campylostelium* retained a sister group relationship to the Grimmiaceae, whereas based on *rps4* data it clustered with the Ptychomitriaceae. The addition of the *rps4–trnT* and

trnT–trnL spacers now joined *Campylostelium* sister to the Ptychomitriaceae, but with low support (MP: 56/- bs, 3/2 dv; ML 74 bs; BI: 82/75 pp). Under these circumstances, it seems more appropriate to consider *Campylostelium* in its own family. The family Campylosteliaceae was described by De Notaris (1869) to include only *Campylostelium* which, according to this author, would differ from Ptychomitriaceae and Grimmiaceae in the shining leaves gradually tapering in a subulate apex. This familial arrangement has been only followed by Limpricht (1885–1890), who also included *Brachydontium* Fürnr., which according to recent studies (Goffinet and Buck, 2004; Hedderson et al., 2004) is not related to *Campylostelium* beyond superficial morphological similarities.

4.3. Ptychomitriaceae

According to our results (Figs. 4 and 5), this family should change its composition rather dramatically. Not only *Campylostelium* and *Glyphomitrium* are excluded from it, but *Jaffueliobryum* and *Indusiella* (includes *Coscinodontella*), formerly considered in the Grimmiaceae s. str. are robustly nested within (Table 1).

Although striking, this proposal is supported by two molecular synapomorphies, the presence of a deletion spanning > seven nucleotides in the *rps4–trnT* spacer (positions 860–868), a > seven nucleotide deletion within the hairpin loop P6 of the *trnL* intron (positions 1827–1835) as well as a insertion of a helical element in P8.4 (Fig. 2). Moreover, there are at least two morphological synapomorphies: (1) the costa with well-differentiated cell layers as seen in cross-section (except *Jaffueliobryum*, whose costa is rather reduced and variable, and never has guide-cells sandwiched between two stereid bands), and (2) the cryptocious sexual condition, first demonstrated for *Ptychomitrium* by Deguchi (1977), and later found in *Aligrimmia* and *Indusiella* (Murray, 1984) and *Jaffueliobryum* (Churchill, 1987; Spence, 2006), but unknown in Grimmiaceae s. str., *Glyphomitrium* and *Campylostelium*.

Although solidly resolved in the Ptychomitriaceae, we have to admit that the placement of *Jaffueliobryum* is a little bit odd in the family. First, morphologically it deviates in having a rather boring costa, and two of its three species have merely mitrate, although large, calyptrae, similar if not identical to the calyptrae found in species of the Grimmiaceae. However, in the Grimmiaceae the calyptrae never have the characteristic lobation at the base, which makes them similar to a Hawaiian skirt in the Ptychomitriaceae. Secondly, the two species studied, morphologically very similar, resulted segregated in our analyses (Figs. 3 and 4), with *J. raui* branching with the morphologically very different *Indusiella thianschanica*. The independence of both genera is firmly fastened on morphological grounds: *Jaffueliobryum* species have broadly ovate leaves ended in a hair-point, and rather indistinct costae, while *Indusiella* species have lanceolate, muticous leaves, and a costa with strongly differentiated cell layers. The phylogenetic relationships of these genera (and *Aligrimmia*) were already raised by Murray (1984) and Churchill (1987). The incongruence we found could derive of incomplete sampling: our original design did not include *J. arsenei* (Thér.) Thér., and all attempts to sequence *Aligrimmia peruviana* and *I. bryanii*, which would help to resolve the relationships of this small group of species were in vain.

4.4. Grimmiaceae

The clade joining the Grimmiaceae s. str. genera is maximally supported in all analyses (Figs. 4 and 5). Morphologically, the family is characterized by leaves with sinuose cell walls and costae of Kawai (1968) type A, B, or C (in *Glyphomitrium*, Campylosteliaceae, and Ptychomitriaceae they are of type D or E), and outer peristome layer thicker than the inner layer (equally thickened in *Glyphomitrium*, Campylosteliaceae, and Ptychomitriaceae). Within the family, MP, ML, or Bayesian methods clearly show that *Racomitrium* and *Schistidium* are well supported monophyletic genera, while *Coscinodon* and *Grimmia* are non-monophyletic taxa. The circumscription of the genera in the family are subject to controversy after the rather revolutionary system proposed by Ochyra et al. (2003), who presented a very detailed account of the history of the taxa they accept at generic rank.

Whatever the taxonomic rank is considered to be, *Racomitrium* is a morphologically well-characterized taxon that in this study appears maximally supported in all analyses. In addition the genus is well defined by several molecular peculiarities such as a synapomorphic inversion of a large P8 fraction in combination with two considerable deletions or a ten nucleotide deletion in the *rps4–trnT* spacer (positions 1027–1047). In addition, *Racomitrium* species share several morphological synapomorphies, like the cladocarpous habit, the sinuose and porose cell walls of the vaginula, and the strongly sinuose-nodulose basal leaf cells. Recently, it was split in four genera (Ochyra et al., 2003; followed by Goffinet and Buck (2004), (cf. Table 1), a proposal that appears to be well supported on morphological grounds. *Racomitrium* has been included most often in the Grimmiaceae, although several authors (e.g., Jones, 1933; Churchill, 1981) have considered it more closely related to *Ptychomitrium* as both share some peristome characteristics, like the divided teeth and the presence of a basal membrane. Our results and the fact that both of these characters are also present in *Grimmia* s. lat. firmly anchor it within Grimmiaceae, though.

Dryptodon has been treated usually as an intermediate genus between *Grimmia* and *Racomitrium* (Crundwell, 1971; Deguchi, 1978; Smith, 1978), sharing with the first the leaf areolation, seta posture and capsule morphology, and with the latter the general habit and the structure of the peristome, deeply divided in two prongs and with a basal membrane. Some authors did not consider it at any rank, but as synonym to *Grimmia* (Nyholm, 1998; Muñoz and Pando, 2000; Greven, 2003; Ignatov and Ignatova,

2003; Hill et al., 2007). After Ochyra et al. (2003), the genus has gained acceptance and included the species formerly treated as *Grimmia* subg. *Rhabdogrimmia* (Goffinet and Buck, 2004; Hedderson et al., 2004). According to Ochyra et al. (2003, pp. 118–121), *Dryptodon* is characterized by the variously curved setae, symmetric and mostly ribbed capsules, recurved leaf margins, and leaf costa protruding in dorsal side, although this definition is not without problems. Our results corroborate the paraphyletic nature of *Grimmia* (Hedderson et al., 2004; Streiff, 2006), which supports the recognition of *Dryptodon* as an independent genus, but considerably more restricted in the number of species included as well as in the characters which define it. The present study is however focused on the familial relationships, and not in resolving the phylogeny of *Grimmia* s. lat. (i.e., *Grimmia*, *Dryptodon*, *Guembelia*, *Hydrogrimmia*, *Orthogrimmia*, and *Streptocolea*, in the sense of Ochyra et al., 2003) that will be treated exclusively and in depth in a forthcoming paper by the same authors.

The results in the present study are in agreement with the view of a genus intermediate between *Grimmia* and *Racomitrium*. When the data are analyzed under MP (Fig. 4), *Dryptodon* branches with *Grimmia–Hydrogrimmia–Schistidium–Coscinodon* complex, although poorly supported. In contrast, when maximum likelihood or Bayesian methods are used (Fig. 5), it is resolved next to *Racomitrium*. Apart from shared substitutions *Racomitrium* and *Dryptodon* are linked by a 13 base insertion in the *trnT–trnL* spacer (positions 1314–1326). As a molecular synapomorphy, all *Dryptodon* share a 16 base insertion at the end of the *trnS* spacer (positions 29–44 in the matrix). In contrast, morphologically the genus is difficult to define beyond the presence of vegetative reproduction by specialized gemmae (Streiff, 2006). Interestingly these are also present in a peculiar *Racomitrium* species (*R. vulcanicola* Frisvoll & Deguchi).

The clade including the *Grimmia–Hydrogrimmia–Schistidium–Coscinodon* complex is strongly supported in all the analyses (Figs. 4 and 5). It includes very similar taxa in terms of sequence variation, and the branch lengths are also similar when analyzed under likelihood methods, which could be the result of rapid radiation processes. Morphologically, they differ in sporophytic traits, but have very similar gametophytes; therefore they have been treated as closely related taxa. Even relatively recent treatments have considered them as members of an encompassing *Grimmia* (Lawton, 1971; Crum and Anderson, 1981; Noguchi, 1988; Sharp et al., 1994), although latter works have split them in at least three genera: *Grimmia*, *Schistidium*, and *Coscinodon*, and included *Hydrogrimmia* in *Grimmia*. As noted above, Ochyra et al. (2003) proposed a radical division of *Grimmia* and offered an outstanding summary on the historical systematic arrangements involving the taxa around this genus. Subsequent authors either embraced this proposal (Goffinet and Buck, 2004) or rejected it (Allen, 2005), and it is here tested for the first time using molecular data. From Figs. 4 and 5, two obvious conclusions arise: *Schistidium* must be considered as an independent genus, while *Grimmia*, *Hydrogrimmia*, and *Coscinodon* must be combined in one for which the former has priority over the other names.

Schistidium represents a monophyletic lineage strongly supported by the molecular data (Figs. 4 and 5). The main DNA sequence synapomorphy involves a fifty one base deletion in the P8 region of the *trnL* intron (Fig. 2), which support the morphological synapomorphies that separate this genus from *Grimmia*, like the reddish-brick color of the plants, the perichaetial leaves larger than the vegetative ones and of different shape, and—specially—the systylius capsules (columella attached to the operculum and falling with it at capsule dehiscence).

Hydrogrimmia has been considered an independent genus including only *H. mollis* on the basis of soft, unistratose, rounded-obtuse and muticous leaves, and straight setae (Abramova, 1969; Churchill, 1981; Ignatov and Ignatova, 2003; Ochyra et al., 2003). However, although its gametophyte is distinctive, caused by the habitat it grows (cold running water), its sporophyte is virtually identical to that of *Grimmia* subg. *Orthogrimmia* (genus *Orthogrimmia* sensu Ochyra et al., 2003), which led other authors to include it in *Grimmia* s. str. (Nyholm, 1998; Muñoz and Pando, 2000; Ignatova and Muñoz, 2004; Norris and Shevock, 2004; Hastings and Greven, 2006). Sequences of cpDNA strongly support the latter view firmly rooting this taxon within *Grimmia* (Figs. 3 and 4).

Coscinodon species have gametophytes identical to species in *Grimmia*, and both genera can only be distinguished by sporophytic traits. Confusions of sterile plants involve thus more often members of different genera: i.e., *Coscinodon cribrosus* is confused with *Grimmia caespiticia*, and *Coscinodon calyptratus* with *Grimmia pulvinata*. Our results suggest that *Coscinodon* has to be merged with *Grimmia*, and also that gametophytic traits are more important than sporophytic to resolve the relationships within *Grimmia*.

Grimmia is a large and difficult genus even after chopping *Dryptodon* and *Schistidium* from it. Inclusion of *Hydrogrimmia* does not add complexity to it, but inclusion of *Coscinodon* increases the variability of sporophyte traits in the encompassing genus considerably. If *Grimmia* should be split in several further genera, as advocated by Ochyra et al. (2003), or maintained as a genus of broader scope, cannot be resolved in the present study: the DNA regions employed were not informative enough at this scale. To clarify the phylogeny of *Grimmia* as proposed in the present study is beyond the scope of a paper like this focused on the familial relationships. A molecular phylogenetic study including more species and more plastid (*trnK-matK*) and nuclear (ITS) genes is now under way.

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